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Full Length Research Paper

Occurrence of storage fungi in jatropha (*Jatropha curcas* L.) seeds

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Atropha is a potential source for production of bio-diesel and an alternative source for fossil fuels. The present study is undertaken for the analysis of storage fungi and its biodiversity in Jatropha seed samples collected from local market and research institutes in Chennai. Standard plating technique was used to study the growth of storage fungi in seeds stored for 12 to 60 months by using selective media called osmophilic media. The analysis of storage fungi in seeds with different treatments to observe as (1) surface mycoflora (2) surface invaders (3) internal fungi (4) kernel fungi. The results show that, the occurrence of different species of Aspergillus*,* Penicillium and *Mucor* species was the dominant mycoflora even from interior portion of the seeds. Among the species of storage fungi encountered, *Aspergillus flavus, Aspergillus niger, Aspergillus glaucus, Aspergillus fumigatus and Penicillium* species were frequently present in the order of dominance. Mucor and Rhizhopus also occur constantly in all the samples. The higher contamination of seeds with storage fungi indicates the possibility of quality losses particularly in lipid, which may affect the quality of bio-diesel. Among the different strains of *A. flavus*, few were found to produce aflatoxin B1. The spoilage of lipids by the named fungi, may affect the quality of bio-diesel. This study will create awareness of fungal spoilage on biodiesel seeds and for remedy to prevent the storage losses by microorganisms.

Key words: Storage fungi, Jatropha seeds, *Aspergillus* spp.*, Penicillum* spp.*, Mucor* sp.*, Rhizopus* sp.*,* Biodiesel, storage losses, aflatoxin.

INTRODUCTION AND SCOPE

Biodiesel is an alternative fuel for motor engines, becoming increasingly important due to diminishing petroleum reserves and the environmental consequences (Zurina, 2009; Verma and Gaur, 2009) . One way of reducing the biodiesel production costs is to use the less expensive feedstock containing fatty acids such as non-edible oils, animal fats, waste food oil and byproducts of the refining vegetables oils (Veljkovic et al., 2006). Fortunately, non- edible vegetable oils, mostly produced by seed-bearing trees and shrubs can provide an alternative source. With no competing food uses, this characteristic turns attention to *Jatropha curcas*, which grows in tropical and subtropical climates across the developing world (Openshaw, 2000). It is a high oil-yield crop grown around the world for "biodiesel production". *J. curcas* can grow well under such adverse climatic conditions because of its low moisture demands, fertility requirements and tolerance to high temperatures (Kaushik et al., 2007; Michael, 2008).

The seed oil extracted is found useful in medicinal and veterinary purpose, as insecticide, for soap production and as fuel substitute (Gubitz et al., 1999). The proximate composition of the Jatropha seeds is i) Moisture 6.20% , ii) Protein 18.00%, iii) Fat 38.00%, iv) Carbohydrates 17.00%, v) Fiber 15.50% and vi) Ash 5.30%. About the oil content, it was found as 35 to 40% in the seeds and 50 to 60% in the kernel. The oil contains 21% saturated fatty acids and 79% unsaturated fatty acids. The composition of *J. curcas* oil consists of mainly fatty acids such as palmitic 18.22%, stearic 5.14%, oleic 28.46% and linoleic

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48.18% (Jumat and Rozaini, 2008). The cake after oil extraction contains as high protein as 19 to 27%, which is suitable as a protein source for either food or non-food products (Akbar et al., 2009). Oilseeds are one of the most difficult commodities to store – because it contain lipids which are more prone to spoilage by microorganisms during storage (Subramnyam and Rao, 1974; Prasad and Sahay, 1986).

Generally, insects, birds, rodents and micro-organisms are mainly responsible for storage losses in food grains including oilseeds (Christensen and Kaufmann, 1969). The storage fungi develop and infest seeds during storage when the moisture is low as less than 16% and it comprised of *Aspergillus glaucus*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus ochraceus*, Aspergillus terreus, *Aspergillus restrictus Penicillum citrinum, Penicillium brevicompactum, Penicillium cyclopium and Penicillium verdidicatum.* Aspergillus species, which grow at a starting relative humidity of 65%, are common initiators and *Pencillium* species require more moisture as over 80% relative humidity (Christensen, 1973). The chief effects of storage fungi on agricultural commodities are (1) Decrease in seed germination, (2) Biochemical changes, (3) Nutritional losses, (4) Heating, (5) Increase of Moisture content, (6) Mustiness (staleness), (7) Caking of grains and (8) Mycotoxin production (Christensen and Kaufman, 1969). The aflatoxins, a group of highly toxic and carcinogenic fungal metabolites produced by *A. flavus.*

Hence, the present study is concentrated on analysis of storage fungi for qualitative and quantitative pattern in Jatropha *(J. curcas.L)* seeds to know the biodiversity with reference to moisture content and storage period. The relatedness of storage period and occurrence of storage fungi were compared with the moisture content of Jatropha seeds.

