

## Summary of Literature on *Jatropha* Toxicity

By Mike Barbee

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### Background

*Jatropha curcas* is currently being grown in Guatemala by farmers as a biodiesel crop with the support of Technoserve-Guatemala. The bi-product of seed-oil extraction is a nutrient-rich seed cake that contains a fraction of the seed's oil that is known to contain elements toxic to mammals and other organisms. There is concern over the application of seed cake as fertilizer due to this toxicity, which effects safe handling/disposal and could have significant impact on the beneficial microbial communities, insects, invertebrates and plant/animal communities.

### Description of toxicity

The toxicity of *jatropha curcas* seeds can be caused by several components, including saponins, lectins (curcin), phylates, protease inhibitors, curcalonic acid, and phorbol esters (PEs) but studies that isolate PEs in toxic and non-toxic strains have determined that PEs are the compounds of concern (Wink et al., 1997). The kernels from seeds contain at least six different PEs (Haas et al., 2002), all of which activate the important cellular target, protein kinase C (PKC), and constitute the most active disruptive components. PEs are diterpenes containing tigliane as a fundamental backbone (Devappa, 2010) and occur naturally in many plants of the family Euphorbiaceae and Thymelaeaceae (Ito *et al.*, 1983). The biological activities of the phorbol esters are highly structure-specific. The phorbol esters, even at very low concentrations, show toxicological manifestations in animals fed diets containing them (see below for references). Due to its interference with cell metabolism, especially with the activators of protein kinase, tumors typically result. Though tumors in general are severely harmful, this activity performed by PEs is vital for cancer research and, indeed, PEs have been extracted for these purposes (Goel et al, 2007).<sup>1</sup>

### What part of the plant is toxic

Esters are among the many compounds that comprise lipids (oils). The oil of *Jatropha curcas* contains the phorbol esters and is concentrated in the seeds, which on average contain approximately 30% oil by mass (Devappa, 2010). Due to the presence of these oils in all parts of the plant, PEs are found in all parts of plant: the plant stem, leaves, and root system. This has make *jatropha* valuable in agriculture as a living fence for keeping cattle and other animals.

### Who/what is it toxic to and what are the effects

The tumor-promoting effect of phorbol esters has been studied in various human and animal cell lines (Goel et al., 2007). Carp (*Cyprinus carpio* L.) were found to be highly susceptible to

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<sup>1</sup> Further Background: The interaction of phorbol ester, TPA (4 $\beta$ -12-*O*-tetradecanoylphorbol-13-acetate) with protein kinase C (PKC) affects activities of several enzymes, biosynthesis of protein, DNA, polyamines, cell differentiation processes, and gene expression. The phorbol esters alter the phosphorylation of specific cellular proteins and increase transcription of certain cellular genes. The mechanism behind tumor production is the interaction with PKC, which further regulates the downstream signaling pathways to induce gene transcription, leading to induction of cell proliferation. The different biological effects of phorbols are structure dependent.

phorbol esters present in *J. curcas* (Goel et al., 2007). The effect of PEs (specifically TPA) was also studied in live guinea pigs whereby the topical application of TPA was reported to induce inflammation and epidermal hyper-proliferation (Goel et al., 2007). PEs were also found to be effective biopesticides against diverse fresh water snails and the diterpene was found to be highly toxic to silkworm larvae after ingestion (Goel et al., 2007). Goats that were fed seeds developed fatty change and necrosis of the liver, hemorrhage in the compound stomach, kidney, lung and heart, catarrhal enteritis, and excessive fluid in the serous cavities (Adam and Maghoub, 1975). Insecticidal activities of oil containing PEs or of concentrated phorbol ester fractions have been recorded for *Manduca sexta*, *Helicoverpa armigera*, *Aphis gossypii*, *Pectinophora gossypiella*, *Empoasca biguttula*, *Callosobruchus chinensis*, *Sitophilus zeamays*, among others (Wink et al., 2007). The isolated toxic fraction of the oil, when applied to the skin of rabbits and rats, produced a severely irritant reaction followed by necrosis (Ghandi et al., 1995). Thus, the ingestion of or contact with jatropha oil poses a severe health hazard to livestock and humans alike, owing to the presence of phorbol ester toxins that are severely irritant to skin and mucous membrane and effect the blood's activities.

