Jatropha curcas is an oil-seed plant with good adaptability to grow in unfavourable conditions like infertile soil with scanty rainfall. It had been exploited for the extraction of oil for bio-diesel. The compressed seed cake, after the oil extraction, is a rich source of protein with certain toxic and anti-nutritional factors. The major toxins in the seed cake are phorbol esters and trypsin inhibitors that lead to various health problems if ingested. Even though the application of the various extracts carries a lot of beneficial advantages, yet the toxicity in oil and the compressed cake does not allow the by-products and the oil to be used elsewhere. Various physicochemical and biological methods have been described for the detoxification of Jatropha seed cake and oil of which the chemical extraction with methanol and ethanol have shown promising results in reducing the toxin contents by 97-100% while UV-irradiation reduced the phorbol esters completely. Submerged fermentation by Bacillus sp. achieved complete detoxification of phorbol esters within a week. A new strain was found to degrade the phorbol esters to phorbol, myristic acid and acetic acid within 12 h of incubation in submerged fermentation process. The detoxified products, in future, can be used as animal feed and food supplement to help utilize the by-products as a healthy diet.

Keywords: Jatropha curcas, Phorbol esters, Biodegradation, Submerged fermentation, Detoxification

INTRODUCTION

Jatropha is an oilseed plant belonging to Euphorbiaceae family which has gained remarkable interest as a raw material for biodiesel industries. It is one of the crops being highly studied for the production of biodiesel for the reason that it can be grown in unfavourable climatic regions like unfertile soils and little rainfall. The Jatropha seed contains approximately 30–35% oil that can be converted into high-quality biodiesel upon transesterification and can be used as a substitute for diesel fuel [1]. The seed cake remaining after oil extraction is an excellent source of plant nutrients which is rich in lignocellulosic compounds, water, minerals and proteins but at the same time it contains many toxic and anti-nutritional compounds such as phorbol esters, phytic acid, trypsin inhibitors, phenolic compounds, lectins (curcin), and saponins in high amounts. Phorbol esters have been identified as one of the main compound responsible for the toxicity that limits the utilisation of Jatropha seed cake for animal nutrition. Different methods have been employed for the detoxification of the Jatropha seed cake that would allow the use of detoxified meal as a protein-rich dietary supplement in the food or feed diets. Many researchers have used physical and chemical treatments to detoxify jatropha seed [2-4]. In this paper, we review the properties of Jatropha toxins and various treatments adopted to detoxify them.

History of Jatropha

The Jatropha curcas plant is a native of Mexico and states of Central America and was later grown by Portuguese traders as a hedge plant and got spread to Asia and Africa [5, 6]. Jatropha curcas belongs to Division- Magnoliophyta, Class-Magnoliopsida, Order-Malpighiales, Family-Euphorbiaceae, Subfamily-Crotonoideae, Tribe-Jatrophaeae, Genus-Jatropha, and Species-Curcas [7]. The genus Jatropha has approximately 175 known species. The generic name Jatropha is derived from the Greek word jatros (doctor), trope (food), which suggests its medicinal uses. Hence the plant has been traditionally used for medicinal purposes [4]. The most common vernacular names of Jatropha curcas in India are Ratanjyot (Hindi), Safedarand, Physic nut (English), Purging nut (English), Katamanak (Malayalam), Kattamanakkku (Tamil), Pepalam (Telugu), Jepal (Gujarati), Kanaranda (Sanskrit), Chandrayot etc [8]. Jatropha is a large shrub or small tree usually 3-5 m in height with a smooth grey bark (fig. 1), which when cut exudes watery and sticky latex. It grows on well-drained soils with good aeration [9] and is well adapted to soils with low nutrient content. They are widely distributed in the tropical and the subtropical Himalayas, the mountains of Western and Eastern Ghats and plains of South India [10]. In India, 18 species are found distributed in various parts of the country which are Jatropha curcas, Jatropha gossypifolia, Jatropha glandulifera, Jatropha heynei, Jatropha integerrima, Jatropha maheshwarii, Jatropha multifida, Jatropha multidendriforma, Jatropha villosa, Jatropha nana, Jatropha podagrica, Jatropha hastate, Jatropha tanjovurensis, Jatropha hatchate, Jatropha macropybala, Jatropha acrocucras, Jatropha diyoka and Jatropha sinera. Out of these, Jatropha curcas gained importance because of its additional features like adaptability to various habitats, larger fruits and seeds, high oil yield, soil conservation capabilities, etc.

