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Influence of Fungi on Carbohydrate and Phenol Content of *Jatropha curcas* Seeds during Storage

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***Jatropha curcas* L. has been considered a potential source of seed oil for the production of biofuel. The aim of this study was to estimate the change in carbohydrate and phenolic content of *Jatropha* seeds after deterioration under storage condition. For estimation of carbohydrate and phenol fresh, stored as well as infested *Jatropha* seeds were used. Whole seed and kernels were infested with six dominant fungi *viz. Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium chlamydosporum* and *Penicillium glabrum* separately. Four different concentrations of seed samples *viz.* 50µl, 100µl, 150µl and 200µl were taken for the carbohydrate and phenol estimation of stored as well as infested *Jatropha* seeds. Carbohydrate content got reduced during storage while the phenolic content increases to show their antimicrobial effect. Maximum carbohydrate found was 50mg/ml at 200µl concentration in *Fusarium chlamydosporum* infested *Jatropha* seeds followed by *Penicillium glabrum i.e.,* 30mg/ml and minimum carbohydrate content found was 1mg/ml at 50µl concentration in *Aspergillus flavus* infested *Jatropha* seeds and 4mg/ml in *Aspergillus fumigatus* at 50 µl concentration in infested *Jatropha* kernels. Maximum Phenol content found was 190mg/100mg dry wt. in *Fusarium chlamydosporum* infested *Jatropha* kernels followed by *Penicillium glabrum* infested *Jatropha* seeds and kernels *i.e.,* 180mg/100mg dry wt. at 200µl concentration while minimum phenol content was found nearly same *i.e.,* 5.33mg/100mg dry wt. and 5.67mg/100mg dry wt. at 50µl and 100µl concentrations in fresh seeds, respectively.**

Key word: *Jatropha curcas*, Seeds, Storage, Carbohydrate, Phenol.

Interest in biodiesel as an alternative fuel for diesel engines has increased in recent years due to environmental concerns on emissions from petroleum based fuels. Biodiesel has therefore attracted extensive attention as a renewable, biodegradable and non-toxic fuel since the past decade[1-3]. Inedible vegetable oils have been identified as the best alternative source with *Jatropha curcas* opined to be the best feedstock for biodiesel production due to its numerous advantages[4]. Apart from non-competition with food as feedstock, biodiesel from *Jatropha curcas* oil provides a commercially viable alternative to diesel as it has comparable desired physico-chemical and performance characteristics[5].

Chemical composition proved that the *J. curcas* seeds are a good source of protein (32.88%),

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oil (27.36%) and carbohydrates (30.11%)[6]. Phenolic compounds occur ubiquitously in plants and a variety of biological activities have been attributed to them. These advantageous properties include antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, anti-neoplastic, mutagenic, antioxidant, anti-hepatotoxic, anti-hypertensive, hypolipidemic, anti-platelet and anti-inflammatory activities. Many of these biological functions corresponded to their free radical scavenging and antioxidant activities[7].

After harvesting, *Jatropha curcas* seeds are stored before the extraction of oil. But storage is done to maintain harvesting quality of product but not to improve it[8]. Storage condition of oil seeds before industrial extraction might influence the quality of the seeds and crude oil[9]. However, in many instances, the quality of *Jatropha* seeds deteriorates gradually due to improper handling and inappropriate storage condition[10]. It seems temperature; moisture and the storage duration are the most important factors which affect stored product quality and quantity[11, 12]. The changes in the level of chemical composition of seeds were also influenced by the growth of seed-borne mycoflora during improper storage period.

The objectives of this study were to carry out preliminary investigation into the influence of seed-borne mycoflora on carbohydrate and phenolic contents of *Jatropha curcas* seeds during storage.

MATERIALS AND METHODS

The whole experiment was carried out during year 2010-11 in the laboratory of the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi.

For estimation of carbohydrate and protein fresh, stored as well as infested *Jatropha* seeds were used. Whole seed and kernels were infested with six dominant fungi *viz. Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium chlamydosporum* and *Penicillium glabrum* separately.

Estimation of Total Carbohydrate

Carbohydrate has been estimated by Anthrone method[13]. The carbohydrate hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides.

Freshly harvested, stored and fungal infested *Jatropha curcas* seeds as well as infested kernels separately were taken for carbohydrate estimation. Weigh 100mg (0.1g) of the sample into a boiling tube. The samples were hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml. of 2.5 N-HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceases. The volume was made to 50 ml. and centrifuged. The supernatant was collected and 1-ml. aliquots were taken for analysis. Prepare the standards by taking 0, 0.5, 1.0, 1.5, 2.0, and 2.5 ml of the working standard. '0' serves as blank. Made the volume to 2.5 ml. in all the tubes including the sample tubes by adding distilled water. Then added 2 ml. of anthrone reagent. Heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630 nm. Drawn a standard graph by plotting concentration of the standard on the X- axis versus absorbance on the Y- axis. From the graph calculated the amount of carbohydrate present in the sample tube.

