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Impact on Seed Quality of Ajwain [*Trachyspermum ammi* (L.) Sprague] Stored under Different Storage Conditions

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ABSTRACT

Background: Ajwain is used as an antioxidant, as preservative and also valued for its medicinal properties. Ajwain seeds undergo deterioration during storage due to various microorganisms and leads to degradation of quality in terms of discolorations and altering the biochemical constituents. Hence, the effect of different types of storage conditions on quality of ajwain seeds was investigated.

Methods: To assess the effect of storage in four different types of containers *viz*. Plastic jars (PJ) of make Pearl Pet, tin containers (TC), gunny bags (GB) and plastic lined gunny bags (PLBG) were used. The duration of storage was one year. External environmental conditions, *i.e.* temperature and relative humidity were recorded quarterly. To study the effect of different storage conditions on quality: (i) Seed quality assessment according to Agmark grade specifications (ii) incidence of mycoflora and (iii) effect on biochemical constituents *viz*. moisture, crude protein, crude fat, crude fiber, ash, carbohydrates, total soluble sugars, iron, total phenols and volatile oil contents were studied. Initial readings served as control for the experiment.

Result: Irrespective of storage conditions, a sharp increase in deterioration was noted in stored samples of ajwain with respect to presence of shrivelled, damaged, discolored and weevilled seeds after 3 months of storage. This shifted the samples from good to substandard grade. Maximum deterioration was noted after 12 months. The incidence of mycoflora was found maximum in samples stored in tin containers and gunny bags followed by plastic lined gunny bags and plastic Jars. A decline in moisture, crude fiber, carbohydrates, total soluble sugars, iron and volatile oil contents and an increase in crude fat and ash contents were observed after 12 months of storage, irrespective of storage containers. Plastic jars were found to be the best of all containers studied for storage purpose.

Key words: Ajwain, Containers, Deterioration, Quality, Storage.

INTRODUCTION

Ajwain is used in small quantities for flavoring numerous Indian foods like *puri*, *mathari*, savory, biscuits, vegetable preparations *etc*. It is also used as an antioxidant and preservative of several food items. It is also valued for its medicinal properties as mainly during lactation and stomach disorders. Ajwain seeds undergo deterioration during storage after the harvest. Among the various microorganism, fungi are important causative factors of deterioration that bring about several undesirable changes during storage, spoiling the quality of seeds by discolouration and altering the biochemical constituents. A perusal of literature revealed that mycoflora associated with ajwain seeds has been studied (Manjari *et al.* 1996; Lal *et al.* 2013; Fagodia *et al.* 2017) but only a meager work has been reported on the qualitative aspect of ajwain related to storage conditions. Hence, the effect of different types of storage conditions on quality of ajwain was investigated.

MATERIALS AND METHODS

Five samples of ajwain, 5 kg each were procured from the local market. One kilogram of each samples in triplicate were stored in four different types of containers *viz*. Plastic jars (PJ) of make Pearl Pet, tin containers (TC), gunny bags (GB) and plastic lined gunny bags (PLBG) for a period of one year. External environmental condition of containers, *i.e.* temperature and relative humidity were also recorded quarterly. Qualitative studies of the ajwain seed under different storage conditions were studied as;

(i) Seed quality assessment according to Agmark grade specifications, (ii) incidence of mycoflora and (iii) effect on biochemical constituents *viz.* moisture, crude protein, crude fat, crude fiber, ash, carbohydrates, total soluble sugars, iron, total phenol and volatile oil contents were studied. Initial readings served as control for the experiment.

Seed quality assessment

The samples were subjected to dry seed inspection and graded as ‘special’, ‘good’, ‘fair’ and ‘substandard’ on the basis of quality parameters laid down by Agmark grade

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specifications (Pruthi, 2001). Each category was weighed and reported by weight per 100 g of sample.

Assessment of incidence of mycoflora

The stored samples of ajwain (200 seeds/ sample) were subjected to standard blotter test (Anonymous, 1976) for isolation and identification of mycoflora on the 8th day. The mycoflora associated with seeds was observed.

Estimation of biochemical constituents

Moisture content was estimated with 100g /sample by distillation with toluene, crude protein using by Kjeldahl method with a conversion factor of 6.25, crude fat by solvent extraction in a soxhlet continuous extraction apparatus and total ash content by standard method (AOAC, 1995). The percent carbohydrate was calculated by subtracting the sum of percentage values of moisture, crude protein, crude fat, crude fiber and ash from 100 and reported as 'carbohydrate by difference'. The estimation of total soluble sugars was done colorimetrically employing anthrone reagent (Sadasivam and Manickam, 1992). Iron content by Wong's method (Raghuramulu *et al.* 1983), the total phenol content by using Folin's Ciocaltean reagent (Sadasivam and Manickam, 1992) and volatile oil content in a Clevenger's distillation apparatus (AOAC, 1995) were estimated.

