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Biochemical and Physicochemical Changes in Seeds and Fatty Oil of *Salvadora oleoides* and *Salvadora persica* by Soil Borne Fungi

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Abstract

Susceptibility of seeds to fungal invasion, proliferation and elaboration of mycotoxins is one of the major factors for the low quality and productivity. The present study was undertaken to investigate the impact of fungal infestation on biochemical content of seeds of two important oil-yielding species namely *S. oleoides* and *S. persica* as well as certain physicochemical characteristics of fatty oil. Seeds from ground lying fruits of the two species were collected and segregated into three groups based on level of infestation. Altogether eight dominant fungi were isolated and identified as *Aspergillus candidus, A. flavus, A. niger, Fusarium oxysporum, Penicillium chrysogenum, Rhizoctonia solani, Rhizopus nigricans* and *Sclerotium rolfsii* based on specified characteristics. Results of the biochemical analysis showed significant decrease in fatty oil, crude protein and carbohydrate contents across the different infestation levels as compared to healthy seeds. The acid values of fatty oil from seeds of *S. oleoides* and *S. persica* constantly increased to a higher value all through at the level of infestation. Further increase in soponification value and decrease in iodine number of the fatty oils was also recorded all over the infestation levels. The results are in agreement with the previous studies on similar aspects with various other oilseeds.

Key Words: Oilseeds, *Salvadora spp*., Biodeterioration, Biochemicals, Physicochemical traits.

Introduction

Despite very harsh climatic condition and immense biotic pressure, flora of Indian arid zone comprises a number of species having great economic potential. The two species of genus *Salvadora* (Family: Salvadoraceae) namely *Salvadora oleoides* Decne. (common name: Jal or Peelu or Khakan) and *Salvadora persica* Linn. (common name: Khara Jal) distributed throughout the arid regions of India are commercially valued for their oilseeds. Both the species are grown as shrub or small tree with short twisted trunk and are considered suitable as shelter-belts and windbreaks on the agricultural fields all over Indian deserts track [1]. Fruits are drupe, globose, smooth which ripen in the month of May-June and acquire a reddish brown colour [2]. Fruits of the two species are sweat and edible and said to increase milk yield when fed to lactating cattle. Seeds forms about 45% of whole fruits and contain 40-50 % of greenish yellow fatty oil [3]. Seed oil of both the species with almost comparable composition though not suitable for edible purpose have wide industrial application. On account of its high saturated fatty acid content, it is used in soap making after purification and is regarded as a potential industrial substitute of coconut oil [4,5]. Medicinally, the oil is used in the treatment of rheumatic pains and as a base for ointments [6,7].

Like all other seeds, oilseeds encounter with various microorganisms in the field as well as during storage. Fungal infection is a hazard to oil-seeds. These microorganisms thrive on the seeds at the expense of their easily digestible components resulting into detrimental changes in physicochemical and biochemical characteristics of seeds apart from the loss of their viability and vigour [8-10]. Ripen fruits of *S. oleoides* and *S. persica* if not harvested on time, often fall on the ground due to high wind or biotic interference. Fruits fallen on the ground and left uncollected for a few days were found to be infected by soil-borne fungi. Fungal infestation in oilseeds causes deterioration in content and quality of fatty oil along with other biochemical changes. In addition, fungal infestation also causes rancidity which is an important quality aspect of seed oil with regard to their industrial applicability and

market value. Several studies have reported reduction in oil, protein and carbohydrate contents, as well as increased free fatty acid and peroxide values in fungal-infected oilseeds [11-16].

The content and quality ascertaining chemical composition as well as physicochemical characteristics of seed fatty oil are significantly affected by agroclimatic conditions [17,18] and microbial infestation [19,20]. Fruits of *S. oleoides* and *S. persica* lying on the ground are infested by fungi if not collected in time, causes deteriorative changes in seeds which affect the quality of seeds leading to customer dissatisfaction and their market price. The present study therefore was aimed to evaluate the deterioration in terms of changes in certain physicochemical and biochemical parameters of *S. oleoides* and *S. persica* seeds under varying levels of fungal infestation.

Materials and Methods

Collection of Fruits

Fruits of *Salvadora oleoides* and *Salvadora persica* were collected from the areas located around Jodhpur district of Rajasthan (India), where trees of the two species are naturally inhabited. Fully mature and healthy fruits of the two species were directly harvested from respective trees. Fungal infested fruits lying on the ground under the trees of *S. oleoides* and *S. persica* were collected separately and segregated into three groups based on visual characteristics like, distortion, spots, wrinkles, discolonisation, etc. Fruits exhibiting 10-20 %, 30-50 % and > 60 % infestations were considered as low, mild and severe infestation respectively. A part of all the three groups of infested fruits were kept for isolation and identification of fungi. Seeds were separated from healthy and infested fruits of both the species by depulping; then thoroughly washed under running tap water and finally dried. All dried seed samples were stored in airtight containers till further analysis.