Calculation

$$\text{Amount of carbohydrate present in 0.5 g. of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Table 1. Carbohydrate content of *Jatropha* seeds during Different Periods of Storage

Concentration of Samples (µl)	Carbohydrate Content (mg/ml)		
	Fresh seeds	One year stored seeds	Two years stored seeds
50	5.33^{i*}	3^{j}	2^{k}
100	10.67^{f}	10^{g}	9^{h}
150	16.67^{c}	15^{d}	14^{e}
200	23.67^{a}	18^{b}	17^{c}

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

Estimation of Phenolic content

Phenolic content has been estimated by Bray and Thorpe[14]. Phenols, the aromatic compounds with hydroxyl groups, are widespread in plant kingdom. Phenols are said to offer resistance to disease and pests in plants. Total phenol estimation was carried out with the Folin-Ciocalteau reagent.

Freshly harvested, stored and fungal infested *Jatropha curcas* seeds as well as infested kernels separately were taken for phenol estimation. Weigh 0.5 g of the sample and grinded it with a pestle and mortar in 10-time volume of 80% ethanol. Centrifuged the homogenate at 10,000 rpm for 20 min. and saved the supernatant. Re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water (5ml.). Pipette out different aliquots (0.2 to 2 ml.) into test tubes. Made the volume 3 ml. in each tube with distilled water. Added 0.5 ml. of Folin-Ciocalteau reagent. After 3 min. added 2 ml. of 20% Na_2CO_3 solution to each tube. Mixed thoroughly. Placed the tubes in boiling water for exactly one minute, cooled and measured the absorbance at 650 nm against a blank reagent. A standard curve was prepared by using different concentrations of catechol.

Data Analysis

All results obtained from the biochemical test of carbohydrate and phenol 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[15].

RESULTS AND DISCUSSION

Table-1 shows estimation of carbohydrate in fresh and stored *Jatropha* seeds and Table-2 shows the carbohydrate content of infested *Jatropha* seeds and kernels at different concentration.

According to Table-1 the maximum carbohydrate was found in fresh *Jatropha* seeds at 200µl concentration ***i.e.,*** 23.67mg/ml. followed by 18 mg/ml in one year stored *Jatropha* seeds at 200µl concentration. Minimum carbohydrate was

Table 2. Carbohydrate content of Infested *Jatropha* Seeds & Kernels at Different Concentrations

Concentration of Samples (µl)	Carbohydrate Content (mg/ml)												
	Infestation on Seed Coat							Infestation on Kernels					
	T_1	T_2	T_3	T_4	T_5	T_6	Control	T_1	T_2	T_3	T_4	T_5	T_6	Control
50	3^{m*}	1^{n}	7^{j}	10^{g}	6^{k}	10^{g}	5.33^{l}	12^{k}	12^{k}	4^{p}	9^{m}	5^{o}	20^{f}	5.33^{o}
100	8^{i}	6^{k}	8^{i}	20^{d}	10^{g}	20^{d}	10.67^{f}	12^{k}	13^{j}	5^{o}	11^{l}	8^{n}	20^{f}	10.67^{l}
150	9^{h}	7^{j}	8^{i}	20^{d}	20^{d}	30^{b}	16.67^{e}	19^{g}	26^{c}	9^{m}	13^{j}	15^{i}	21^{e}	16.67^{h}
200	9^{h}	8^{i}	9^{h}	20^{d}	50^{a}	30^{b}	23.67^{c}	21^{e}	29^{a}	13^{j}	21^{e}	19^{g}	27^{b}	23.67^{d}

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

T_1= Seeds/Kernels infested with *Alternaria alternata*
T_2= Seeds/Kernels infested with *Aspergillus flavus*
T_3= Seeds/Kernels infested with *Aspergillus fumigatus*
T_4= Seeds/Kernels infested with *Aspergillus niger*
T_5= Seeds/Kernels infested with *Fusarium chlamydosporum*
T_6= Seeds/Kernels infested with *Penicillium glabrum*

found in two years stored *Jatropha* seeds at 50µl i.e., 2mg/ml.

Table-2 reveals the carbohydrate content of fresh and infested *Jatropha* seeds and kernels. Maximum carbohydrate found was 50mg/ml at 200µl concentration in *Fusarium chlamydosporum* infested *Jatropha* seeds followed by *Penicillium glabrum i.e.,* 30mg/ml at 200µl concentration and in *Aspergillus flavus* and *Penicillium glabrum* infested *Jatropha* kernels it was 29mg/ml and 27mg/ml, respectively at 200µl concentration and minimum carbohydrate content found was 1mg/ml at 50µl concentration in *Aspergillus flavus* infested *Jatropha* seeds and 4mg/ml in *Aspergillus fumigatus* at 50 µl concentration in infested *Jatropha* kernels. In control maximum carbohydrate concentration found was 23.67mg/ml at 200µl concentration and minimum was 5mg/ml at 50µl concentration.

Carbohydrates increase the severity of the infection and that they may serve as easily metabolized carbon substrates for the pathogen[16-18]. The gradual loss of protein and carbohydrate content of sesame and sunflower seeds due to *A. flavus* and *A. niger* during storage[19]. The sharp decline in the levels of soluble carbohydrates during the desiccation of seeds[20]. Sucrose and raffinose level declined in stored seeds of maize although the monosacharides, glucose fructose and galactose diminished faster[21].