MATERIALS AND METHODS

Sample collection

The Jatropha seed samples were collected from i) Southern Railway, Logo works, Biodiesel production plant, Perambur, Chennai, ii) M.S. Swaminathan Research foundation, Chennai; iii) M/s.Perumal Chetty and Sons (Herbal medicine merchants), Chennai and iv) Local market of Chennai. The samples were collected in cleaned PET bottles, tightly capped, labeled and brought to the laboratory for further analysis.

Determination of moisture content

The samples were crushed in to coarse powder before it is taken for analysis of moisture content. About 5 g of sample was taken in to a pre-weighed small glass dish and dried in the hot air oven at 60±5°C for 3 h period. After cooling the sample, it was weighed in an analytical balance for the weight loss during drying. In the same way, the procedures were repeated until the same weight was obtained after drying. The weight values were recorded and substituted in the following formula for calculating the moisture content of the seed samples.

$$\text{Moisture content (\%)} = \frac{(W1-W2)}{W2} \times 100$$

Where,

W1 - original weight of sample before drying
W2 - weight of sample after drying.
X 100 - multiplication for % of moisture content

Analysis of storage mycoflora

Cleaning of glassware

About 25 g of potassium dichromate was dissolved in 50 ml of distilled water and made up to 1000 ml by adding commercial grade sulphuric acid (Salle, 1948) and care was taken to keep the solution cool while mixing with acid. The glasswares were first washed with detergent solution which was thoroughly washed off with running tap water. Then, they were soaked in the cleaning solution for a few hours, washed thoroughly with running tap water and finally with distilled water. The washed glasswares were dried with 60°C.

Culture media preparation

For analysis of storage mycoflora, the highly osmotic culture as CDA (Czapek's Dox Agar) media contain 50% sucrose was used throughout the study for the growth of micro-organism (Rao and kalyanasundaram, 1983 and Sheila et al., 1978). For isolation and sub culturing the storage fungi, the CDA containing 3% sucrose was used. To avoid the contamination of bacteria and yeast during the growth of storage fungi, the antibiotics (streptomycin and penicillin) were used 25 unit per ml of the molten medium.

Composition of CDA (50% sucrose) media

Sucrose - 500.00 g for (50%) and 30 g for (3%)
Sodium nitrate - 3.00 g
Dipotassium hydrogen phosphate - 1.00 g
Magnesium sulphate - 0.50 g
Potassium chloride - 0.50 g
Ferrous sulphate - 0.01 g
Agar - 20.00 g
Distilled water - 1000 ml
H adjusted to 6.5.

To observe the specific character of storage fungi for identification, the following media were used (Raper and Fennel, 1965).

Composition of malt agar

Malt extract - 20.0 g
Sucrose - 30.0 g
Agar - 20.0 g
Distilled water - 1000 ml
H adjusted to 6.5.

Identification

To identify the individual species of storage fungi, The Manual of Aspergillus after Rapen and Fennel (1965), and The Manual of Penicillia after Raper et al. (1945) were followed. The hyphomycete fungi were identified after Barnett and Hunter (1977).

Table 1. Details of sample collection.

S. no.	Sample details	Place of collection	Period of storage (months)	No. of samples
1	Sample lot No. 1	Southern Railway, Logo works, Biodiesel production plant, Perambur, Chennai	30 to 60	5
2	Sample lot No. 2	M.S. Swaminathan Research foundation, Taramani, Chennai	24	4
3	Sample lot No. 3	M/s.Perumal Chetty and Sons (Herbal medicine merchants), Chennai	18	6
4	Sample lot No. 4	Local Herbal products stores, Chennai.	12	5

Pre-plating treatment of grains

The sample was mixed thoroughly and a sub sample was drawn for plating. The following treatment on samples was given before plating for enumerations of the storage mycoflora.

Untreated seeds: The seeds were plated directly without any treatment. By this method, the "surface mycofloras" (SC) of the seeds were allowed to grow.