Composition of Jatropha

Jatropha curcas is an economic tree that cultivates well in the tropical and tropical climate, and therefore plays an essential role in controlling soil erosion and land restoration [11-13]. It is considered to be a good source of proteins and lipids indicating good nutritional value.

Fig. 1: Jatropha tree
The study of *Jatropha curcas* seeds showed that it contains; moisture 6.62 %; protein 18.2%; fat 38.0%; carbohydrates 17.30%; fibre 15.50% and ash 4.5% [14]. The oil from the seed of *Jatropha curcas* can serve as a good source of oil for biodiesel production and for this purpose several tones of seed are utilised which results in the production of de-oiled seedcake. *Jatropha* proteins can be obtained from the press cake which contains around 25% w/w proteins. The defatted pressed *Jatropha* seed cake has high protein content, and the essential amino acid contents are higher than the FAO amino acid reference except for lysine [15]. In terms of functional properties and extraction conditions, *Jatropha* protein may be considered as good as to other well-known oilseed proteins such as rapeseed, canola, or sunflower proteins. Proteins from plants are less expensive than animal proteins so they can be effectively used for fortification and formulation of food products with desirable functional properties. *Jatropha* proteins could be produced as the existing industrial proteins e.g. soy proteins, casein, or wheat gluten. However, toxic compounds like curcin and phorbol esters restrict the use of *Jatropha* proteins for food applications. *Jatropha* proteins after detoxification may serve as potential components for animal feed, while without detoxification their use will be limited to technical applications only. At the industrial level, for extracting proteins from *Jatropha* press cake with higher yields and protein recovery, a more efficient method is a prerequisite which should be able to extract proteins with good functional properties e.g. water absorption, viscosity, solubility, foaming properties, flavour binding and emulsifying properties which are essential for food/technical applications. As large volumes of biodiesel are generated, so will be the seed cake, which could support livestock production.

**Benefits of Jatropha**

Bioactive compounds from natural sources contain substances including phytochemicals and antimicrobials which are known to have the enormous therapeutic potential [16-18]. The increased resistivity of certain pathogens to antibiotics could be because of non-selective use of synthetic antimicrobial drugs which is a major cause of concern to the global population [19]. This, in turn, demands for an urgent need of developing the new generation antibiotics and antimicrobial agents. Many parts of the *Jatropha* plant are used for the curing human and veterinary diseases for eg. The white latex works as a disinfectant in mouth infections in calves, goats, human and chickens [3, 42-44]. The leaves of *Jatropha* plant (fig. 2) have certain compounds which make them effective against malaria [25], rheumatic and muscular pains [23, 26]. The roots of the plant are known to contain an antidote for snake venom [24, 27] and are also used for treating eczema, scabies, ringworm and gonorrhea [28]. Antibiotic activity of *Jatropha* has also been reported against certain microorganisms like *Staphylococcus aureus* [24, 29-35], *Escherichia coli* [24, 30-34], *Enterobacter aerogenes* [36], *Streptococcus pyogenes* [33, 34, 37], *Candida albicans* [31, 33, 37], *Salmonella typhimurium*, *Shigella dysenteriae*, *Psuedomonas aerugunosa* [31, 35, 38-40], *P. flourescens*, *Klebsiella pneumonia* and *K. ozauena* [38], *Aspergilus flavus*, *Neisseria gonorrhea* [31], *Erwinia carotovora*, *Xanthomonas sp.* [40], *Aspergillus niger*, *Penicillium julltanum* [33]. In addition to this, the seeds of *Jatropha curcas* comprise of about 20 to 39% oil which makes them as an important source for bio-fuel production. Also, the oil from its seeds has been found to be useful in cosmetic industry, for the production of candles, soaps and also for medicinal purposes [14, 41].