### **How toxic is the seed cake containing phorbol esters**

Seeds are often consumed raw and after roasting without ill effects in Mexico (from varieties noted for being non-toxic) and shows that the body can tolerate or detoxify low amounts of phorbol esters. PE concentrations can range from 0.27 (Mexican, non-toxic varieties) to 2.49 (Cape Verde variety) mg g<sup>-1</sup> in defatted kernel meal (Makkar et al., 1997). The higher the amount of phorbol esters, the lower the acceptance of jatropha seeds by animals (Makkar et al., 1997). Studies that show exposure limits for humans working with oil or seed cake have not been conducted and conclusions must be drawn from live experiments done with other animals, which have not been exact on lower threshold limits. A study by Ghandi et al. showed that the acute oral LD<sub>50</sub> of the oil was found to be 6 ml/kg body weight in rats. The isolated toxic fraction, when applied to the skin of rabbits and rats at a dose of 50 µl produced a severely irritant reaction followed by necrosis; in mice, this fraction had a dermally toxic and lethal effect. The oil and the toxic fraction at 25 and 1 mg, respectively, in 10 ml saline disrupted red blood cells. In a study by Ahmed et al., powdered seed was fed to six calves at doses of 2.5 and 0.25 g/kg body mass once and to two other calves at 0.025 g/kg body mass up to 14 days. The onset of toxic manifestations in the six calves fed a single dose was rapid and death occurred within 19 hours of administration. The two calves that received daily the lowest dose of *J. curcas* showed signs of poisoning and died within 10 to 14 days. The goats which received 1 g/kg/day or more (5 and 10 g/kg/day) of *Jatropha* seeds died within 2 to 9 days, while those which received the plant at 0.25 g and 0.5 g/kg/day were either killed in extremis or died within 11 to 21 days (Adams, 1975).

Toxicological databases, such as TOXNET maintained in the United States by the National Library of Medicine does not have information specifically on the intake of PEs by humans. Therefore, the information listed in peer-reviewed literature is the best source for any information on exposure levels. In summary, those studies that show the limits for non-toxic and human tolerable exposure amounts serve as the only lower threshold limit for ingesting jatropha and those studies on lab mammals give the lower limit for topical exposure. There

are no studies known that show the exposure effects of handling jatropha seedcake in any type of agricultural setting.

Further studies have been recommended to evaluate the eco-toxicity of PEs (particularly the extent of leaching of PEs from different soils), as well as the effects of PEs and their degraded products on water channels and water bodies (Goel et al., 2007).

### **How can it be detoxified**

The naturally occurring phorbol esters are unstable and are susceptible to oxidation, hydrolysis, transesterification, and epimerization during isolation procedures (Haas et al., 2002). The most simple and cost effective method is the biochemical degradation process during the aerobic composting process. A study by Devappa demonstrated that toxicity of seedcake exposed to air diminished to negligible levels after 30 days in all experiments (2010).

#### *Solid State Fermentation*

Under optimized solid state fermentation conditions deoiled seed cake at a water ratio of 1:1, inoculum size 1.5 ml/5 g of deoiled *J. curcas* seed cake, a temperature of 30°C at 65% relative humidity, *P. aeruginosa* PseA completely degraded phorbol esters in nine days.

#### *Heat Treatment*

Solvent extraction of phorbol esters followed by heat treatment to inactivate lectins in *Jatropha* seed meal was reported to convert the nontoxic meal to a high-quality protein source for livestock (Makkar et al., 1997). Heat treatment of defatted seed cake at 121°C did not effect phorbol ester concentrations and structure (Aderibigbe et al., 1997).

#### *Bioconversion by *Hyles euphorbiae* larvae*

*Hyles euphorbiae* larvae are reported to detoxify TPA when administered orally; these larvae were able to metabolize nearly 70% to 90% of the phorbol ester and about 10% to 30% was retained and recovered in the feces (Hundsdoerfer et al., 2005).

#### *Digestion and bioconversion by Liver Enzymes*

Liver carboxylesterase are reported to detoxify the phorbols (Mentlein, 1986).

#### *Aerobic composting*

PEs are completely biodegradable in soil and their degraded products appear to be innocuous (Devappa, 2010). The degradation of PEs in the soil depends on the temperature and the moisture levels. In all samples, PEs were fully degraded by day 23, with a minimum of 12 days for seedcake mixed with soil at both high moisture level temperature (42°C). In addition, spectra of the prominent peaks from the chemically oxidized (chromic acid) and enzymatically oxidized (in soil) were similar. *These observations suggest that the PEs in soil are degraded by microbial enzymes involving oxidation as one of the intermediate steps.* In the soil, these intermediate oxidized products are further degraded with increase in incubation time (Devappa, 2010).

### **Plant uptake of toxicity and transfer to fruits**

The uptake and exhibition of phorbol esters in fruit-bearing plants that have received jatropha seed cake as a fertilizer with active PEs has not been studied. Jatropha seed cake has been widely used as a fertilizer in various parts of the world but because there is no mention and systematic study of its toxicity being transferred and concentrated in plants, there can be no conclusions drawn.

