Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 2341-2345

Received: 18-07-2020
Accepted: 21-08-2020

Bittu Ram
Ph.D., Scholar, Department of Seed Science and Technology, CCS HAU, Hisar, Haryana, India

Satbir Singh Jakhar
Assistant Scientist, Department of Seed Science and Technology, CCS HAU, Hisar, Haryana, India

Axay Bhuker
Assistant Scientist, Department of Seed Science and Technology, CCS HAU, Hisar, Haryana, India

Satpal
Assistant Agronomist, Forage Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

Jagdeep Singh
Assistant Professor, Department of Plant Pathology, CCS HAU, Hisar, Haryana, India

Corresponding Author:
Bittu Ram
Ph.D., Scholar, Department of Seed Science and Technology, CCS HAU, Hisar, Haryana, India

Effect of plant oil treatments and containers on electrical conductivity, dehydrogenase activity and Mycoflora incidence of sorghum seed during storage

Bittu Ram, Satbir Singh Jakhar, Axay Bhuker, Satpal and Jagdeep Singh

Abstract
The present investigation was carried out at Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar to assess the effect of plant oils treatments and storage containers on seed quality and storability in sorghum. Seeds of forage sorghum variety HJ 541 were treated with plant oils and stored in three containers. The two factor experiment comprising of ten different treatments including plant oils were given to seeds (T_0 – Untreated, T_1 - Castor oil @ 2 ml/kg seed, T_2 - Neem oil @ 2 ml/kg seed, T_3 - Aonla oil @ 2 ml/kg seed, T_4 - Til oil @ 2 ml/kg seed, T_5 - Linseed oil @ 2 ml/kg seed, T_6 - Karanj oil @ 2 ml/kg seed, T_7 - Akhrot oil @ 2 ml/kg seed, T_8 - Ajwain oil @ 2ml/kg seed, T_9 - Carbendazim @ 2 g/kg seed) and kept in different containers (C_1: Cloth bag, C_2: Polythene bag and C_3: Metal box) under ambient conditions in laboratory in three replicates. All the treatment combinations were evaluated for seed quality parameters *viz.* electrical conductivity, dehydrogenase activity and seed mycoflora. The neem oil (2 ml kg^{-1}) performed better than others and, among containers polythene bag was proved better followed by metal box and cloth bag for storability at ambient condition. The dehydrogenase enzyme activity of seeds found decreased with the passage of time in all the containers and treatments. The electrical conductivity and the percentage frequency of seed mycoflora showed increased values with the progress of storage period. Carbendazim and neem oil were found most effective against the different fungal genera of sorghum seed in all the three containers. Among the containers less incidence of mycoflora was observed in seed stored in polythene bag followed by metal box in the all the treatments along with control.

Keywords: Plant oil, container, storability, mycoflora, sorghum, seed quality

Introduction
Sorghum an important food and fodder crop of India belongs to family Poaceae. It is the fifth major cereal crop in the world after wheat, rice, maize and barley. Lack of availability of quality seeds led to a decline in production caused by the use of low-quality seeds and adaptation in the field is reduced (Jyoti and Malik, 2013) [9]. The maintenance of seed quality during storage is becoming challengeable due to problem of quick loss of seed quality. So, many biotic and abiotic factors influenced the storage potential of seeds and results in gradual seed deterioration (Kumar *et al.*, 2014) [12]. Seed containers or packaging materials are considered as one of the most important factors influencing longevity of seeds in storage.

There are many biotic and abiotic factors which affect the seed longevity and quality during storage period. Seed is a living body and it loses its viability even under optimum storage conditions because it is a natural and continues process (Kapoor *et al*., 2010; Hartmann *et al*., 2016) [10, 8]. Sorghum has been found to be associated with seed-borne pathogen *viz*., *Fusarium moniliforme*, *Gloecercospora sorgi*, *Sphacelotheca* sp., *Ascochyta sorghina* and *Esepohilium turcicium*. Among all of this *Fusarium moniliforme* is reported to be most serious in sorghum as in rice and maize. Moldy ears, stalk rot and top rot were caused by this pathogen which reduces plant stands and depreciate yield of sorghum (Osunlaja, 2005) [14]. The seed treatment has been reported to reduce the leaching of inhibitors from the seed coverings and may restrict oxygen diffusion to embryo to enhance the (Vanagamudi *et al*. 2003; Simon, 1974) [22, 19]. Uses of chemicals for controlling of the pathogenic mycoflora can harmful for the other beneficial micro organism present in our ecosystem.