Statistical analysis

The values of all the parameters taken in this study are as mean value of five samples. To compare the difference in between samples due to different storage period or storage condition, ANOVA (two-way) was carried out. Further, the difference in between two samples was checked by computing critical difference following t-test.

RESULTS AND DISCUSSION

Seed quality

Irrespective of the storage conditions, the change in quality of ajwain was affected noticeably showing presence of shrivelled damaged, discoloured and weevilled seeds during storage. In samples which were initially graded as good, a sharp increase in deterioration was observed after 3 months upto 9 months of storage (Table 1). This shifted the samples from good to substandard grade. Insect infestation noted by the end of this storage period also contributed towards deterioration. A higher deterioration was found in samples stored in tin container (TC), closely followed by gunny bag (GB), plastic lined gunny bag (PLGB) and plastic jar (PJ). Higher deterioration in samples stored in TC, GB and PLGB may be attributed to their higher accessibility to external environmental conditions. In earlier studies among the different packaging materials, polythene bags as storage container has been reported to be the best in soybean and sunflower (Sajjan *et al.* 2013; Meena *et al.* 2017) and in blackgram (Malarkodi *et al.* 2017). In PJ, deterioration may be attributed to phenomenon of heating occurring due to poor ventilation (Neergaard, 1977).

Incidence of mycoflora

Mycoflora associated with stored seed samples of ajwain in different containers is presented in Table 2. Prior to storage, a total of 14 fungal species belonging to 10 genera were predominant. 17 species of 12 genera were of very less occurrence and recorded in 1-3 seed samples with 1-2% incidence. At this stage, field fungi dominated which require relatively higher moisture content for their growth. They were *Alternaria* spp., *Chaetomium* spp., *Cladosporium oxyoporum, Curvularia* spp., *Drechslera* spp., *Phoma* spp., *Rhizopus nigricans* and *Actinomycetes* (bacteria). A gradual shift in qualitative and quantitative spectrum of fungal population was observed on storage in different storage containers. As the storage period progressed (6 months), the moisture content of stored samples showed a decline and conspicuously decline in population of field fungi but increase in storage fungi was noted. Irrespective of storage containers after 9 months of storage, a sudden rise in number of fungal species was observed. Insect infestation might also have played a role in increased fungal incidence.

On further storage (12 months), fungal incidence declined with reduction in moisture content of stored samples. As compared to other containers, the quantum of fungi was found less in PJ followed by PLBG containers. Accessibility of stored samples in TC and GB to atmospheric moisture and surrounding microclimate may be attributed to higher incidence in these containers. Besides being imperious to atmospheric conditions, increased concentration of CO_2 within air tight storage containers produced by respiring samples and microbes could be a reason of low fungal incidence in stored sample of PJ. Studies with concern of moisture content, incidence of visible moldiness and germinability of melon seeds stored in jute bags and polythene bags revealed percent seed germination declined from 98% to 37.3% in jute bags and 48.7% in polythene bags after 12 months and increase in storage fungi *viz*. *Aspergillus, Penicillium* and *Rhizopus* with prolonged storage (Bankole *et al.* 1999). A gradual decrease in field fungi with simultaneous increase in storage fungi accompanied by a reduction in seed germination was recorded in studies on deterioration of species of mustard, sesame and linseed when stored in a storehouse (Mondal *et al.* 1981). Soybean seeds when stored in metalized film bags and aluminum foil bags showed high germination and seed vigour and kept water activity and seed moisture level low which delayed seed quality and deterioration caused by storage fungi such as *Aspergillus* sp., *A. flavus, A. glaucus, A. niger, A. terreus* and *Penicillium* sp. followed by polypropylene bags and woven bag (Chuansin *et al.* 2006).

Malaker *et al.* (2008) studied the effect of different storage containers as dole (container made of bamboo), earthen pitchers, tin containers, polythene bags and refrigerators (10°C) and different time on seed quality of wheat among which high moisture content and black point severity and lowest percent of seed germination reported in seeds stored in 'dole' and population of storage fungi

Table 1: Effect of different storage containers on seed quality parameters of ajwain as assessed by Agmark grade specifications (Mean of five samples).

