International Journal of Basic and Applied Sciences Vol. 4. No. 3 2015. Pp. 142-145

Full Length Research Paper

Effect of Mycoflora on Quantity and Quality of Oil Produced from *Pongamia pinnata* seeds during Storage

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Abstract

Pongamia pinnata(L.) Pierre is a fast-growing leguminous tree with a variety of applications and enormous economic potentials. Oil from the seeds can be used as alternative fuel and for making biodiesel which aims to overcome energy crisis problems. The objective of the present study was to quantify the Pongamia oil after fungal infestation during storage. Eleven dominant fungi viz. Penicillium sp., Aspergillus oryzae, Aspergillus ustus, Aspergillus sydowii, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Rhizopus sp., Fusarium sp., Colletotrichum gloeosporioide and Absidia sp. were found to be associated with the seeds. Oil was extracted from seeds collected from trees, fallen on ground and storage depots. The oil was extracted from all the samples after every three months and compared with fresh seeds oil for quantity and oil quality. Fresh seeds produced high oil yield and oil quality while stored and ground Pongamia pinnata seeds showed decline in their oil content as well as oil quality. The present study also revealed that oil content of Pongamia pinnata declined due to the infection of seed mycoflora and caused deterioration of seeds during storage.

Key words: *Pongamia pinnata, Biochemical changes, oil quality, oil quantity, seed deterioration.*

Introduction

An ever increasing demand of energy resources has been a challenge as the fossil fuel resources are dwindling day by day. Biodiesel seems to be a solution for future as it is an environmentally viable alternative. Several researchers have made systematic efforts to use plant oil and their esters (biodiesel) as a fuel in compression ignition (CI) engines. There is various types of raw material like *Jatropha curcus L, Pongamia pinnata* (Karanja), Moha, Undi, Castor, Saemuruba, Cotton seed etc. An non- edible oil seeds and various vegetable oils including palm oil, soybean oil, sunflower oil, rapeseed oil and canola oil have been used to produce biodiesel fuel and lubricants. Out of these *Pongamia pinnata* is a better source of raw material due to its easy availability in wild. *P. pinnata* is drought resistant, semi-deciduous, nitrogen fixing leguminous tree that grows to about 15–25 meters (15–80 ft) in height with a large canopy which spreads equally wide. Oil made from the seeds of *Pongamia pinnata* is known as honge oil and has been used as lamp oil, in soap making, and as a lubricant for thousands of years. This oil is rapidly gaining popularity as a source of feedstock for bio-diesel production. Acid value, peroxide value, iodine value and saponification value are also important in assessing the quality of natural oils (Choe and Min, 2006). The determination of Refractive index furnishes the most and easily applied preliminary means of judging the quality of samples. Jamaluddin *et al*., (1985) estimated the oil content in infected and healthy seeds of *P. pinnata*. Pandey and Prasad (1993) reported biochemical changes in seeds of *P. pinnata* during storage. The objective of the present study is to quantify the *Pongamia pinnata* oil after fungal infestation during storage and check the quality of the extracted oil by comparing with the oil extracted from fresh healthy *Pongamia* seeds.

Materials and Methods

Sample Preparation

The seeds of *Pongamia pinnata* were obtained directly from the trees, fallen on the ground and seed storage depots. The oil from these seeds was extracted by standard method (Fresh wt. basis and moisture free basis) as described here. The samples from ground and storage were infected by various mycoflora. For moisture free basis (dry wt.), 50 gm of *Pongamia pinnata* kernels were dried in a petridish and put in a oven at 30^{0}C for 20 minutes. The dried *Pongamia pinnata* kernels were cooled down to room temperature and then ground through a food chopper by feeding kernels slowly to prevent expression of oil (Sadasivam and Manickam, 2008).

Oil Extraction

The oil was extracted using petroleum ether as solvent by soxhlet extraction apparatus in at 3 months interval *viz*., 3, 6, 9 and 12 months. Fine grounded *Pongamia pinnata* powder samples were weighed and transferred to the thimble and placed into fat extraction tube of the soxhlet apparatus. The bottom of the extraction tube was attached to a soxhlet flask. Approximately 150 ml of petroleum ether was poured in the tube in the soxhlet flask. The extraction of sample was continued for 6 h at 60^{0}C on mantle heater. After the extraction solvent was removed and the weight of oil was recorded. Oil yield (percentage) was measured by following formula:

$$\text{Oil yield (\%)} = \frac{Wt.of\ oil \times 100}{Wt.of\ seed\ sample\ (g)}$$

Refractive Index

The refractive index (Rf) of the oil was measured by refractometer make Erma Tokyo.