So, use of plant extract is a best alternative for controlling the plant pathogens (Gulter, 1988; Tripathi and (Gulter, 1988; Tripathi and Dubey 2004) [7, 21]. Against a wide range of fungi plant extracts showed antifungal activity (Masoko *et al.*, 2007; Abd-Alla *et al.*, 2001) [13, 1]. Therefore, seed treatment with different plant extracts was done to reduce the spread, development and infestation of storage mycoflora and consequently check the deterioration of seeds.

Materials and Methods

The study was carried out on sorghum seed (variety: HJ 541) produced in *kharif* 2017 having seed germination 92 per cent (above Indian Minimum Seed Certification Standards). The two factor experiment comprising of ten different seed treatments and three different containers was conducted in three replicates in CRD design. The sorghum variety seed treated as per the ten different treatments including plant oils [(T_0 – Untreated, T_1 - Castor oil @ 2 ml/kg seed, T_2 - Neem oil @ 2 ml/kg seed, T_3 - Aonla oil @ 2 ml/kg seed, T_4 - Til oil @ 2 ml/kg seed, T_5 - Linseed oil @ 2 ml/kg seed, T_6 - Karanj oil @ 2ml/kg seed, T_7 - Akhrot oil @ 2 ml/kg seed, T_8 - Ajwain oil @ 2ml/kg seed, T_9 - Carbendazim @ 2 g/kg seed)] were kept in different containers (C_1: Cloth bag, C_2: Polythene bag and C_3: Metal box) under ambient conditions in laboratories of Department of Seed Science & Technology, CCS Haryana Agricultural University, Hisar (Haryana). Seeds were taken from each of the different containers at three months interval up to 15 months and the seeds were used for evaluating various seed quality parameters.

Electrical conductivity

Electrical conductivity of the seed leachates was measured to know the status of membrane permeability as per ISTA (Anon., 1999) [4]. For this, 50 seeds selected randomly replicated thrice were soaked in separate beakers each containing 75 ml of distilled water and kept in the germinator at 25±1°C for 24 h. The electrical conductivity of seed leachates was measured by 60 direct reading conductivity meter. The conductivity was expressed in µS/cm/g.

Dehydrogenase activity

The Dehydrogenase activity (DHA) of the seeds of all treatments was measured according to method suggested by Kittock and Law (1968) [11]. The seeds from each container, replicated thrice were grounded to pass through 20-mesh screen. The 200 mg flour was soaked in 5 ml of freshly prepared 0.5 per cent triphenyltetrazolium chloride solution having pH 7.0 and incubated at a temperature of 38 °C for 2 h. Then it was centrifuged at 10,000 rpm for 3 minutes. The formazan was extracted with 10 ml acetone for 16 h at room temperature, then centrifuged for 3 minutes at 10,000 rpm and acetone solution containing formazan was transferred to the cuvette. The absorbance reading of the solution was taken at 480 nm wave length using systronic spectrophotometer 169. These observations were expressed as change in OD g^{-1} ml^{-1}.