Agmark grades (%)	0 month	Duration of storage							
		6 months				12 months			
		Storage containers				Storage containers			
		PJ	TC	GB	PLGB	PJ	TC	GB	PLGB
Inorganic foreign matter									
Special (≤ 0.25)									
Good (≤ 0.50)	0.35	0.36	0.36	0.39	0.38	0.39	0.40	0.39	0.41
Fair (≤ 1.00)									
Substandard (> 1.00)									
Organic foreign matter									
Special (≤ 0.50)									
Good (≤ 0.75)	0.64	0.62	0.65	0.67	0.69	0.61	0.58	0.68	0.68
Fair (≤ 1.00)									
Substandard (> 1.00)									
Shrivelled, immature, damaged, discolored and weevilled seeds									
Special (≤ 1.0)									
Good (≤ 2.0)	1.17								
Fair (≤ 3.0)									
Substandard (> 3.0)		10.93	13.77	13.05	12.84	16.78	19.87	19.80	19.47
Moisture									
Special (≤ 11.0)	8.08	6.40	5.42	5.54	5.21	6.14	6.09	6.07	6.15
Good (≤ 11.0)									
Fair (≤ 11.0)									
Substandard (> 11.0)									
Temperature (^{o}C)									
Max.	36.0	36.5	36.5	36.5	36.5	34.6	34.6	34.6	34.6
Min.	19.0	21.1	21.1	21.1	21.1	20.5	20.5	20.5	20.5
Relative humidity (%)									
Max.	55.5	53.5	53.5	53.5	53.5	77.0	77.0	77.0	77.0
Min.	12.5	13.5	13.5	13.5	13.5	23.0	23.0	23.0	23.0

*PJ = Plastic jar, TC = Tin container, GB = Gunny bag, PLGB = Plastic lined gunny bag.

viz.Aspergillus, Chaetomium, Nigrospora, Penicillium and *Rhizopus* increased with the increase in storage duration. During isolation and characterization of filamentous fungi in different stages of harvest, fermentation, drying and storage of coffee beans, *Aspergillus* species found predominant during storage period (Silva *et al.* 2008).

Effect on biochemical constituents

The impact of different storage conditions on biochemical constituents (Table 3) was viewed from two lines - incidence of mycoflora and insect infestation during storage. Fungi are heterotrophic in their nutrition. They secrete a large number of digestive enzymes which hydrolyze macro-molecules to simple molecules which can be easily utilized by the fungus. Insect infestation exposes the seed to increased fungal infection. The combined effect has additive effect in altering the quality of seed.

Irrespective of storage conditions, the moisture content reduced as compared to initial value. Maximum reduction was observed in samples stored in GB (-24.87%) closely followed by TC (-24.62%), PJ (-24.00%) and PLGB (-23.88%) after 12 months of storage (Table 3). The decline in moisture content during storage may be attributed to prevailing low relative humidity and high temperature conditions for most part of the storage study (TC, GB and PLGB). In PJ, the decline may be due to gradual build up of temperature because of poor ventilation. Microbial infection may also be responsible for most of the heating (Neergaard, 1977).

Some fungi species like *Alternaria, Cephalosporum, Chaetomium, Fusarium, Penicillium, Rhizopus etc*. were observed in stored sample of ajwain and have been reported to contain pectinolytic, cellulytic, ignolytic and hemicellulolytic enzymes (Singh, 1984). These enzymes degrade components of cell walls so that fungi would reach the protoplasm of the cell lowering the fiber content of the sample. The decline observed in crude fiber content during storage may be attributed to these reasons. Maximum reduction was noted in samples stored in GB (-16.93%) followed by TC (-15.93%), PLGB (-15.50%) and PJ (-14.56%). Similar pattern of reduction was observed with

Table 2: Per cent incidence of mycoflora in ajwain in different storage containers in standard blotter test (Mean of five samples).

Fungi	0 month	Duration of storage							
		6 months				12 months			
		Storage containers							
	-	PJ	TC	GB	PLGB	PJ	TC	GB	PLGB
Alternaria alternata	11		2				4	4	2.5
Aspergillus flavus		13.5	10.5	12.5	10.5	20	27	29.5	25
A.fumigatus	1.5	10	7	9.5	8	19	23	21	19
A.nidulans	1	7	12	15	6	13	31	34	29
A.parasiticus			1.5	2		3.5	19	17	15
A.terreus			3	2	2	4	18	19	11
Chaetomium bostrychodes	1								
C.globosum	2								
C.murorum	2								
Cladosporium oxysporum	9.5						1.5		
Curvularia lunata	16						5	7	8
C.pallescens	6.5		2	1			2	5	4
Drechslera tetramera	5						2	1	2
Fusarium oxysporum		3	5	4	2	5	16	19	17
Memnoniella echinata	2					1		3	
Penicillium citrinum		1	2	2	1	7	21	19	17
Phoma sp.	4						1		
Rhizopus nigricans	5.5		1.5	1			3	4	2
Stachybotrys parvispora						1		2	1
Trichothecium roseum							8.5	8.5	5
Actinomycetes**	7								
Total No. of genera	10	3	6	5	3	5	10	10	9
Total No. of species	14	5	10	9	6	9	15	15	14