Isolation and Identification of Fungi

The isolation of fungi from the infested fruits of *S. oleoides* and *S. persica* was carried out by blotter test method [21]. A pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized petridishes of 9 cm diameter. Fungal infested fruits of *S. oleoides* and *S. persica* were separately placed at equidistance on moist blotters in autoclaved separate petridishes. All petridishes were incubated at room temperature (28±2^{0}C) for 6 days and fungi were isolated and identified using standard protocols [22,23].

Measurement of Seed Weight

In order to find out the impact of fungal infestation on seed quality, 100-seed weight of healthy and infested seeds of and *S. persica* were measured and changes in 100-seed weight of seeds with different level of infestations were determined. All measurement were replicated thrice for accuracy and results were expressed as mean± standard error of M (SEM).

Biochemical Analysis of Seeds

Determination of fatty oil content in healthy and infested seeds of S. oleoides and *S. persica* was done as per standard method [24]. The percentage of oil was determined and expressed as dry-weight basis. Crude protein was determined by estimating nitrogen content by microkjeldahl technique [24]. The amount of N content was multiplied by 6.25 factors which gave crude protein content of the samples. Carbohydrate was estimated by Anthrone method [25]. All estimations were replicated thrice and results were expressed as mean± standard error of M (SEM).

Statistical Analyses

The SPSS 10 package was used for the statistical analysis the data of biochemical changes on different level of infections in the seeds. The two way analyses of variance models were used to study variation of each quality ascertaining physicochemical properties viz., acid value, saponification value, iodine value etc. with respect to level of infestation. LSD, Duncun Multiple range test were also performed for entire analysis.

Results and Discussion

Susceptibility of seeds to fungal invasion, proliferation and elaboration of mycotoxins is one of the major factors for the low quality and productivity. Deterioration in biochemical composition of seeds in general and oilseeds in particular due to storage fungi has been studied to a large extent. However, studies on impact of soil-borne fungi on biochemical content and oil quality of oilseeds from ground-lying fruits have been very limited. The present study was undertaken to investigate the fatty oil, crude protein and carbohydrate content as well as certain physicochemical characteristics of fatty oil of seeds obtained from fungal infested fruits of two important oil-yielding species of Indian arid zone namely *S. oleoides* and *S. persica*. The fungi infesting the fruits of the two species lying on the ground were isolated and identified. Biochemical content of seeds and physical values of fatty oil derived from seeds with different levels of fungal infestations were determined and compared with the respective the values of healthy seeds of the two *Salvadora* species.

Identification of Fungi on Infested Seeds

Altogether eight dominant fungi were isolated and identified as *Aspergillus candidus, A. flavus, A. niger, Fusarium oxysporum, Penicillium chrysogenum, Rhizoctonia solani, Rhizopus nigricans* and *Sclerotium rolfsii* based on growth characteristic, mycelial morphology, spore morphology and other important characters. An increase in the fungi with progression of infestation was observed.

Changes in Seed Weight

It is evident from the results presented in Table 1 and 2 that infection caused considerable loss in seed weight. Reduction in 100-seed weight in *S. oleoides* seeds with different level of infection was recorded in the range of 2.68±0.08 to 1.47±0.12 gm as compared to 2.68±0.08 gm of the healthy seeds. In case of *S. persica* seeds the reduction in 100-seed weight ranged from 2.32±0.02 to 1.38±0.03 in seeds with different level of infection as compared to 2.63±0.02 gm of the healthy seeds (Figure 1).

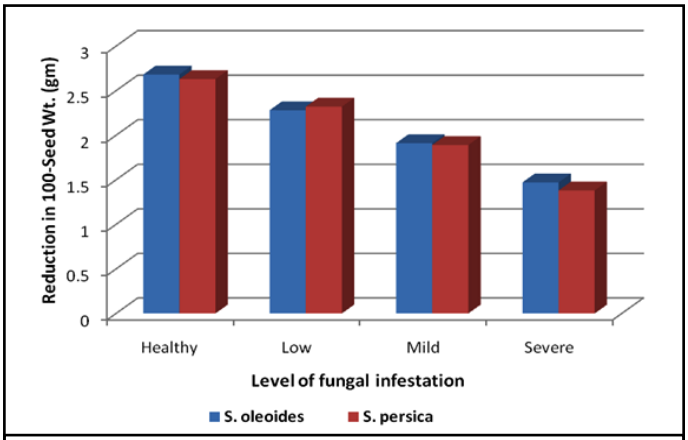

Figure 1: Reduction in weight of fungal infested seeds of *S. oleoides* and *S. persica*

It is amply clear from Fig.1 that there is a decreasing trend in 100-seed weight of both *Salvadora* species as compared to healthy seeds with increasing level of infestation. Reduction in 100-seed weight found to be highly significant at $p < 0.000$ level of significance.

