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RESEARCH ARTICLE

EFFECT OF MYCOFLORA ASSOCIATED DURING THE SEED STORAGE ON QUANTITY AND QUALITY OF *JATROPHA CURCAS L.*OIL

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ABSTRACT

Jatropha curcas L. is a wonder plant 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 is to quantify the *Jatropha* oil after fungal infestation during storage. Oil was extracted from fresh, stored and infested *Jatropha* seeds. Seeds were infested with six dominant fungi *viz. Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium chlamydosporum* and *Penicillium glabrum* as well as fresh seeds were used for the extraction of *Jatropha* oil. The seeds were powdered and extracted thoroughly with light petroleum ether (60-80°C) in a Soxhlet extractor for 24-48 h in each case. Fresh seeds produced 47.189% oil while stored *Jatropha curcas L.*L. seeds showed decline in their oil content. The present study also revealed that oil content of *Jatropha curcas L.*L. was declined due to infestation of seed mycoflora and it is also the main cause of deterioration of seeds during storage.

INTRODUCTION

*Jatropha curcas L.*a member of the *Euphorbiaceae* family is a multipurpose tree of significant economic importance because of its several industrial uses including bio-diesel production (Makkar *et al*. 2008). Diesel is the major fuel used in transportation especially for vehicles such as trucks and trains. There is a growing demand for transportation fuel in most countries with the manifestation of the current world energy crisis. Thus it is important to explore the feasibility of substituting diesel with an alternative fuel that can be produced on a massive scale for commercial utilization. The non-edible vegetable oil of *Jatropha curcas L.*promises a commercially viable alternative to diesel as it has the desired physiochemical and performance characteristics comparable to diesel to facilitate continuous operation without much change in design (Sayyar *et al.* 2009).

Solid liquid extraction is a common and efficient technique in producing oil for biodiesel production (Forson *et al.* 2004). Solid liquid extraction, sometimes called leaching, involves the transfer of a soluble fraction (the solute or leachant) from a solid material to a liquid solvent. The solute diffuses from the solid into the surrounding solvent. Normally, solid liquid extraction is dependent on the nature of the solvent and oil, reaction time between solvent and seeds, temperature of the process, particle size of the meal and the ratio of solvent to the meal.

By increasing the temperature approaching to the boiling point of the solvent, both the diffusion coefficient and the solubility of the oil in the solvent are enhanced, thus improve the extraction rate (Richardson and Harker, 2002). Less oil is extracted from the larger particles (>0.75 mm) compared to the smaller size particles because less amount of oil will be transferred from inside the larger particles to the surrounding solution in comparison with the smaller ones (Tzia and Liadakis, 2003). The objective of the present study is to quantify the *Jatropha* oil after fungal infestation during storage by comparing with the oil extracted from fresh *Jatropha* seeds.

MATERIALS AND METHODS

Oil was extracted from fresh, stored and infested *Jatropha* seeds. Seeds were infested with six dominant fungi *viz. Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium chlamydosporum* and *Penicillium*

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glabrum as well as fresh seeds were used for the extraction of *Jatropha* oil.

Sample Preparation

Place about 50g of *Jatropha* kernels in a drying dish and dry at 130°C for not more than 20 minutes in a forced-draft oven. Cool to room temperature and then grind through the food chopper, feeding the kernels slowly to prevent expression of oil (Sadasivam and Manickam, 2008).

Oil Extraction

The collected ripe seeds were collected and the damaged seeds were discarded. The seeds were cleaned and dried in an oven at 105°C for 30 minutes. The seeds were powdered and extracted thoroughly with light petroleum ether (60-80°C) in a Soxhlet extractor for 24-48 h in each case. Once more the remaining powdered seed was extracted to collect all oil in the seeds. Combined petroleum ether (60-80°C) extract was dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40°C by using rotary evaporator to recover oil (Link, 1975).

