

Toxicity levels in treated oil cakes of *Simarouba glauca* DC.

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Abstract

Simarouba glauca an exotic species introduced to India has potential to yield oil, used for Biofuel production and oil cake for feed and manure. The cake is rich in protein, amino acids and other organic and inorganic nutrients. It is unfit for use as it contains toxic compounds like alkaloids, Phenolics, Phytic acid and Saponin and other anti-nutritional factors. Therefore experiments were conducted to remove the toxins and make oil cake fit for use as animal feed or for human consumption. Different treatments, boiling, roasting, autoclaving, soaking with water, methanol extraction, fermentation, acetic acid, sodium hydroxide, roasting + ammonia, hydrochloric acid were given to the cake and effect of these on toxic contents of the cake were studied. There was significant decrease in the levels of toxic constituents due to treatments. Methanol and roasting + ammonia treatments were most effective in removing the alkaloids from the cake. Phenolics were reduced to a very low level by roasting + ammonia and fermentation treatments. Acetic acid treatment reduced the phytic acid to the lowest level over all other treatments. Saponins were completely removed by hydrochloric acid treatment.

Results showed that it is possible to remove the toxic compounds from the cake and retain nutrients by different treatments by a programmed treatments schedule.

Introduction

Simarouba glauca is tolerant to extremes of agro-ecological conditions and performer better growth and yield. Introduced to India during 1960s, was brought to the University of Agricultural Sciences GKVK, Bangalore in 1986 and developmental activities began from 1992 onwards (Joshi and Joshi, 2002). Its potential seed yield is 4 tonnes per ha. Seeds having 45-55 per cent oil (Jaipuria (1996), account for 2.6 tonnes of oil and 1.4 tonnes cake. This oil is used at present for industrial purposes Rath *et al.* (1987). But oil was being consumed by some people in Orissa (Jaipuria, 1996).

The cake containing about 50 per cent protein is rendered unfit for consumption as animal feed or in human diet, because of presence of toxic anti-metabolites like, alkaloids, phenolics, phytic acid, saponin and others (Govindaraju *et al.* (2009). It is used as manure (Altschul, 1950). Some attempts were made to remove these toxic components earlier (Abou-Arab and Abu-Salem, 2010). The levels and types of toxic chemicals, varies depending upon the agro-climatic and agro-ecological conditions (Ramachandran *et al.*, 2006). Therefore

experiments were conducted to detoxify the oil cake of the *Simarouba glauca* seeds obtained from Karnataka. The present paper deals with the results of detoxification experiments.

Materials and methods:

Seeds of *Simarouba glauca* plants growing in University of Agricultural Science, GKVK, campus, Bangalore were collected. Oil was extracted using an oil expeller and cake was collected for further studies. The following treatments were given to the oil cake as done earlier (Behura *et al.*, 2008). Cake material was finely ground in mortar and pestle and subjected to treatments as follows.

Autoclaving:

Powdered oil cake of *Simarouba glauca* (100g) weighed put in a container, covered suitably and autoclaved at 121⁰C and 15 psi for 30 minutes. It was followed by drying at 60⁰C and free flowing powder was obtained.

Soaking with Water:

Twenty grams of defatted oil cake powder was mixed with 200ml of distilled water (1:10 w/v) for 24 hours followed by heating for an hour and excess water was decanted. Water treated powder was dried at 60⁰C to a moisture level of 6-8 % w/w.

Fermentation:

Samples of finely ground SOC was soaked in water, 1:10 (w/v) at room temperature. The clear mixture was stirred once in the beginning and left undisturbed. The supernatant water was carefully decanted without disturbing the sediments after 72 hours and the sample was sun dried.

Boiling:

Samples of SOC (50g) were treated with 400 milli liters of water for 16 hours. The slurry was warmed and then agitated for 3 hours at 70-75⁰C. The hot mixture was filtered and the residual material was washed with 50ml of hot water and sun dried.

Hydrochloric acid treatment:

Twenty grams of the finely ground SOC sample was soaked in 1% HCl in 1:10 ratio with intermittent stirring. After 24hours the supernatant was carefully decanted without disturbing the sediments. Then the sample was washed two times with distilled water and supernatant decanted, treated sample was sun dried and the fine powder was collected.

Roasting:

Twenty grams of finely ground samples of SOC were taken in petriplates and spread uniformly. This was kept in a hot air oven at 200⁰C for 15 minutes. Later the sample was removed from the oven and cooled and stored.

Acetic acid treatment:

Samples of SOC twenty (g) were treated with 160 ml of water for 16 hours. The slurry was warmed and then agitated for 3 hours at 70-75°C. The hot mixture was filtered and the material was washed with 20 ml of hot water. The material was extracted for the second time with 150 ml of water containing 1.7 ml of glacial acetic acid following the same procedure and sun dried.

Sodium hydroxide treatment:

Twenty (g) of finely ground sample of SOC was soaked in 0.1N NaOH (1:10, w/v) with intermittent stirring. After 24 hours, the supernatant was carefully decanted without disturbing the sediments. Then the sample washed two times with fresh water to remove excess alkali and sun dried.