**Phorbol esters in Jatropha**

Phorbol esters are tetracyclic diterpenes with a tigliane skeletal structure [45, 46] and are highly toxic, cathartic and skin irritant. A number of plants have been reported to contain the toxic phorbols like *Sapium indicum*, *Sapium japonicum*, *Euphorbia frankiana*, *Jatropha curcas* [47], Haas et al. [48] characterised six phorbol esters (*Jatropha* factors C1-C6) from *Jatropha curcas* seed oil where all complexes have the same diterpene moiety, namely, 12-deoxy-16-hydroxyphorbol (fig. 3). Phorbol esters are widely distributed in different parts of the *Jatropha* plant, but they are mainly concentrated in the seed kernel [49] and during extraction of oil.
Phorbol esters reduced by 55% in meal

Phorbol esters reduced by 97.9%

Phorbol ester content reduced to 0.05

Phorbol esters reduced 75.26%

from *Jatropha* seeds, being lipophilic in nature 70-75% of phorbol esters go with the oil, and 25-30% remain in the seed meal [50]. *Jatropha curcas* seeds are toxic to humans and animals due to the toxicity of phorbol esters. In humans, phorbol esters intoxication may lead to mouth pain, vomiting, muscle shock, high pulse or even death [51]. Erythema, oedema, necrosis, loss of weight, reduced water intake are some of the toxicity symptoms found in rodents, rabbits and goats when they were fed with phorbol ester containing feed. Becker and Makkar [52] carried out the toxicological study of phorbol esters in fish and observed lower metabolic rate, rejection of feed and increase in fecal mucus production as adverse effects of phorbol esters.

**Detoxification methods**

A lot of research has been done on the extraction of oil from *Jatropha* seeds. A conventional method like solvent extraction is the most extensively used technique for the extraction of oil as it results in higher oil yields along with large amount of press cake which is rich in proteins, fibre, carbohydrates and some minor components; but for its utilisation as feed, the press cake should be detoxified. Several methods have been tried for detoxifying defatted cake and kernel meal (table 1) that include physical, chemical and biological methods.

**Physical and chemical treatments**

The physical methods for the detoxification of *Jatropha* involve the heat treatment that employs the use of moist heat. Research has been done using moist heat alone for the reduction in phorbol esters content with not much breakthrough [53, 54]. Moist heat along with other treatments resulted in a partial decrease in phorbol ester contents [55]. The combination of physical and chemical treatments has been demonstrated with better efficiency of detoxification [55-57].

The polar nature of phorbol esters has made the use of organic solvents such as ethanol or methanol for detoxification of *Jatropha* cake [58, 59]. Treatment of *Jatropha* seed cake with methanol as organic solvent gave better results but the process is time-consuming as it requires a number of extractions and also the toxicity of methanol makes the process non-ecofriendly. In a study done by Rakshit and Makkar *et al.* [57], 99% reduction in phorbol esters content in oil was achieved using eight litres of methanol for one kg of oil, with a total extraction time of 60 min. Pighinelli *et al.* [60] reported that methanol treatment of *Jatropha* seed cake resulted in 100% reduction of phorbol esters as compared to the treatment with ethanol and sodium bicarbonate which resulted in 64% decrease. Vittaya and Rayakorn [61] have observed in their study that treatment of *Jatropha* meal with 90% methanol and 85% ethanol has resulted in a complete reduction in the phorbol esters.