Fungi, which were less abundant (in 1-3 seed samples with 1-2% incidence) were *Alternaria dauci, A.dianthicola, A.longipes, A.tenuissima, Aspergillus ochraceus, Cephalosporium nigricans, Curvularia brachyspora, Drechslera halodes, D.sorghicola, Epicoccum purpurascens, Fusarium equiseti, F. solani, Macrophoma* sp., *Macrophomina* sp., *Stachybotrys atra, Torula herbarum, Ulocladium oudemansii, Verticillium albo-atrum.*

* PJ = Plastic jar, TC = Tin container, GB = Gunny bag, PLGB = Plastic lined gunny bag

** A group of filamentous bacteria.

respect to total soluble sugars content, reduction being -60.62%, -57.26%, -52.38% and -49.45% in GB, TC, PLBG and PJ respectively. The reduction in total sugar content in all storage containers differed significantly (P<0.05) when compared in between 6 and 12 months. Loss of carbohydrate content was more pronounced in samples stored in GB (-31.47%) followed by PLBG (-26.85%), TC (-10.62%) and PJ (-2.98%) after completion of storage period.

There appears to be a definite correlation between fungal infection and reduction in carbohydrate and total soluble sugars during storage (Srivastava and Roy, 1994; Purushottam *et al.* 1996; Oladimeji and Kolapo, 2008). In ajwain samples stored in TC, GB and PLBG lesser depletion was found as compared to those samples stored in PJ. Utilization of sugars as a result of activation of carbohydrate hydrolysis in the infected tissue or due to their direct assimilation by the fungi could also result in carbohydrate depletion. In the absence of proper storage conditions, spices lose this characteristic aroma, owed to its volatile oil content. In umbelliferous species, a decrease in volute oil content has been reported on storage for one year (Balcar and Kozlowski, 1962). Similar findings have been reported in the present study wherein the average volatile oil content of the samples was 3.62 ml/100g initially. After completion of storage period the samples stored in PJ and TC showed maximum reduction (-55.80%) each followed by GB (-44.73%) and PLBG (-22.65%). The difference in oil content in between samples stored in PJ-PLGB and TC-PLGB was found significant. The values of samples stored in PJ, TC and GB also differed significantly (P<0.05) when compared in between 6 and 12 months.

During storage, the iron content also showed a decline; the percent reduction was -54.38%, -41.97%, -37.57% and -17.94% for PLGB, GB TC and PJ respectively. The difference in iron content in between samples stored in PJ and PLGB at 12 months was found significant (P<0.05). The difference was also significant between time of storage *i.e.* 6 and 12 months for samples stored in TC, GB and PLGB.

Table 3: Changes in biochemical constituents of ajwain in different storage conditions (values reported per 100g sample).

0 month	Duration of storage								C.D. at 5% Due to Containers
	6 months				12 months				
	Storage containers								
	PJ	TC	GB	PLGB	PJ	TC	GB	PLGB	
Moisture (g)									
8.08	6.40	5.42	5.54	5.21	6.14	6.09	6.07	6.15	0.55
	(-20.79)	(-32.92)	(-31.43)	(-35.51)	(-24.00)	(-24.62)	(-24.87)	(-23.88)	
Crude protein (g)									
13.95	13.00	14.36	17.25	15.76	12.56	17.50	22.42	18.87	9.77
	(-6.81)	(+2.94)	(+23.65)	(+12.97)	(-9.96)	(+25.44)	(+60.71)	(+35.26)	
Crude fat (g)									
19.75	29.54	27.82	29.79	28.97	27.12	25.53	27.14	28.47	2.69
	(+49.56)	(+40.86)	(+50.83)	(+46.6)	(+31.64)	(+29.26)	(+37.41)	(+44.15)	
Crude fiber (g)									
16.00	14.40	15.40	15.28	15.54	13.67	13.45	13.29	13.52	2.70
	(-10.00)	(-3.75)	(-4.50)	(-2.87)	(-14.56)	(-15.93)	(-16.93)	(-15.50)	
Ash (g)									
5.62	5.39	4.86	4.91	5.26	5.00	4.72	6.00	6.22	1.17
	(-4.09)	(-13.52)	(-12.63)	(-6.40)	(-11.03)	(-16.01)	(+6.76)	(+10.67)	
Carbohydrates (g)									
36.60	31.27	32.14	27.23	29.26	35.51	32.71	25.08	26.77	12.61
	(-14.56)	(-12.18)	(-25.60)	(-20.05)	(-2.98)	(-10.62)	(-31.47)	(-26.85)	
Total soluble sugars(g)									
16.38	12.98	13.15	10.68	11.85	8.28	7.80	6.45	7.00	3.13
	(-20.75)	(-19.71)	(-34.79)	(-27.65)	(-49.45)	(-52.38)	(-60.62)	(-57.26)	
Iron (mg)									
32.71	32.78	38.43	38.83	32.51	26.84	20.42	18.98	14.92	8.33
	(+0.21)	(+17.48)	(+18.70)	(-0.61)	(-17.94)	(-37.57)	(-41.97)	(-54.38)	
Total phenol (g)									
5.58	3.16	1.18	2.20	1.44	4.11	4.08	3.55	4.10	1.31
	(-43.36)	(-76.85)	(-60.57)	(-74.19)	(-26.34)	(-26.88)	(-36.37)	(-26.52)	
Volatile oil (ml)									
3.62	3.00	3.00	3.20	3.40	1.60	1.60	2.00	2.80	1.10
	(-17.13)	(-17.13)	(-11.60)	(-6.07)	(-55.80)	(-55.80)	(-44.75)	(-22.65)	