Roasting after ammonia treatment:

30 grams of finely ground sample of SOC was treated with 3% aqueous ammonia with 30% moisture and kept in airtight plastic bag. After seven days the poly bag was opened and the sample was roasted in the hot air oven at 200 °C for 15 minutes.

Methanol extraction:

Ten (g) of defatted *Simarouba glauca* oilcake was soaked in methanol, 1:5(W/V), mechanically stirred for 15 minutes then crushed well methanol was filtered and the sample washed two times by distilled water to remove the methanol and the sample was sundried.

Control:

The defatted cake twenty (g) was kept as control sample and no treatment was given to it.

Extraction and estimation of alkaloids (Narasimhan and Mehrotra, 2003), phenolics (Singleton *et al.*, 1999), phytic acid (Thompson and Erdman, 1982 and Taussky and Shorr, 1953) in the treated samples and control, were done. Saponin contents from the treated and untreated samples were extracted (Gestetner *et al.*, 1966) and estimated by using HPLC method (Govindaraju *et al.*, 2009). The values obtained were expressed on per cent basis.

Results and Discussion:

Results showed the effects of different treatments on toxic contents of *Simarouba glauca* oil cake. Percentage of alkaloids was reduced from 0.27 per cent of controls to lowest level of 0.01 per cent by methanol and roasting after ammonia treatments. This shows their solubility in methanol and destruction by the latter. Boiling and water soaking were effective, but retained higher alkaloids levels. Other treatments also were effective to a lesser degree, which is also reported earlier (Basu, 2010).

Phenolics (Table 1) were reduced from 0.005 per cent in control samples to 0.002 per cent by both roasting after ammonia and fermentation treatments, Though other treatments also reduced phenolics, it was not very conspicuous, which was also reported earlier (Basu, 2010).

Percentage of phytic acid (Table 1) the anti-nutritional factor, which was high in the untreated samples (2.52 %) was further enhanced by hydrochloric acid treatment (3.46 %). Acetic acid was most effective in reducing it to the lowest level (0.88 %), followed by fermentation, water soaking and others. Earlier reports (NOVOD., 2007) on lesser percentage reduction of phytates in Pongamia cake by hydrochloric acid and alkali (calcium hydroxide) treatments also fall in line with the present observations.

Saponins (Table 1) were reduced to a maximum extent by hydrochloric acid (100.00%), whereas only 75.80 per cent of saponins were removed by boiling with water, as per earlier report (Basu, 2010). Roasting (92.81 %) followed by boiling (66.90 %), water soaking (59.30 %) and other treatments were also effective. This shows that saponin is acid and heat sensitive to level. Similar observations have been reported earlier also (Basu, 2010)

However higher levels of alkaloids, phenolics, saponins and phytates in untreated oil cake samples from seeds collected from same location as at present, reported earlier (Govindaraju *et. al.*, 2009) may be due to different storage conditions and time of collection of seeds.

The results obtained also show the feasibility of using different treatments in reducing alkaloids, phenolics, phytic acid and saponin contents, toxic compounds in *Simarouba glauca* oil cake. However the present results do suggest, keeping in view earlier reports also (Govindaraju *et. al.*, 2009, Basu, 2010 and NOVOD., 2007) that the oil cake may be subjected to acetic acid, methanol, hydrochloric acid and ammonia + roasting treatments, sequentially. This may help to reduce the toxic compounds like alkaloids, phenolics, phytic acid and saponin. However this needs to be tested by adopting permutation and combination of treatments, keeping in view the cost and time. This would be a great step in making *Simarouba* oil cake fit for food and feed in this age of decreasing forest cover escalating cost of living of the down trodden and the farmers.

Table 1. Toxic compounds of Simarouba glauca oil cake after (g/100g) detoxification

Sl.NO.	Treatments	Alkaloid	Phenolics	Phytic acid
1	Control	0.27	0.005	2.52
2	Boiling	0.04	0.003	2.47
3	Roasting	0.21	0.003	1.66
4	Autoclaving	0.20	0.003	1.91
5	Soaking with water	0.04	0.004	1.22
6	Methanol extraction	0.01	0.003	2.51
7	Fermentation	0.08	0.002	0.99
8	Acetic acid	0.09	0.004	0.88
9	NaOH Treatment	0.05	0.004	1.62
10	Roasting after Ammonia treatment	0.01	0.002	3.01
11	HCl Treatment	0.06	0.003	3.46
	F-value	*	*	*
	Sem±	0.01	0.0002	0.07
	CD at 5%	0.03	0.0007	0.21
	CV%	16.65	13.27	6.00

*Significant at 5% level

Table 2. Reduction (per cent) of saponin contents in detoxified *Simarouba glauca* oil cake.

Sl.No	Treatments	Reduction per cent
1	Boiling	66.9
2	Roasting	92.81
3	Autoclaving	2.40
4	Soaking with water	59.30
5	Methanol extraction	42.38
6	Fermentation	33.82
7	Acetic acid	45.17
8	NaOH treatment	9.14
9	Roasting after Ammonia	16.25
10	HCl treatment	100.00

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