Seed mycoflora

Seed health test was conducted by blotter method as recommended by ISTA (Anon., 1999) [4]. A set of 400 seeds from the each container were tested for seed mycoflora. Twenty five seeds each in three replications were placed equidistantly in a circle (sixteen in outer circle, eight in middle circle and one at the centre) in Petri dish of 9 cm diameter containing two moist blotters. Sufficient moisture was maintained by wetting blotters with sterilized distilled water. The seeds were incubated for seven days in an incubator at 25°±1 °C temperature, with 12 hours light and 12 hours dark alternate cycles. After seven days, the seed were examined by a low power stereo binocular microscope and different fungi found on the seeds were recorded and expressed in percentage. The type of mycoflora growing on each seed was identified and their percentage frequency (PF) of occurrence was calculated by applying the following formula:

$$PF = \frac{\text{Number of seeds on which fungus appears}}{\text{Total number of seed}} X\ 100$$

Results and Discussion

Electrical conductivity

Electrical conductivity is a good measure for assessing seed quality. The less damage to seed coat will result in less electrical conductivity which ultimately reflexes a good condition of seed. At the end of storage period (fifteen month), the maximum electrical conductivity (2.037) was recorded in control. The lowest electrical conductivity (1.523) was found in the seed treated with (T_9) carbendazim followed by seed treated with (T_2) neem oil (1.776). Among containers, polythene bag (1.842) was found better. Interaction effect of polythene bag with (T_9) carbendazim (1.204) was found superior in comparison to others (Table 1). Among all combinations least electrical conductivity was found in polythene bag stored seed treated with carbendazim. It was followed by combination of carbendazim treatment and cloth bag. Among oils the neem oil showed the least electrical conductivity which reflexes the better quality of seed. This variation in electrical conductivity indicated increased membrane permeability and declining compactness of seed coat and cellular membrane deterioration (Simon, 1974) [19]. Reddy *et al.*, (2017) [16] found that brinjal seeds treated with neem oil was observed less electrical conductivity and also effective in controlling the seed borne pathogen as compared to carbendazim. Similar results were reported by Arati (2000) [5] in bengal gram, (Anon., 2004) [3] in wheat and Varaprasad *et al.* (2005) [23] in brinjal.

Dehydrogenase activity

Among the treatments, (T_9) carbendazim (0.177) gave better results followed by (T_2) neem oil (0.176). Among containers, polythene bag (0.144) proved better. Interaction effect of cloth bag with (T_9) carbendazim (0.184) was found superior in comparison to others (Table 2). The effect of neem oil on cloth bag stored seed (0.183) was found significant among all other treatments. Singh *et al.* (2007) [20] studied the effect of treatment, container and storage period of longevity of lentil seed and found that all the treatments were found significantly superior over control for germination percentage, seedling vigour index, dehydrogenase activity and electrical conductivity during entire 10 months of storage. Shashibhaskar (2012) [17] reported that seeds pelleted with carbendazim and stored in polyethylene bag maintained higher germination, seedling length, vigour index, dry matter production and dehydrogenase activity (0.303 OD value).

Table 1: Effect of seed treatment with plant oils and containers on electrical conductivity (µS/cm/seed) in sorghum seeds

Treat.	3 Months				6 Months				9 Months				12 Months				15 Months			
	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean
T_0	0.768	0.790	0.801	0.786	0.921	0.929	0.905	0.918	1.141	1.136	1.171	1.149	1.545	1.534	1.505	1.528	2.029	2.045	2.038	2.037
T_1	0.662	0.643	0.711	0.672	0.735	0.716	0.734	0.728	1.090	1.050	1.073	1.071	1.360	1.224	1.357	1.314	1.786	1.851	1.891	1.843
T_2	0.586	0.609	0.563	0.586	0.735	0.615	0.757	0.702	1.021	0.982	0.988	0.997	1.156	1.138	1.102	1.132	1.738	1.756	1.835	1.776
T_3	0.727	0.741	0.740	0.736	0.784	0.733	0.892	0.803	1.074	1.023	1.081	1.059	1.219	1.251	1.257	1.242	1.872	1.916	1.911	1.900
T_4	0.659	0.685	0.703	0.682	0.770	0.748	0.766	0.761	1.110	1.044	1.106	1.087	1.230	1.233	1.289	1.251	1.889	1.931	1.908	1.909
T_5	0.643	0.608	0.652	0.634	0.820	0.822	0.862	0.835	1.098	1.079	1.100	1.092	1.339	1.249	1.344	1.311	1.897	1.964	1.965	1.942
T_6	0.682	0.688	0.593	0.654	0.893	0.862	0.735	0.830	1.121	1.070	1.074	1.088	1.364	1.464	1.298	1.375	1.835	1.917	1.823	1.858
T_7	0.660	0.621	0.744	0.675	0.832	0.882	0.801	0.838	1.140	1.092	1.118	1.117	1.368	1.184	1.323	1.292	1.993	1.901	1.987	1.960
T_8	0.693	0.668	0.618	0.660	0.885	0.898	0.884	0.889	1.101	1.111	1.109	1.107	1.270	1.334	1.254	1.286	1.853	1.932	1.827	1.871
T_9	0.562	0.576	0.588	0.575	0.697	0.734	0.623	0.685	0.978	0.942	0.988	0.969	1.053	1.038	1.070	1.054	1.638	1.204	1.726	1.523
Mean	0.664	0.663	0.671		0.807	0.794	0.796		1.087	1.053	1.081		1.290	1.265	1.280		1.853	1.842	1.891	
CD (P=0.05)		C	T	CX T		C	T	CX T		C	T	CX T		C	T	CX T		C	T	CX T
		0.001	0.002	0.003		0.001	0.001	0.002		0.001	0.001	0.003		0.001	0.001	0.002		0.001	0.001	0.002