The high extraction yields of phorbol esters according to Sevura *et al.* [62] are due to hydrogen bonding interactions between methanol and hydroxyl groups of phorbol esters. The nontoxic nature of ethanol as compared to methanol has an added advantage if the detoxified meal has to be used as animal feed. Although using a combination of solvent systems is effective in reducing the phorbol ester contents but they are less suitable for large-scale use as this could complicate the extraction process and make the solvent recovery difficult. Also, the various stages involved in the processing of the biomass up to the detoxification levels increases the expenditure and make the process highly expensive. These processes may also involve the use of a combination of some chemicals that result in the presence of other undesirable residues. Combinations of treatments are used by different researchers [42, 55, 63, 64] for the removal of phorbol esters from *Jatropha* (table 1).

Some of the other methods of detoxification include hydrothermal processing techniques [15], ionising radiation, supercritical fluid extraction (SCFE) [65] etc. Detoxification by SCFE using CO$_2$ would allow the use of aqueous/organic solvent extracts from *Jatropha* seed as an effective insecticidal and antimicrobial agent [65]. Diwani *et al.* [66] reported that the ozone treatment can be the best and less expensive method for removing toxic phorbol esters from *Jatropha* seed cake compared to other methods as the treatment removes 75.26% phorbol esters and consumes less time and chemicals.

**Table 1: Physico-chemical treatments for the removal of toxins in *Jatropha* press cake**

| S. No. | Process/treatment                                                                 | Effects on phorbol esters and other anti-nutritional factors                                      | Reference |
|-------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|-----------|
| 1     | Methanol/ethanol (50/50 during 8h)                                                | Phorbol esters reduced by 97.30% in cake                                                        | [63]      |
| 2     | NaHCO$_3$ or NaOH (3%)                                                            | Phorbol esters reduced by 95% in meal                                                            | [71]      |
| 3     | 90% methanol and 85% ethanol                                                       | Phorbol esters reduced 100%                                                                     | [61]      |
| 4     | Plasma generated by helium gas at high voltage and input power of about 50 Win water and methanol | Complete degradation of Phorbol esters in methanol was achieved.                                 | [72]      |
| 5     | Heat treatment+bentonite+zn oxide+NaHCO$_3$ (4%)                                  | Phorbol ester content reduced to 0.05-0.04 mg/g                                                | [57]      |
| 6     | Methanol                                                                          | Phorbol ester reduced by 100%                                                                   | [60]      |
| 7     | Ethanol (unknown dose) followed by NaHCO$_3$ treatment                             | Phorbol esters reduced by 97.9%                                                                   | [42]      |
| 8     | NaHCO$_3$.0.075%ozone treatment for (2,3,6,9 and 12) minutes at dose 50 mg/land 300m A | Phorbol esters reduced 75.26%                                                                  | [66]      |
| 9     | Alkaline methanol+heat treatment                                                   | Phorbol ester reduction and trypsin inhibitor inactivation                                        | [56]      |
| 10    | Solid-liquid extraction with a sequential combination of hexane and methanol      | Phorbol esters reduced by 99.6%                                                                  | [64]      |
| 11    | CaOH (2%)                                                                         | Phorbol esters reduced by 90% in meal                                                            | [73]      |
| 12    | Ethanol (95%) at 35 °C+heating with pressurised steam at 90 °C for 30 min+sun-drying and further autoclaving at 121 °C during 30 min | Phorbol esters and anti nutrients were still active                                                | [59]      |
| 13    | Petroleum ether extraction                                                        | Phorbol esters reduced by 67.7% in kernels                                                       | [55]      |
| 14    | Double solvent extraction followed by moist heat treatment                         | Phorbol esters reduced by 70.8% in kernels                                                       | [55]      |
| 15    | Double solvent extraction+wet extrusion, re-extraction with hexane and moist heat treatment | Phorbol esters reduced by 87.7% in kernels                                                       | [55]      |
| 16    | Ethanol (80%) or methanol (92%)                                                    | Saponins and phorbol esters reduced by 95% after four extractions                               | [58]      |
| 17    | Moist heating at 121 °C for 20 min                                                | No effect on phorbol esters, Trypsin inhibitors total inactivation                              | [58]      |