There was 1.5 to 3 fold decreases in protein, carbohydrate and phenol levels and 2-3 fold increase in reducing sugar were observed in moderately infected and highly infected seeds compared to healthy seeds[22]. The results therefore indicate the degradation of protein and carbohydrates and accumulation of reducing sugar due to degradation of carbohydrates, by the pathogen.

Table-3 shows estimation of phenol content of fresh and stored *Jatropha* seeds and Table-4 shows the phenol content of infested *Jatropha* seeds and kernels at different concentration.

Data presented in Table-3 shows that the maximum phenol content was found in two years stored seed *i.e.,* 35mg/100mg dry wt. followed by one year stored seeds *i.e.,* 30mg/100mg dry wt. at 200µl concentrations. Minimum phenol content was found nearly same *i.e.,* 5.33mg/100mg dry wt. and 5.67mg/100mg dry wt. at 50µl and 100µl concentrations in fresh seeds, respectively.

There was an increase in the phenolic compounds from first day onwards[23]. A rapid increase in phenolics contents was observed in case of seeds stored at open condition up to 7th day and later decreased. The phenolic content in the seeds stored in 21±2°C increased up to 16.185µg mL^{-1} by 17th day and later decreased. There was a steady increase in the phenolic contents in seeds stored at 14±2°C up to 15th day and a slight decrease was seen afterwards. The phenolic contents increased gradually in the seeds stored at 28±2°C in sealed polythene bags.

Table-4 reveals the phenol content of fresh and infested *Jatropha* seeds and kernels. Maximum Phenol content found was 190mg/100mg dry wt. in *Fusarium chlamydosporum* infested *Jatropha* kernels followed by *Penicillium glabrum* infested *Jatropha* seeds and kernels *i.e.,* 180mg/100mg dry wt. at 200µl concentration. Minimum phenol content was found nearly same *i.e.,* 5.33mg/100mg dry wt. and 5.67mg/100mg dry wt. at 50µl and 100µl concentrations in fresh seeds, respectively.

The antimicrobial property of phenolic compounds and especially those of the flavonol group is well documented[24, 25]. The first stage of

Table 3. Phenol content of Jatropha seeds during Different Periods of Storage

Concentration of Samples (µl)	Phenol Content (mg/100mg dry wt.)		
	Fresh seeds	One year stored seeds	Two years stored seeds
50	5.33^{g*}	10^{f}	15^{e}
100	5.67^{g}	15^{e}	15.33^{e}
150	10.33^{f}	20^{d}	25^{c}
200	15.33^{e}	30^{b}	35^{a}

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

Table 4. Phenol content of Infested Jatropha Seeds & Kernels at Different Concentrations

Concentration of Samples (µl)	Phenol Content (mg/100mg dry wt.)												
	Infestation on Seed Coat							Infestation on Kernels					
	T_1	T_2	T_3	T_4	T_5	T_6	Control	T_1	T_2	T_3	T_4	T_5	T_6	Control
50	15^{o*}	30^{m}	10^{p}	15^{o}	20^{n}	35^{l}	5.33^{q}	65^{o}	50^{q}	25^{r}	50^{q}	70^{n}	55^{p}	5.33^{u}
100	45^{j}	50^{i}	40^{k}	30^{m}	40^{k}	80^{d}	5.67^{q}	120^{j}	100^{k}	50^{q}	85^{l}	145^{f}	115^{j}	5.67^{u}
150	50^{i}	65^{g}	50^{j}	60^{h}	50^{j}	170^{b}	10.33^{p}	165^{d}	135^{g}	80^{m}	125^{h}	180^{c}	145^{f}	10.33^{t}
200	75^{e}	135^{c}	75^{e}	70^{f}	75^{e}	180^{a}	15.33^{o}	185^{b}	155^{e}	100^{k}	155^{e}	190^{a}	180^{c}	15.33^{s}

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

T_1= Seeds/Kernels infested with *Alternaria alternata*
T_2= Seeds/Kernels infested with *Aspergillus flavus*
T_3= Seeds/Kernels infested with *Aspergillus fumigatus*
T_4= Seeds/Kernels infested with *Aspergillus niger*
T_5= Seeds/Kernels infested with *Fusarium chlamydosporum*
T_6= Seeds/Kernels infested with *Penicillium glabrum*

defense mechanism involves a rapid accumulation of phenols at the infection site which restricts or slows the growth of the pathogens[26]. The changes in the level of total phenolic compounds in chickpea as influence by *Aspergillus flavus, A. niger, Fusarium maniliforme* and *Pencillium oxalicum* infection[27]. The four days after treatment, infected pods always contained the highest amount of phenolics followed by the wounded, and finally by intact pod[28]. The results showed that the increase in phenolic content could be correlated to the aging, the wounding and the infection of the pods. This could account for a higher synthesis of disease-related molecules like phenols and hydroxyproline-rich glycoproteins which generally accumulate in fungus infected plants[29]. The increase amount of phenol might be responsible for resistance response in plant[30].

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