* PJ = Plastic jar, TC = Tin container, GB = Gunny bag, PLGB = Plastic lined gunny bag.

Figure in parentheses indicates per cent deviation from healthy samples.

Phenols play an important role as a constituent of the biochemical defense mechanisms of the host plants. Irrespective of storage conditions, the phenol content reduced after 6 months of storage. On further storage, the content increased but the value of reduction being maximum in sample stored in GB (-36.37%), followed by TC (-26.88%), PLGB (-26.52%) and PJ (-26.34%). The difference in phenol content was significant ($P<0.05$) in between sample stored in PJ and TC and PJ and PLGB at 6 months. It also differed significantly in samples stored in TC, GB and PLGB when compared in between 6 and 12 months. The trend of phenol content observed in the present study should be viewed in perspective of incidence of mycoflora at different durations of storage; low incidence till 6 months lowered the phenol content later on increase in incidence elevated the phenol content. The decline in phenol content may also be due to use by the pathogens or polymerization by oxidative enzymes (Chourasia and Kumari, 1989; Srivastava and Roy, 1994).

The crude protein content of samples stored in PJ (-9.96%) reduced progressively with increase in storage duration, whereas a higher content was noted in those stored in TC (+25.44%), PLGB (+35.26%) and GB (+60.71%) after 12 months. The difference in protein content in between samples stored in PJ and GB at 12 months differed significantly ($P<0.05$). Low fungal incidence as well as incidence of insect pests observed in PJ as compared to other containers may be causative factor of reduced protein content in the seeds. Moderate to heavy insect infestation as noted in samples stored in TC, GB and PLGB resulted in increase in crude protein, non-protein nitrogen and uric acid as reported that insects bore seeds leaving excreta, body fragments and metabolites like uric acid decreasing the true

protein content of seeds (Samuels and Modgil, 1999). Mycelium protein might also increase the protein content. A decrease in ash content of ajwain samples was observed after 6 months of storage irrespective of the storage conditions.

The ash content in between samples stored in PJ and PLGB; TC and GB and TC and PLGB at 12 months differed significantly ($P<0.05$). The increase in ash content may be attributed due to consumption of endosperm by insects and as a result husk portion rich in ash was left behind leading to increase in total ash content of samples (Girish *et al.* 1974).

As compared to initial values an increase in crude fat content was noted in all the storage containers by the end of storage period. The fat content remained the maximum in samples stored in PLGB (+44.15%) followed by GB (+37.41%), PJ (+31.64%) and TC (+29.26%). The difference in fat content of samples stored in TC and PLGB for 12 months was found significant ($P<0.05$). Similar increase in fat content has also been reported by several researcher in different crops (Ibraheem *et al.* 1987; Kamble and Gangawane, 1987; Ekundayo and Idzi, 1990; Mathur, 1990).

CONCLUSION

An impact of different storage conditions was assessed on seed quality of ajwain samples. Plastic jar was found to be the best among all other containers studied for seed storage of ajwain. Air tight containers are suitable for storage of spices for a longer duration as the product stored in this situation prevented from exposure to external environmental conditions as compared to other containers namely tin containers, gunny bags and plastic lined gunny bags.

Hence, Plastic jars can be used for the storage of ajwain at the domestic level and also for storage of seeds for sowing purposes.

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