Chemical parameters

Acid value, saponification value, peroxide value and iodine value of oil were analyzed using standard methods of analysis described in AOAC (1970).

Results

Oil yield

Reduction in oil content and quantity, was recorded with increase in seed storage period. The fresh seeds collected from tree showed high oil yield (34.92%) in comparison to stored (28.03%) and ground seeds (30.75%) with respect to their fresh weight. The oil content on dry weight basis, seeds collected from trees, ground and storage were (37.94%), (33.39%) and (32.48%) respectively (Table 1 and Fig 1). At the end of first quarter, seeds collected from trees showed high oil yield (31.23%) in comparison to the stored (29.85%) and ground seeds (26.6%) when compared on fresh weight basis, whereas, on dry weight basis in tree seeds (33.92%), ground seeds (30.77%) and storage seeds (30.43%) decrease in oil yield content was recorded. After the 2nd quarter seeds collected from trees showed high oil yield (32.57%) in comparison to the storage (29.50%) and ground seeds (27.88%) when compared on fresh weight basis. On dry weight basis, in tree seeds (35.50%), storage seeds (33.50%), and ground seeds (30.95%) decreasing trend was recorded. In the 3rd quarter seeds collected from trees showed high oil yield (30%) in comparison to the storage (28.73%) and ground seeds (25.63%) when compared on fresh weight basis, whereas, on dry weight basis in tree seeds (35.29%), storage seeds (31.60%), and in ground seeds (28.47%) decreasing oil yield was recorded. After the 4th quarter, seeds collected from trees showed high oil yield (28.35%) in comparison to the storage (27.54%) and ground seeds (24.65%) when compared on fresh weight basis. Whereas, on dry weight basis, in tree seeds (32.73%), storage seeds (29.23%), and in ground seeds (25.33%) decreasing trend was recorded.

Physicochemical composition of Pongamia pinnata oil

Refractive index (RI)

The refractive index of the oil increased after prolonged storage of *P. pinnata* seeds. The refractive index of seeds collected from trees, was 1.46601, from stores 1.46711, and1.4667, collected from ground. After 3 months RI for the storage was 1.4662 followed by 1.4670 (6 months), 1.4671 (9 months) and 1.4678 (12 months). An increase in (RI) was also recorded in seeds collected from ground. i.e. 1.4669 after 3 months of storage followed by 1.4673 (6 months), 1.4675 (9 months) and 1.4679 (12 months). In case of seeds collected from different seed stores the refractive index also increased i.e. 1.4672 after 3 months storage followed by 1.4673 for 6 months, 1.4676 for 9 months and 1.4683 for 12 months. (Table 2)

Acid value

The acid values increased with increase in storage period of *P. pinnata* seeds. Initially the fresh seeds collected from tree showed 24.26mg/gm, from store 27.49mg/gm, and ground samples 25.52mg/gm from. However, after 3 months of storage the tree sample showed 25.21 mg/gm followed by 27.32 mg/gm (6 months), 28.73 mg/gm (9 months) and 30.39 mg/gm (12 months). In case of ground seeds at 3 months intervals, it was 26.64 mg/gm (3 months storage) 28.56 mg/gm (6 months), 29.46 mg/gm (9 months) and 34.75 mg/gm (12 months). The seeds collected from different seed stores recorded 27.52 mg/gm (3 months) 32.54 mg/gm (6 month), 34.92 mg/gm (9 month) and 37.54 mg/gm (12 month) (Table 2).

Saponification value

Saponification number (SN) is an indicator of molecular weight of the fatty acid or chain length of fatty acid in the oil. The saponification values also increased with increase in storage period. The SN for seeds collected from tree were 182.64 in fresh seeds, 187.79 mg/gm after 3 months of storage followed by 189.16 mg/gm (6 months), 192.34 mg/gm (9 months) and 196.45 mg/gm (12 months). In case of ground seeds it was 189.20, initially, then 190.78 mg/gm after 3 months storage followed by 193.56 mg/gm (6 months), 195.24 mg/gm (9 months) and 198.78 mg/gm (12 months). The seeds collected from different seed stores was found to have SN value of 193.27, initially followed by 193.72 mg/gm (3 months), 195.27 mg/gm (6 month), 197.78 mg/gm (9 month) and 199.27 mg/gm (12 month) (Table 2).