A rotary evaporator (or rotavapor) is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation **(**Harwood and Moody, Illustrated Edition). The seed oils were filtered through Whatman filter paper No.1 to remove any foreign particles and pure oil preserved in cold storage properly. Official and tentative methods (1993) of AOCS Chicago were followed for the determination of physicochemical characteristics of seed oil (Mukherjee, 2002). The percent oil content of the seeds was calculated with the help of the following formula:

$$\text{Oil (\%)} = \frac{W_2 - W_1}{\text{Weight of the seed sample (g)}} \times 100$$

Where,
W_2 = Weight of bottle with oil
W_1 = Initial weight of the bottle

Statistical Analysis

Mean value with standard error was calculated to check the variation of extracted oil from seeds and kernels of *Jatropha curcas L.*L. and loss in weight after extraction of oil (Chandel, 2002).

$$\text{S.E.} = \frac{S}{\sqrt{n}}$$

Where, S = Standard Deviation of Sample
n = Sample size
All results obtained after the extraction of *Jatropha curcas L.*oil were subjected to analysis (ANOVA) using statistical packaged for social sciences (SPSS). The (DMRT) Duncan multiple range test at 5% level of probability was used to ascertain the significance between the different treatments used (Levesque, 2007).

RESULTS AND DISCUSSION

Table-1 reveals the effect of storage on the per cent oil content of *Jatropha curcas L.*L. extracted from fresh and stored seeds.

Table 1 Per cent oil obtained from *Jatropha* kernels during different period of storage

Sample	Wt. of *Jatropha* kernels (g)	Wt. of Bottle	Wt. of Bottle with oil	Oil (%)	Oil (ml)
				(Mean±S.E.)	
Fresh seeds	50	22.4	45.977±0.19 a*	47.189±0.27 a	30.167±0.29 a
One year stored seeds	50	22.4	42.363±0.54 b	39.513±0.45 b	27.333±0.58 b
Two years stored seeds	50	22.4	41.600±0.39 b	38.444±0.46 c	24.333±0.58 c

*Means on the same column with same superscripts are not significantly different (P>0.05)

Fresh seeds (control) produced 47.189% oil while stored *Jatropha curcas L.*L. seeds showed decline in their oil content. The percent oil content of one year stored and two years stored *Jatropha curcas L.*L. seeds were 39.51% and 38.44%, respectively (Fig.1a). Neergaard (1977) reported that freshly harvested seeds are more likely to be free from fungal infection and will express maximum seed oil. Similar results were observed by Gupta and Rao (2008) that on keeping the *Jatropha* seeds for long time, oil content of the seeds declines.

Table 2 Per cent oil obtained from *Jatropha* kernels from fungal infested seeds

Sample	Wt. of *Jatropha* kernels (g)	Wt. of Bottle (g)	Wt. of Bottle with oil (g)	Oil (%)	Oil (ml)
				(Mean±S.E.)	
T_1	50	22.4	36.430±0.45 c*	28.200±0.35 e	21.167±0.29 e
T_2	50	22.4	38.380±0.39 b	31.560±0.49 d	23.433±0.40 d
T_3	50	22.4	35.160±0.19 d	25.647±0.56 f	19.067±0.12 f
T_4	50	22.4	39.223±0.39 b	34.697±0.43 b	26.200±0.20 b
T_5	50	22.4	38.500±1.32 b	32.993±0.01 c	25.200±0.35 c
T_6	50	22.4	35.133±0.23 d	26.110±0.19 f	19.400±0.36 f
Control	50	22.4	45.977±0.19 a	47.189±0.27 a	30.167±0.29 a

*Means on the same column with same superscripts are not significantly different (P>0.05)
T_1= Oil extracted from seeds infested with *Alternaria alternata,* **T_2**= Oil extracted from seeds infested with *Aspergillus flavus*
T_3= Oil extracted from seeds infested with *Aspergillus fumigatus,* **T_4**= Oil extracted from seeds infested with *Aspergillus niger*
T_5= Oil extracted from seeds infested with *Fusarium chlamydosporum*, **T_6**= Oil extracted from seeds infested with *Penicillium glabrum*

Cost of bio-diesel is sensitive to storage time of seeds as well as raw oil. This also supports to Oladimeji and Kolapo (2008) who observed decrease in oil content of sunflower after a period of storage. Sayyar *et al.* (2011) reported that the preheating the seeds for 4 minutes by microwave before the extraction improved the oil recovery up to 2%. Ultrasonication and microwave did not affect the oil quality in terms of free fatty acid content. Table-2 reveals the effect of seed

mycoflora on the percent oil content of *Jatropha curcas L.*L. extracted from infested and fresh seeds.

weight loss of *Jatropha* kernels after oil extraction in fresh *Jatropha* seeds is due to high oil content.