Changes in biochemical composition

The healthy and infested seed samples of *S. oleoides* and *S. persica* were subjected to estimation of fatty oil, crude protein and carbohydrate contents. The results of all estimations were statistically analyzed and levels of significance were calculated for each of the biochemical estimations as summarized in Table 1.

Table 1: Biochemical content of healthy and infested seeds

Level of infestation	Fatty oil content (%)		Protein content (%)		Carbohydrate content (%)	
	S. oleoides	*S. persica*	*S. oleoides*	*S. persica*	*S. oleoides*	*S. persica*
Healthy	43.86±0.43	40.14±4.72	26.21±3.23	23.96±0.21	25.31±0.08	23.96±0.22
Low	38.25±0.24	34.38±0.43	25.08±0.64	21.16±0.20	23.19±0.17	22.16±0.15
Mild	26.53±0.27	24.75±0.47	19.71±0.4	18.38±0.05	17.31±0.22	16.24±0.24
Severe	17.95±0.4	16.20±0.21	14.08±0.15	12.43±0.05	11.87±0.20	10.79±0.23
P value	**0.001**	**0.001**	**0.001**	**0.001**	**0**	**0**

The values are Mean ± SEM of triplicates (n=3)

Changes in Fatty Oil Content

The healthy seeds of *S. oleoides* and *S. persica* recorded the highest percentage of fatty oil which considerably declined with the increasing level of fungal infestation in seeds (Table 1). The yield of fatty oil in seeds with different level of infestation ranged from 38.25±0.24 to 17.95±0.4 % for S. oleoides and 34.38±0.43 to 16.20±0.21 % for *S. persica* which are much less than fatty oil content of healthy seeds of *S. oleoides* (43.86±0.43 %) and *S. persica* (40.14±4.72 %). Deterioration in fatty oil content in seeds has been recorded throughout the different levels of infestation. The analysis of results showed that the oil contents differs significantly ($p < 0.001$) with the level of infestation in *S. oleoides* and *S. persica* seeds. The reduction in fatty oil content of oilseeds can be attributed to utilization of total fat by the colonizing fungi [27]. The results are in agreement with the previous research findings on similar aspect [28,29].

Changes in Crude Protein Content

The crude protein content of 26.21±3.23 and 23.96±0.21 % were found to be highest for the healthy seeds of *S. oleoides* and *S. persica* respectively which progressively declined with the increasing level of fungal infestation. Seeds of *S. oleoides* and *S. persica* with different level of infestation recorded protein content ranging from 25.08±0.64 to 14.08±0.15 % and 21.16±0.20 to 12.43±0.05 % respectively. The deterioration in crude protein contents across the level of infestations were found highly significant at $p < 0.001$ level. For both the species the protein contents decreases as the level of infection increases i.e 26.21% to 14.08 % in the case of *S. oleoides* and 23.96 % to 12.43 % in the case of *S. persica.* Decreasing trend in protein content of infested seeds as compared to the healthy seeds may be due to breaking down of protein into amino acids under the influence the fungal action [30]. Similar observations were found in vegetable seeds and red and black gram seeds [31].

Changes in Carbohydrate Content

Data presented in table 1 clearly indicated a significant decline in the carbohydrate contents in seed of *S. oleoides* and *S. persica* across the different levels of infection. Considerable decline in carbohydrates contents from 25.31±0.08% to 11.87±0.20 % and

from 23.96±0.22 % to 10.79±0.23 have been recorded in seeds of *S. oleoides* and *S. persica* respectively with regard to different infestation levels. The differences in contents of carbohydrates found to be highly significant at p<0.000 level. Decrease in carbohydrate may be due to utilization of sugar by fungi as a substrate for their growth [10].

Changes in Physicochemical Values of Fatty Oil

The fatty oil extracted from healthy and infested seeds of *S. oleoides* and *S. persica* were analyzed for some quality ascertaining physicochemical characteristic including free fatty acid (FFA) value, saponification value and iodine value. The results of all determinations were statistically analyzed and levels of significance were calculated for each of the biochemical estimations as summarized in Table 2.