Washed seeds: The seeds, were held in a nylon-meshed tea strainer, washed in running tap water for 2 to 3 min and rinsed with sterile water, before being transferred to a sterile Petri dish lined with filter paper to absorb excess moisture. By this procedure the "Surface Invaders" (SI) were allowed to grow. It was surmised that these fungi represented either very closely adherent spores, or those that had made some growth on the seed surface.

Surface sterilized seeds: For surface sterilization of the seeds, 0.1% of mercuric chloride was used and sterile 7.5% sodium chloride solution as the washing fluid. These had been arrived at in our laboratory, after experimenting with standard surface sterilising agents such sodium hypochlorite (Sheila et al., 1978). The strainer with washed seeds was dipped in mercuric chloride solution and shaken well for 60 s. Immediately after the given time, the strainer was lifted off and the seeds were washed repeatedly with sodium chloride solution. The solution was then drained off and the seeds transferred to a sterile Petri dish lined with filter paper. The fungi growing out of these seeds were recorded as "Internal Fungi".

De-shelled seeds: Some of the surface sterilized grains were plated after aseptically removing the shell using sterile forceps and scalpel. This was done to observe the extent of invasion of the kernel by fungi. This group of fungi was termed as "Kernel Fungi" (KF) in the present study.

Plating, incubation, observation and identification

From the seeds spread out in the filter-paper lined Petri dish, 20 seeds were picked randomly one by one using sterile forceps and plated on the plates of medium. A paper template was used under the plates for even distribution of 4 seeds in each plate. The plates were incubated at 30±1°C and observed daily from the 3rd to 5th day and in some cases up to the 7th day after inoculation. The morphological and microscopical characters of the fungal colonies that grew out were observed for identification of the individual species. The fungi were isolated in 3% CDA for further examination.

RESULTS

Sample collection

The details of sample collection are presented in Table 1. In total, about 20 samples were collected from 4 different locations. The period of storage of Jatropha seeds is from 2 to 60 months.

Qualitative picture of storage mycoflora on seeds

The individual species of storage fungi encountered from different Jatropha seed samples from the present study are:

1. *A. glaucus (*Mangin*)* Thom and Church,
2. *A. flavus* Link,
3. *A. niger* Van Tieghem,
4. *Aspergillus flavipus* Link,
5. *Aspergillus nidulan* Eldams,
6. *Aspergillus fumigatus* Fresenius,
7. *A. candidus* Link,
8. *P. citrinum* Thom,
9. *Mucor* sp., and
10. *Rhizhopus* sp.

The quantitative pattern of storage fungi in different Jatropha seed samples at various levels of contamination over Jatropha seeds were presented in Table 2 and Figure 1 (surface mycoflora), Figure 2 (Surface Invaders), Figure 3 (Internal fungi) and Figure 4 (Kernal fungi). With reference to the surface mycoflora, the grains plated directly on agar plates, *Mucor* sp. was present in all the

Table 2. Observation of Storage mycoflora in Jatropha seeds at different treatments.

S. no.	Name of the fungi	% of fungal distribution			
		SM	SI	IF	KF
1	*A. niger*	90	58	75	10
2	*A. flavus*	85	75	68	20
3	*A. fumigatus*	25	0	50	0
4	*A. glaucus*	5	37	50	0
5	*A. candidus*	25	25	0	0
6	*A. nidulans*	5	0	0	25
7	*A. flavipes*	5	0	25	0
8	*P. citrinum*	10	0	0	0
9	*Mucor* sp.	100	100	100	0
10	*Rhizopus* sp.	32	0	16	0

SC – Surface Mycoflora, SI – Surface Invaders, IF – Internal Fungi, KF – Kernel Fungi.

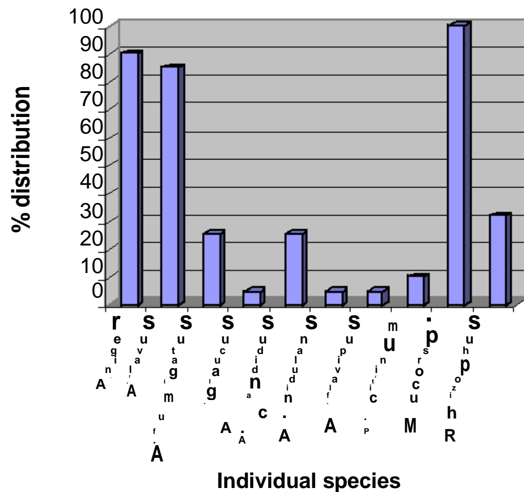

Figure 1. Pattern of surface storage mycoflora in Jatropha seeds.