**Radiation treatments**

| 1     | Gamma irradiation of cake (30kGy) at the rate of 2.5kGy/hr                        | Phorbol ester reduction by 75.8%                                                                 | [50]      |
| 2     | UV irradiated (220 to 400 nm) *Jatropha* oil at 25 °C for 40 min                  | 100% reduction of phorbol esters                                                                | [70]      |
| 3     | TPA (phorbol-12-myristate-13-acetate) degradation process by microbubbles enhanced ozonolysis | 12 ppm of TPA in mixture with distilled water can be fully decomposed within 10 min              | [74]      |
| 4     | Ozonation of the moist seedcake                                                   | Reduction of the phorbol esters concentration by 80%                                              | [75]      |
Use of radiations

Years of observations on various species for many generations led to conclude that the irradiated foods are safe and non-toxic to consume [67]. Irradiation has the advantages of very high performance and less secondary pollution and therefore can be considered as an additional processing method for removing both heat-stable and heat-labile anti-nutrients [67]. Radiation techniques have been employed for the degradation and transformation of anti-nutritional compounds and several suspicious carcinogens [68, 69]. γ-Radiations have been tried by Gogoi et al. [50] to detoxify the seed cake and 75% reduction was achieved within 12 h of treatment. The combination of UV irradiation and chemical treatments has also experimented for the reduction of phorbol esters. Xiao et al. [70] irradiated Jatropha oil at 25 °C for 40 min and the wavelengths ranged from 220 to 400 nm which resulted in 100% reduction of the phorbol esters. Not much research is documented as far as radiation therapy for detoxifying the phorbol esters.

Biological detoxification

On the contrary, bio-detoxification does not involve the application of any chemicals or mixes and taking into consideration the safety and energy concerns; the biological methods are more advantageous than the others. But at the same time bio-detoxification may be inconvenient and time-consuming. Biodetoxification of Jatropha seed has been done using white rot fungi [76], Aspergillus niger, Penicillium chrysogenum, Rhizopus oligosporus, Rhizopus nigricans and Trichoderma longibrachitum [77] and many other microorganisms have been used to inactivate the toxins and anti-nutritional factors in Jatropha kernel meal (table 2).

| S. No. | Process/treatment | Effects on phorbol esters and other anti-nutritional factors | Reference |
|-------|-------------------|-------------------------------------------------------------|-----------|
| 1.    | Fermentation in broth cultures by Trichoderma harzianum, T. harzianum, Pseudomonas aeruginosa, Gladosporium cladosporioides, Fusarium chlamydosporum, F. chlamydosporoides and P. chlamydosporum | Phorbol esters are removed by 88.9%–99.7% after 30 d of incubation. | [78] |
| 2.    | Solid state fermentation of Jatropha seed cake with white rot fungi for 20 d | Phorbol esters are totally removed | [79] |
| 3.    | Submerged fermentation by bacillus strains | 100% phorbol esters degradation achieved in 7 d. | [80] |
| 4.    | Solid state fermentation of seed cake with Streptomyces Ririllarum (YUCM 310038) in 9 d | Phorbol esters are reduced by 97% in seed cake | [81] |
| 5.    | Solid state fermentation of seed cake with Cunninghambella echinula Japanese (QS-90) in 12 d. | Degradation of phorbol esters to the extent of 75-100% | [82] |
| 6.    | Solid state fermentation of Jatropha kernel cake with Abisiaispinosus, Mucororussi | Tannin, Saponin and phytate contents decreased | [83] |
| 7.    | Jatropha curcas fermented by consortium of Aspergillus niger and Neurospora strophila (3 g inoculum and 3 h of fermentation time) | Phorbol esters reduced by 79.6% | [84] |
| 8.    | Solid state fermentation of seed cake with white-rot fungi Bjerkanderaaerubidae or Phlebia aerubidae | Phorbol esters reduced by 91-97% in seed cake | [76] |
| 9.    | Solid state fermentation of seed cake with Pseudomonas aeruginosa PseA strain within 9 d under 30 °C, pH = 7 and 65% relative humidity | Phorbol esters reduced to undetectable level | [85] |
| 10.   | Solid state fermentation of Jatropha curcas kernel cake with Aspergillus niger, Penicillium sp., Trichoderma harzianum and Trichoderma longibrachitum in 10 d. | Tannin and phytate contents were reduced | [86] |
| 11.   | Solid state fermentation of Jatropha seed cake with P. ostreatus for 45-days incubation | 99% degradation of phorbol esters | [87] |
| 12.   | Fermentation of Jatropha seed cake with P. ostreatus for 60 d | Phorbol ester concentration was reduced by 99% | [88] |