Iodine value

Wij's method was adopted for the estimation of iodine value for oil. The iodine value decreased with increase in storage period. The fresh seeds collected from tree recorded as iodine value of 98.46, from store 94.28, and 95.27 from ground samples. However, the seeds collected from tree was recorded to have 98.43 gm/100gm seeds after 3 months of storage followed by 92.90 gm/100gm seeds (6 months), 88.06 gm/100gm seeds (9 months) and 84.82 gm/100gm seeds (12 months). In case of ground seeds it was 95.38 gm/100gm seeds after the storage of 3 months followed by 83.46 gm/100gm seeds (6 months), 76.69 gm/100gm seeds (9 months) and 74.94 gm/100gm seeds (12 months). The seeds collected from different seed stores showed 93.59 gm/100gm seeds after 3 months storage followed by 88.44 gm/100gm seeds (6 month), 71.83 gm/100gm seeds (9 month) and 67.94 gm/100gm seeds (12 month) (Table 2).

Specific gravity

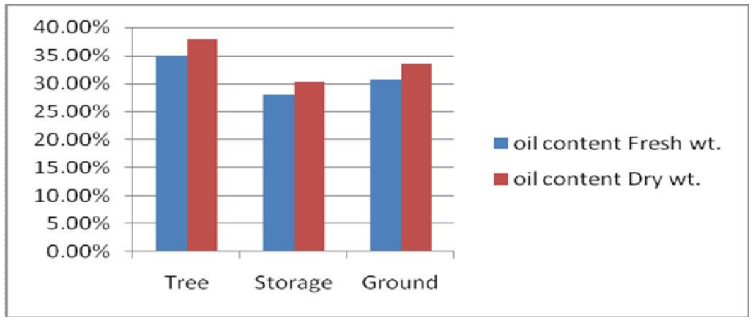
The specific gravity of oil increased with storage period. The fresh seeds collected from tree showed 0.9146, from store, 0.9357, and 0.9238 from ground samples. However, the seeds collected from tree, was recorded to have specific gravity of 0.9273 after 3 months of storage followed by 0.9328 (6 months), 0.9538 (9 months) and 0.9567 (12 months). In case of ground seeds it was 0.9328 for 3 months followed by 0.9634 (6 months), 0.9732 (9 months) and 0.9823 (12 months). The seeds collected from different seed stores showed 0.9588 in 3 months stored seeds followed by 0.9736 (6 month), 0.9789 (9 month) and 0.9845 (12 month) (Table 2).

Table1: Oil yield of *P. pinnata* seeds stored for different period.

Sample	Oil content (ml)	
	% yield on fresh wt. basis	% yield on moisture free basis
Fresh		
Tree	34.92%	37.94%
Storage	28.03%	30.43%
Ground	30.75%	33.39%
1st quarter		
Tree	31.23%	33.92%
Storage	29.85%	32.48%
Ground	26.6%	30.77%
2nd quarter		
Tree	32.57%	35.50%
Storage	29.50%	33.50%
Ground	27.88%	30.95%
3rd quarter		
Tree	30%	35.29%
Storage	28.73%	31.60%
Ground	25.63%	28.47%
4th quarter		
Tree	28.35%	32.73%
Storage	27.54%	29.23%
Ground	24.65%	25.33%

Table 2. Chemical parameters of *P. pinnata* seed oil after different storage period in the seeds collected under different condition.

Storage Duration	Sample	Refractive Index	Acid Value (mg/g)	Saponification value (mg/g)	Iodine number (g/100g)	Specific Gravity
Fresh	Tree	1.4660	24.26	182.64	98.46	0.9146
	Storage	1.4671	27.49	193.27	94.28	0.9357
	Ground	1.4667	25.52	189.20	95.27	0.9238
3month	Tree	1.4662	25.21	187.79	98.43	0.9273
	Storage	1.4672	27.52	193.72	93.59	0.9588
	Ground	1.4669	26.64	190.78	95.38	0.9328
6month	Tree	1.4670	27.32	189.16	92.90	0.9378
	Storage	1.4673	32.54	195.27	88.44	0.9736
	Ground	1.4672	28.56	193.56	83.46	0.9634
9month	Tree	1.4671	28.73	192.34	88.06	0.9538
	Storage	1.4676	34.92	197.78	71.83	0.9789
	Ground	1.4675	29.46	195.24	76.64	0.9732
12month	Tree	1.4678	30.39	196.45	84.82	0.9567
	Storage	1.4683	37.54	199.27	67.94	0.9845
	Ground	1.4679	34.75	198.75	74.94	0.9823