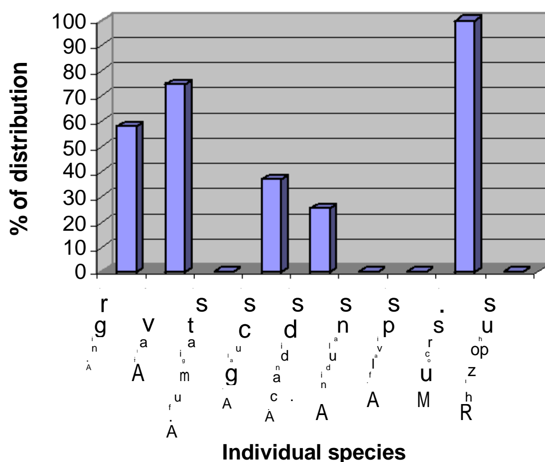

Figure 2. Pattern of storage fungi in Jatropha seeds as surface invaders.

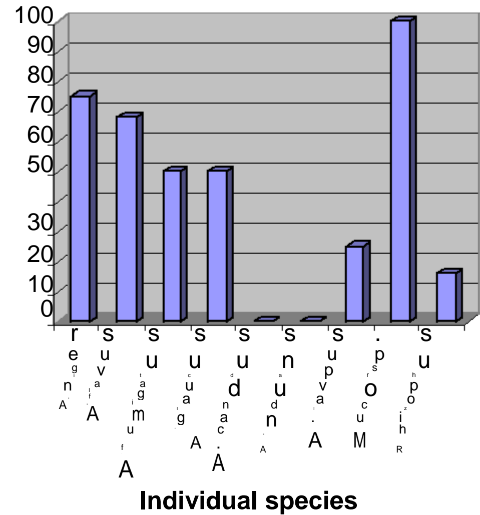

Figure 3. Pattern of storage mycoflora in Jatropha seeds as internal fungi.

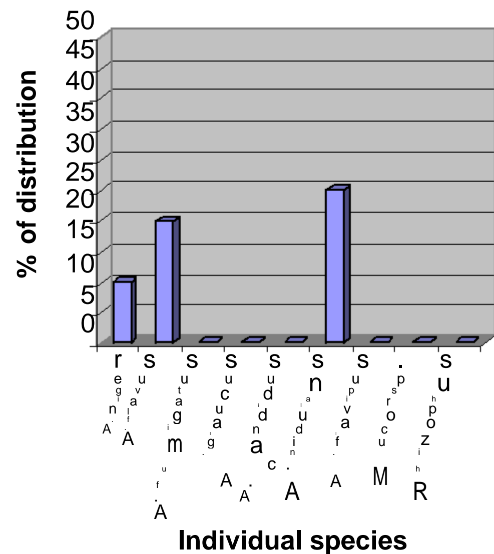

Figure 4. Pattern of storage fungi in Jatropha seeds as kernel fungi.

grains (100%) and *A. niger* (90%) occurred predominantly followed by *A. flavus* (85%), *Rhizopus* sp. (32%) , *A. fumigatus* (25%), *A. candidus* (5%), *A. glaucus* (5%), *A. nidulans* (5%), and *Aspergillus flavipes* (5%) which is represented in Figure 1.

With reference to the surface invaders, the seeds plated after washing, *Mucor* sp. was present in all the grains (100%) followed by *A. flavus* (75%) and *A. niger* (58%), *A. glaucus* (37%), *A. fumigatus* (25%), *A. candidus* (25%), *A. glaucus* (5%), *A. nidulans* (5%), and *A. flavipes* (5%) and no *Rhizopus* sp. (0) found in grains which is represented Figure 2. With reference to the Internal fungi, the seeds plated after surface sterilization,

Table 3. Moisture content on different samples of Jatropha seed samples.