C_1: Cloth bag C_2: Polythene bag C_3: Metal box

T_0: Untreated (Control); T_1: Castor oil; T_2: Neem oil; T_3: Aonla oil; T_4: Til oil; T_5: Linseed oil ; T_6: Karanj oil; T_7: Akhrot oil; T_8: Ajwain oil; T_9: Carbendazim (control)

Table 2: Effect of seed treatment with plant oils and containers on dehydrogenase activity (O.D value) in sorghum seeds

Treat.	3 Months				6 Months				9 Months				12 Months				15 Months			
	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean	C1	C2	C3	Mean
T_0	0.509	0.508	0.507	0.508	0.412	0.409	0.408	0.410	0.301	0.307	0.301	0.303	0.207	0.238	0.208	0.218	0.107	0.111	0.104	0.107
T_1	0.511	0.517	0.514	0.514	0.445	0.471	0.460	0.459	0.312	0.323	0.310	0.315	0.212	0.214	0.232	0.219	0.162	0.164	0.182	0.169
T_2	0.518	0.530	0.528	0.525	0.468	0.468	0.465	0.467	0.346	0.319	0.320	0.328	0.250	0.218	0.220	0.229	0.183	0.172	0.173	0.176
T_3	0.513	0.532	0.509	0.518	0.467	0.465	0.451	0.461	0.309	0.331	0.310	0.317	0.227	0.229	0.214	0.223	0.124	0.142	0.131	0.132
T_4	0.515	0.519	0.515	0.516	0.436	0.456	0.458	0.450	0.307	0.329	0.329	0.322	0.225	0.214	0.218	0.219	0.119	0.140	0.148	0.136
T_5	0.512	0.537	0.524	0.524	0.426	0.423	0.421	0.423	0.319	0.321	0.318	0.319	0.213	0.239	0.220	0.224	0.120	0.136	0.117	0.124
T_6	0.511	0.511	0.513	0.512	0.461	0.434	0.442	0.446	0.308	0.312	0.312	0.311	0.207	0.229	0.246	0.227	0.124	0.139	0.136	0.133
T_7	0.514	0.538	0.515	0.522	0.439	0.448	0.451	0.446	0.279	0.317	0.332	0.309	0.219	0.221	0.218	0.219	0.136	0.141	0.115	0.131
T_8	0.510	0.515	0.499	0.508	0.436	0.450	0.443	0.443	0.297	0.327	0.310	0.311	0.208	0.212	0.212	0.211	0.145	0.125	0.139	0.136
T_9	0.520	0.540	0.551	0.537	0.478	0.465	0.467	0.470	0.354	0.323	0.329	0.335	0.223	0.254	0.221	0.233	0.184	0.174	0.174	0.177
Mean	0.513	0.525	0.517		0.447	0.449	0.447		0.313	0.321	0.317		0.219	0.227	0.221		0.140	0.144	0.142	
CD (P=0.05)		C	T	C X T		C	T	C X T		C	T	C X T		C	T	C X T		C	T	C X T
		0.001	0.003	0.005		0.001	0.002	0.004		0.001	0.002	0.003		0.001	0.001	0.002		0.001	0.001	0.002