Fresh seeds

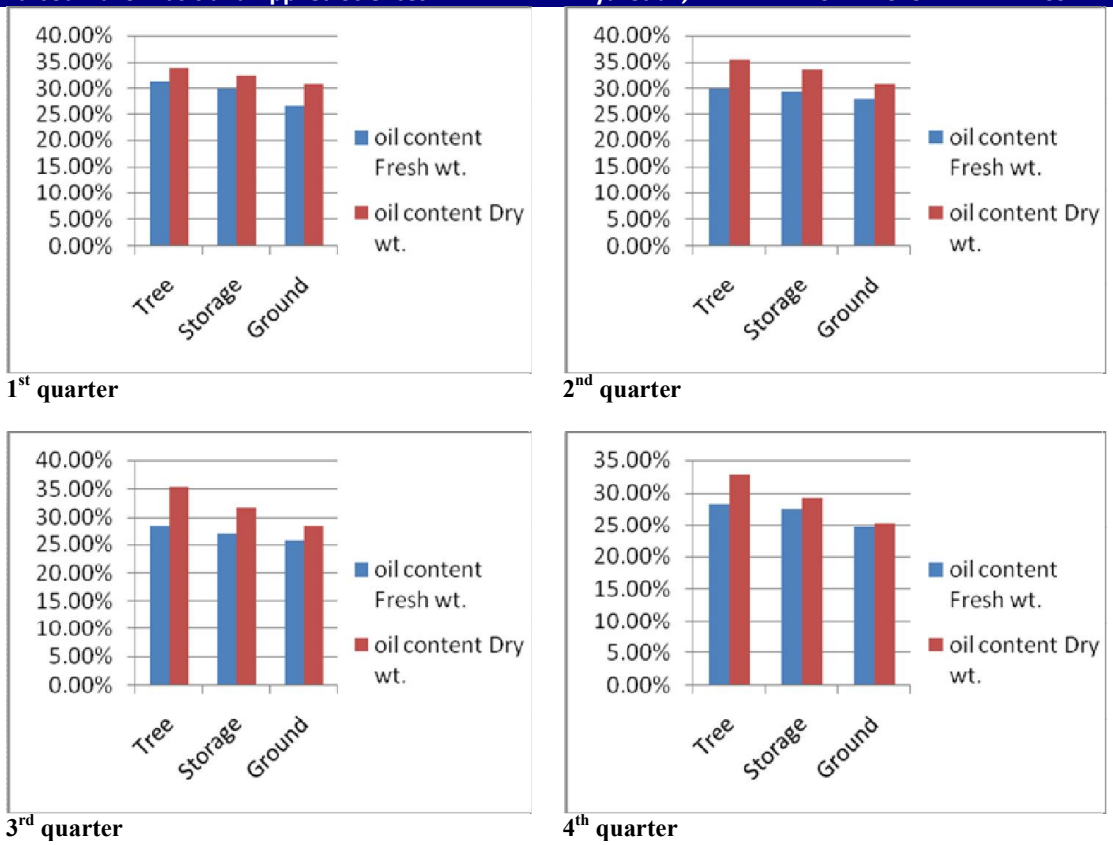


Fig 1: Oil yield of *P. pinnata* seed samples with respect to their fresh and dry weight after different storage duration.

Discussion

Pandey and Prasad (1993) also reported biochemical changes in seeds of *P. pinnata* during storage. The significant reduction in oil yield was obtained in the seeds stored for more than a year. High seed infection with different seed mycoflora was found to be associated with the deterioration. Sharma (1977) also observed earlier that bio-deterioration of seeds were common under tropical conditions due to microbial infestation. These microbes affected biochemical composition of seed as well as properties of extracted oil leading to chemical changes in *Pongamia pinnata* seed oil as observed after different storage durations. In fresh seeds oil yield and oil quality was high, but after storage the oil content declined in all the different quarters due to the presence of storage and seed borne fungi while the quality of oil also declined. Seed depot samples and ground *P. pinnata* seeds showed decline in their oil content as well as oil quality.

The refractive index of the oil also increased with increase in storage period. The acid value of deteriorated *P. pinnata* oils showed increased. In order to know the role of storage fungi with reference to the saturation or unsaturation of oil, saponification number of fungal deteriorated *P. pinnata* oils was calculated, and found increase in saponification number with seed deterioration. Wij's method was adopted for the estimation of iodine number of all the oil and results revealed decrease in iodine number of *P. pinnata* oil. The specific gravity of *P. pinnata* oil increased in seed samples, after different storage period. It was concluded that after the fungal infection the *P. pinnata* seeds deteriorate rapidly showing decrease in oil yield and quality with increase in storage duration.

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