Sl. no.	Sample no.	Storage period	No. of samples	Average moisture content (%)	Standard deviation (±)	Standard error (±)
1	Sample lot No. 1	60 months	5	11.6	0.380	0.1699
2	Sample lot No. 2	24 months	4	9.45	0.4358	0.2179
3	Sample lot No. 3	18 months	6	8.63	0.225	0.091855
4	Sample lot No. 4	12 months	5	8.28	0.27748	0.1240

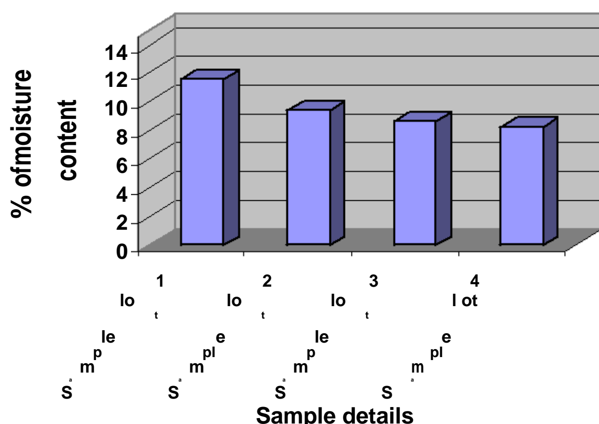

Figure 5. Average moisture content of Jatropha seeds at different sample lots.

Mucor sp. was present in all the grains (100%) followed by *A. niger* (75%), *A. flavus* (68%) and *A. fumigatus* (50%) , *A. candidus* (25%), *A. glaucus* (50%), *A. nidulans* (5%), and *A. flavipes* (25%) and absence of *Rhizopus* sp. (0) found in seeds which is represented in Figure 3. Figure 4 represents the distribution of storage fungi in Jatropha seeds, after the removal of shell in the seeds after surface sterilization. Most of the fungi were absent and less than 25% of seed samples were contaminated with *A. niger* (10%), *A. flavus (*20% and *A. nidulans* (25%).

Details of moisture content

The moisture content of Jatropha seed samples collected from different sources at various storage periods were presented in the Table 3 and Figure 5. The moisture content ranges from 8.0% in the sample from 12 months storage period to a maximum of 12.0% from the sample of a maximum of 60 months period. Here, the reduction of moisture content observed in seeds stored for long period. Figure 5 represents the average moisture content of Jatropha samples from different lots like lot 1 up to 12 months storage, lot 2 up to 18 months storage, lot 3 up to 24 months storage and lot 4 stored up to 60 months of storage which shows consistent reduction of moisture during storage period which is from 11.6 to 8.28% (Figure 5).

DISCUSSION AND CONCLUSION

It was observed from the present study that, as different species of storage fungi all the samples even at low moisture content shows that, the xerophilic nature of storage fungi and grow at low water activity substrate. It is also ascertained that, when the seeds are stored, there is a possibility of continuous growth in certain species of the storage microorganisms and increase in the moisture content (Chakrabarty, 1987). However, in the present study, there is no significant increase in the individual species of fungi as observed, which may be due to a very minor level of variation in the moisture content even after long storage. The slight moisture content increase against long term storage period might be due to the hydrolysis or breakdown of lipid, carbohydrate and proteins and release of water molecules. The release of free fatty acids from lipid degradation by fungi is well known and hydrolysis of starch and proteins for utilization by the microorganisms also, has been studied from the earlier findings (Christensen and Kaufmann, 1969).

The reported losses in oilseeds and oils are the increase of Free Fatty Acids (FFA), which degrades the fatty acid fraction and decrease of viscosity also was comparable with earlier studies (Abulude et al., 2007; Hanny et al., 2008). Hence, the energy value of biodiesel may be expected to lose during the storage period and due to storage fungi (Ashwani and Satyawati, 2008). Therefore, suitable storage conditions and period of storage have to be ascertained and recommended through research evidences to avoid these losses. The important observation in the present investigation is the predominant occurrence of *Mucor* sp., *A. niger* and *A. flavus* in the order of dominance, which shows that these fungi may be having the ability of utilization of oil present in the Jatropha seeds and further losses on biodiesel value in seeds, if stored in poor conditions and after improper drying.

Therefore, it is concluded that from the present study, as there has been the possibility of lipid breakdown and release of free fatty acids (FFA) may occur during the growth of storage fungi in Jatropha seeds and oil seeds

(Bothast, 2009; Hanny et al., 2008). Even after the extraction of oil from the seeds, the oilcake may contain unwanted microbial products like mycotoxins and further development of fungi due to contamination in the oil mills during the operation of oil extraction process indicates the spoilage of nutrients like protein, carbohydrate, fats which pose health hazard for use of these byproducts for poultry and animals.