### **Toxicity through inhalation**

There are no studies about jatropha the fraction of phorbol esters that may be present during pressing, composting, or burning. There is not enough information about the volatility to estimate the partitioning of oil that may contain esters in the air during the processing and application

### **Handling *Jatropha Curcas* seed cake**

There are currently no guidelines for handling *Jatropha curcas* seedcake, nor is the seed cake or phorbols listed as toxic substances or chemicals/substances of concern. Due to the findings presented above, jatropha seed cake that has not been stripped of its phorbol esters needs to be treated with the utmost caution. Acute toxicity levels in humans has not been studied but the many studies performed on animals demonstrates that the seedcake is likely to cause serious problems when ingested even at low doses of approximately .27 mg/g (see above). The handling of seedcake so that it comes in contact with bare skin needs to be taken seriously since there have been no formal studies performed using jatropha seedcake or soil mixed with the soil as the source of the phorbol esters on skin. Based on experiments with the topical application of the oil, acute levels of dermal exposure to humans appear to be of little concern but no studies have been conducted on chronic exposure due to prolonged exposure of handling seedcake or oil. ***It is recommended at this point, that those who are working directly with oil and seed cake during the pressing process wear protective eye and skin covering and, if possible, surgical masks to reduce inhalation.***

### **References**

1. Adams, E.I. and Magzoub, M. (1975). Toxicity of *Jatropha curcas* on goats. *Toxicology*: 4347-354.
2. Aderibigbe A.O., Johnson, C., Makkar, H.P.S., Becker, K., Foidl, N. (1997). Chemical composition and effect of heat on organic matter- and nitrogen-degradability and some antinutritional components of *Jatropha* meal. *Animal Feed Science and Technology*, 67: 223-43.
3. Ahmed, O.M.M. and Adam, S. E. I (1979). Effects of *Jatropha curcas* on calves. *Vet Pathol.* 16: 476.
4. Devappa, R.K. (2010). Biodegradation of *Jatropha curcas* phorbol esters in soil. *Journal of the science of food and agriculture*, v. 90, no. 12, pp. 2090.

1. Duke, J.A. (1988). CRC Handbook of Medicinal Herbs. CRC Press, Boca Raton, FL, pp. 253–254
2. Gandhi, V.M., Cherian, K.M., Mulky, M.J. (1995). Toxicological studies on ratanjyot oil, Food and Chemical Toxicology, Volume 33, Issue 1, January, Pages 39-42.
3. Goel, G., Makkar, H.P.S., Francis, G. and Becker, K., (2007). Phorbol esters: Structure, biological activity and toxicity in animals. *Int J Toxicol* 26:279–288.
4. Haas, W., Sterk, H., and Mittelbach, M., Novel (2002). 12-deoxy-16-hydroxyphorbol diesters isolated from the seed oil of *Jatropha curcas*. *J Nat Prod*, 65:1434–1440.
5. Hundsdoerfer, A. K., Tshibangul, J.N., Wetterauer, B., and Wink, T. (2005). Sequestration of phorbol esters by aposematic larvae of *Hyleseuphorbiae* (Lepidoptera: Sphingidae). *Chemoecology*, 15:261–267.
6. Ito Y., Yanase S., Tokuda H., Krishishita M., Ohigashi H., Hirata M. and Koshimizu K. (1983). Epstein Barr virus activation by tung oil, extracts of *Aleuritesfordii* and its diterpene ester 12-O-hexadecanoyl-16-hydroxyphorbol-13-acetate. *Cancer Letters* 18, 87-95.
7. Joshi, C, Mathur, P., and Khare, S.K. (2011). Degradation of phorbol esters by *Pseudomonas aeruginosa* PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. *Bioresource Technology*, Volume 102, Issue 7.
8. Kingsbury, J.M., (1964). Poisonous plants of the United States and Canada, Prentice-Hall.
9. Makkar, H. P. S., and K. Becker. (1997). Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *J. Agric. Food Chem.* 45:3152–3157.
10. Mentlein, R. (1986). The tumor promoter 12-O-tetradecanoyl phorbol 13-acetate and regulatory diacylglycerols are substrates for the same carboxylesterase. *J. Biol. Chem.* 261:7816–7818.
11. Sastri B. N. (1959) *The Wealth of India, Ran' Materials*. pp. 293 295. Council of Scientific and Industrial Research, New Delhi.
12. Wink, M., C.Koschmieder, M. Sauerwein, and F. Sporer. (1997). Phorbol esters of *Jatropha curcas*—Biological activities and potential applications. *Biofuel and industrial products from Jatropha curcas*, ed. by Ubitz, 160–166. Graz, Austria: Dbv-Verlag University of Graz.