We hypothesised that there are such microorganisms in nature which have the potential of degrading all kinds of the toxins of the kernel cake at the same time. We obtained such a strain in our lab by a high throughput strain isolation and screening approach from soil and detoxified (100%) the kernel cake by an ecologically friendly submerged fermentation (SmF) by the newly isolated strain (unpublished data). A pilot scale detoxification was carried out using our strain Pseudomonas in submerged fermentation with a fresh batch of Jatropha seed cake for a period of 3, 6, 9 and 12 h. It was observed with an increase in incubation time there was a decrease in the amount of phorbol esters in the seed cake because of the release of esterolytic enzymes by the strain Pseudomonas. The esterolytic enzymes break the compound into phorbol, myristic acid and acetic acid. Since these compounds are non-toxic, the aim of detoxifying the seed cake was fulfilled. Complete degradation of phorbol esters from the seed cake takes 12 h, which might be considered long for the industry. So, optimisation studies will be carried out to extract maximum phorbol esters faster and more efficiently. Many research groups across the globe are involved in parallel research to develop biotechnological strategies to overcome the challenges of phorbol esters toxicity.

After detoxification, Jatropha proteins can be extracted as they are present in high amounts and can be used as potential components for animal feed, while without detoxification Jatropha proteins are limited to technical applications. In a study conducted by Malviya et al. [89], the protein from the seed extract of Jatropha curcas was isolated by column chromatography which had a solubility of about 90% above pH 9 and suggested it can be used as a good protein source in food applications, in improving dietary supplement products and protein energy product. Jatropha proteins were also extracted from the Jatropha seeds using the principle of isoelectric precipitation [90, 91]; Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), elemental analysis and Fourier transform infrared spectroscopy (FTIR) were used to analyse the obtained proteins. Liana et al. [92] extracted Jatropha protein by alkaline extraction followed by isoelectric precipitation method by which they were able to obtain a protein isolate with 93.21% of proteins.

CONCLUSION

Detoxifying the seed cake with multifaceted toxic constituents is still a great challenge to the Jatropha industry. Several chemical and physical methods have failed to make headway to fully degrade phorbol esters from Jatropha curcas seed cake to convert it to animal feed. However, methanol and ethanol extractions have the potential to completely remove (not degrade) the phorbol esters from the kernel cake. But, handling or the disposal of the toxins raises an...
environmental and health concern. In addition, the use of an organic solvent is expensive and could have a residual effect on the animals and human beings consuming the feed. However, among three detoxification techniques, for environmental awareness with safety and energy concerns, the biological method would be more advantageous than the others.

ACKNOWLEDGEMENT

The first two authors acknowledge DTU and UGC for providing research fellowships.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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\textbf{How to cite this article}

- Shilpi Ahluwalia, Rajkumar Bidlan, Jai Gopal Sharma, Pushpendra Singh. Review on phorbol ester degradation of \textit{Jatropha} seed cake for its use as animal feed. Int J Pharm Pharm Sci 2017;9(1):7-13.