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REFERENCES

Ashwani K, Satyawati S (2008). An evaluation of multipurpose oil crop for industrial uses (Jatropha curcas.L): A review, p. 350.

Barnett HL, Hunter BB (1977). Illustrated genera of imperfect fungi. 3rd ed . Burgess publishing company, Minnesota, p. 245

Bothast RJ (2009). Fungal deterioration and related phenomena in cereals, legumes and oilseeds. Northern Regional Research Center Agricultural Research Service U.S. Department of Agriculture Peoria, IL 61604.

Christensen CM (1969). Influence of moisture content , temperatute and time of storage upon invasion of rough rice by storage fungi. Phytopathl., 59: 145-148.

Christensen CM (1973). Loss of viability in storage : Microflora. Seed Sci. Technol., 1: 547-562.

Chakrabarti DK (1987). A review of deterioration of oil-seeds by fungi with special reference to India. Int. Bioderior., 23: 137-157.

Emil A, Zahira Y, Siti KK, Manal I, Jumat S (2009). Characteristic and Composition of Jatropha curcas oil seed from Malaysia and its potential as biodiesel feedstock. ISSN 1450-214X, Eur. J. Sci. Res., 29(3): 396-403.

Abulude FO, Ogunkoya MO, Ogunleye RF (2007). Storage properties of oils of two Nigerian oil seeds *Jatropha curcas* (Physic Nut) and Helianthus annus (Sunflower). Am. J. Food Technol., 2: 207-211.

Gübitz GM, Mittlebach M, Trabi M (1996). Exploitation of the tropical oil seed plant *Jatropha curcas*. L. Int. Bioresour. Technol., 58: 77-82.

Hanny JB (2008). “Biodiesel production from crude Jatropha curcas.L seed oil with high content of free fatty acids”, Bioresour. Technol., pp. 1716-1721.

Jumat S, Rozaini A (2008). Physicochemical properties of Malaysian *Jatropha curcas* seed oil. Sains Malaysian, 37(4): 379-382.

Kaushik N, Kumar S, Kaushik N, Roys S (2007). Genetic variabiality and divergence studies in seed traits and oil content of *Jatropha (Jatropha curcas. L.)* accessions. Biomass and Bioenergy, 31: 497-502.

Makkar HP, Becker S, Sporer K, Wink M (1997). Studies on nutritive potential and toxic constituents of different provenances of Jatropha curcas . J. Agric. Food Chem., 45: 3152-3157.

Michael W (2008). “Biodiesel produced from *Jatropha* seed oil”, p. 480.

Openshaw K (2000). A review of *Jatropha curcas*: An oil plant of unfulfilled promise. Biomass and Bioenergy, 19: 1-15.

Prasad T, Sahay SS (1986). Aflatoxin elaboration in Brassica campesteris under storage. J. Indian Bot. Soc., 66: 54-57.

Rao GJ, Kalyanasundram I (1983). Osmophilism in food grain storage fungi. Mycopathologia, 83: 3-7.

Raper KB, Fennel DI (1965). The genus *Aspergillus*. The William and Wilkians Company, Baltimore.

Raper KB, Thom C, Fennell DI (1949). A manual of the *Penicillia*. The William and Wilkins Company, Baltimore.

Salle AJ (1948). Laboratory manual of fundamental principles of Bacteriology (3rd ed.). Mc-Grew-Hill Book Company, Inc.

Sheila V, Vijiya K, Kalyanasundaram I (1978). Fungal spoilage of stored food grains –A preliminary study with rice. J. Madras Univ. (sec. B), 41: 149-160.

Subramanyam P, Rao AS (1974). Occurrence of aflatoxins and citrinin in groundnut at harvest in relation to pod condition and kernel moisture content. Curr. Sci., 43: 707-710.

Verma KC, Gaur AK (2009). *Jatropha curcas L.*: Substitute for Conventional Energy, ISSN 1817-3047. World J. Agric. Sci., 5(5): 552-556.

Veljkovic VB, Lakicevic SH, Stamenkovic OS, Todorovic ZB, Lazic KL (2006). Biodiesel production from tobacco (Nicotiana tabacum L.) seed oil with a high content of free fatty acids. Fuel, 85: 2671-2675.

Zurina ZA (2009). “Extraction of oil from *Jatropha* seed-optimization and kinetics”, Am. J. Appl. Sci., 6(7): 1390-1395.