Table 3 Loss in kernel weight of *Jatropha* seeds after extraction of oil during different period of storage

Sample	Wt. of *Jatropha* kernels before extraction of oil (g)	Wt. of *Jatropha* kernels after extraction of oil (g)	Loss in kernel wt. (g)
Fresh seeds	50	37.467±0.42 c*	12.533±0.42 a
One year stored seeds	50	42.457±0.44 b	7.543±0.44 b
Two years stored seeds	50	44.233±0.25 a	5.767±0.25 c

*Means on the same column with same superscripts are not significantly different (P>0.05)

Fresh seeds (control) produced 47.189% oil while infested *Jatropha curcas L.*L. seeds showed decline in their oil content. The per cent oil content of infested *Jatropha curcas L.*L. seeds were 28.30% (seeds infested by *Alternaria alternata*), 31.84% (seeds infested by *Aspergillus flavus*), 25.97% (seeds infested by *Aspergillus fumigatus*), 32.99% (seeds infested by *Aspergillus niger*), 34.55% (seeds infested by *Fusarium chlamydosporum*) and 26.17% (seeds infested by *Penicillium glabrum*). The present study revealed that oil content of *Jatropha curcas L.*L. was declined due to infestation of seed mycoflora and it is also the main cause of deterioration of seeds during storage (Fig.1b).

Table-4 reveals that maximum weight loss of *Jatropha* kernels after oil extraction was observed in fresh *Jatropha* seeds *i.e.,* 12.30g. Weight loss of *Jatropha* kernels after oil extraction in infested *Jatropha* seeds were 3.7g (seeds infested by *Alternaria alternata*), 5.1g (seeds infested by *Aspergillus flavus*), 2.95g (seeds infested by *Aspergillus fumigatus*), 3.65g (seeds infested by *Aspergillus niger*), 5.9g (seeds infested by *Fusarium chlamydosporum*) and 3.64g (seeds infested by *Penicillium glabrum*). The present study reveals that infested seeds also show less weight loss of kernels after extraction of oil because there is less oil content remains in infested seeds

Table 4 Loss in kernel weight of *Jatropha* seeds after extraction of oil from fungal infested seeds

Sample	Wt. of *Jatropha* kernels before extraction of oil (g)	Wt. of *Jatropha* kernels after extraction of oil (g)	Loss in kernel wt. (g)
T_1	50	46.233±0.25 b*	3.767±0.25 d
T_2	50	46.167±0.29 b	3.833±0.06 d
T_3	50	47.133±0.23 a	2.600±0.20 e
T_4	50	44.233±0.25 d	5.767±0.25 b
T_5	50	45.077±0.13 c	4.923±0.13 c
T_6	50	46.463±0.45 b	3.537±0.45 d
Control	50	37.467±0.42 e	12.533±0.42 a

*Means on the same column with same superscripts are not significantly different (P>0.05)
T_1= Seeds infested with *Alternaria alternata*, **T_2**= Seeds infested with *Aspergillus flavus*
T_3= Seeds infested with *Aspergillus fumigatus,* **T_4**= Seeds infested with *Aspergillus niger*
T_5= Seeds infested with *Fusarium chlamydosporum* **, T_6**= Seeds infested with *Penicillium glabrum*

Mathur and Sinha (1978) reported that fungi association bring certain biochemical changes in seeds during storage by decreasing reducing sugars and oil content. Basha and Pancholy (1986) reported a decrease in oil, iodine value, soluble carbohydrates and protein contents in groundnut seed infested with *Aspergillus* spp. Reduction in seed oil content at the end of the storage period may be attributed to the effect of the storage conditions, the seed moisture content and the fungi associated with the seed as found by Neergaard (1977) and Simic *et al*. (2007). Ramamoorthy and Karivaratharaju (1986) found that groundnut pods stored in cloth bag showed decrease in oil content and protein content with increase free fatty acid content and fungal infection with advancement of storage period. Vaidya and Dharemvir (1989) reported loss in oil content of stored groundnut due to *A. flavus* and *A. niger*. Decrease in oil content was positively correlated with increase in lipase activity on account of fungal infection (Saraswat and Mathur, 1985; Saxena *et al.* 1998 and Taung and McDonald, 1995). Data presented in Table-3 reveals that maximum weight loss of *Jatropha* kernels after oil extraction was observed in fresh *Jatropha* seeds *i.e.,* 12.30g whereas weight loss of kernels after extraction of oil in one year and two years stored *Jatropha* seeds were 7.65g and 6.15g, respectively. More

a = Oil Extracted from Fresh and Stored *Jatropha curcas* Seeds

b = Oil Extracted from Fresh and Infested *atropha curcas* Seeds

Figure 1

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