Table 2: Physicochemical values of fatty oil from healthy and infested seeds

Level of infestation	FFA values (mg KOH/g)		Sap Values (mg KOH/g)		Iodine value (g/100 g)	
	S. oleoides	***S. persica***	***S. oleoides***	***S. persica***	***S. oleoides***	***S. persica***
Healthy	0.98±0.02	1.24±0.13	249.03±0.47	245.53±0.24	3.55±0.08	5.59±0.08
Low	1.96±0.08	1.96±0.12	281.93±0.39	260.91±0.26	3.18±0.13	5.12±0.07
Mild	7.89±0.19	6.99±0.09	300.72±0.81	276.71±0.29	2.43±0.09	4.5±0.067
Severe	9.43±0.19	9.99±0.47	323.72±0.53	305.55±0.67	1.57±0.09	2.57±0.05
P value	0.000	0.000	0.614	0.614	0.000	0.000

The values are Mean ± SEM of triplicates (n=3)

Changes in free Fatty Acid Value

It has been observed from the data summarized in table 2 that the free fatty acid value or simply acid value of fatty oils from healthy and infested seeds of *S. oleoides* and *S. persica* increased with augmented level of infestation. The acid value of fatty oil from healthy seeds of *S. oleoides* and *S. persica* were found as 0.98±0.02 and 1.24±0.13 mg KOH/g respectively which increased to 9.43±0.19 and 9.99±0.47 mg KOH/g respectively for the oil from severely infested seeds. The acid value of the fatty oil from fungal infested seeds constantly increased to a higher value all through the infestation levels (Table 2). The increase in acid values with respect to infestation levels found to be highly significant at p > 0.000 levels of significance for both species. The increase in acid value could be due to the conversion of the oil into fatty acids [32]. It could also be due to production of the free fatty acids at a rate faster than that utilized by the associated fungi growing on the seeds. Also, fungi produced lipases which tend to hydrolyze the ester linkages of the oil to free the linked fatty acids thereby increasing the level of free fatty acid. The results are in agreement with similar findings recorded with seeds of melon [29] and palm [33].

Changes in Saponification Value

The data summarized in table 2 clearly revealed that saponification value of fatty oils from seeds of both *Salvadora* species increases with enhanced level of infestation which showed a reverse trend in comparison to rest of the biochemical composition and other physicochemical traits (Table 2). Analysis of data suggested a significant difference in saponication values of fatty oils from seeds of both the species affected by low to severe level of infection. An increase in saponification value from 249.03 to 300.73 mg KOH/g in case of *S. oleoides* seed oil and 245.23 to 298.90 mg KOH/g in case of seed oil of *S. persica* were recorded.

Changes in Iodine Value

Iodine value or iodine number is indicative of the quantity of unsaturated acids present in the oil. However, the reports on iodine value of fungal infested seeds have been incongruous. Lalithakumari (1971) observed a low iodine number in oil extracted from infested groundnut seeds [34] whereas Ward and Diener (1961) recorded an increase in iodine number in peanut owing to fungal invasion [35]. In the present study the iodine value of fatty oil from healthy and infested seeds of *S. oleoides* continually decreased from 3.55±0.08 g/100g (healthy seeds) to 1.57±0.09 g/100g (severely infested seeds) whereas that of *S. persica* the iodine value decreased from 5.59±0.08 g/100g (healthy seeds) to 2.57±0.05 g/100g (severely infested seeds). Like other biochemical and physicochemical characteristics of seeds of both *Salvadora* species, iodine value also exhibited a decreasing trend with increasing level of fungal infestation which found to be significant at p<0.000 over the level of inflections (Table 2). Increase in saponification value and decrease in iodine number of the fatty oil from seeds of the two *Salvadora* species with increasing level of infestations may be due to the formation of number of short chain fatty acid glycerides during the lypolysis of oil by the enzyme lipase. The relation between iodine number and soponification values is an important criterion for the assessment of quality of oils.

Several reports reveal that fungal infestation in the field and storage conditions cause changes in physicochemical and biochemical characteristic of seeds [36-38]. Oil yielding plants are very important for economic growth of the industrial and energy sectors. Oilseeds containing polyunsaturated fatty acids are important source of biodiesel. These seed oils are better than diesel fuels in terms of physicochemical properties and biodegradability. The quality of fatty oils is dictated by several physical and biochemical parameters that are dependent on the source of oil, geographic, agroclimatic, and agronomic conditions as well as harvest and post harvest handling [39,40].

Conclussion

On account of their high oil content and suitability as an alternate source of edible oil for industrial utilization and well as for fuel and energy, seeds of both *S. oleoides* and *S. persica* have a great commercial importance. However, deteriorated oilseeds cannot be reasonably used for industrial purposes. The study led to the conclusion that fungal infestation induced bio-deteriorative changes in the biochemical constituents such as, total fat, crude protein, carbohydrates of both *S. oleoides* and *S. persica* seeds as well as free fatty acid, saponification and iodine values of seed oil. All these together reduce the economic value of these seeds and hence the growth and development of fungi in seeds may be prevented through timely harvesting and adopting better harvesting and post-harvest handling of *Salvadora* seeds. Therefore, in order to attain an increased production of good quality oil, special attention on deterioration of the oilseed is crucial to met quality standard of seeds and industrially important fatty oil.

Conflict of Interest

The authors declare that there is no conflict of interest.

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