C_1: Cloth bag C_2: Polythene bag C_3: Metal box

T_0: Untreated (Control); T_1: Castor oil; T_2: Neem oil ;T_3: Aonla oil;T_4: Til oil; T_5: Linseed oil ; T_6: Karanj oil; T_7: Akhrot oil; T_8: Ajwain oil; T_9: Carbendazim (control)

Table 3: Mycoflora average in sorghum seed (variety: HJ 541) treated with plant oil kept in different storage containers

Treat.	Per cent frequency in 3 month			Per cent frequency in 6 month			Per cent frequency in 9 month			Per cent frequency in 12 month			Per cent frequency in 15 month		
	Cloth bag	Polythene bag	Metal box	Cloth bag	Polythene bag	Metal box	Cloth bag	Polythene bag	Metal box	Cloth bag	Polythene bag	Metal box	Cloth bag	Polythene bag	Metal box
T_0	0.86	0.61	0.66	1.08	0.83	0.91	1.58	0.63	0.77	2.00	1.08	1.41	2.08	1.33	1.81
T_1	0.72	0.42	0.53	0.89	0.81	0.78	1.28	0.50	0.58	1.69	0.83	1.19	1.78	1.08	1.44
T_2	0.28	0.25	0.39	0.61	0.36	0.61	0.61	0.28	0.39	1.14	0.61	0.69	0.94	0.56	0.86
T_3	0.69	0.53	0.56	0.94	0.69	0.81	1.33	0.36	0.47	1.61	0.75	1.19	1.75	1.08	1.28
T_4	0.75	0.50	0.58	1.06	0.75	0.86	1.25	0.53	0.61	1.67	0.92	1.31	1.86	1.17	1.47
T_5	0.72	0.39	0.58	1.03	0.78	0.89	1.28	0.56	0.56	1.42	0.97	1.22	1.67	1.08	1.50
T_6	0.81	0.42	0.61	1.08	0.72	0.86	1.25	0.42	0.61	1.69	0.94	1.42	1.89	0.97	1.19
T_7	0.75	0.53	0.64	1.00	0.81	0.92	1.19	0.56	0.67	1.64	0.92	1.39	1.92	1.06	1.42
T_8	0.61	0.53	0.64	0.94	0.67	0.72	1.17	0.61	0.50	1.64	1.00	1.03	1.64	1.08	1.61
T_9	0.30	0.13	0.27	0.97	0.25	0.27	0.41	0.16	0.27	0.97	0.41	0.55	0.86	0.39	0.69
Avg.	0.65	0.43	0.55	0.96	0.67	0.76	1.14	0.46	0.54	1.55	0.84	1.14	1.64	0.98	1.33

T_0= Control (untreated);T_1=Castor oil;T_2=Neem oil;T_3=Aonla oil;T_4=Til oil;T_5=Linseed oil; T_6=Karanj oil; T_7=Akhrot oil; T_8=Ajwain oil; T_9= Carbendazim (Control)

Seed mycoflora

Seed mycoflora presence or absence is a sign of seed health. Treated sorghum seed developed less mycoflora particularly *Alternaria*, *Helminthosporium*, *Curvularia* and *Penicillium* while more association of *Asperigillus*, *Fusarium* and *Mucor* were observed with the seeds during storage period in different containers. The storage fungi if develop in seed will deteriorate the seed quality as well as vigour. Among the all the treatments, (T_9) carbendazim (0.39) was found most effective against all the eight genera (Table 3) after 15 month of storage followed by (T_2) neem oil (0.56) treatment in the container polythene bag. More incidence of mycoflora was observed in the untreated seeds in all the three containers. However if we compare the overall effect of containers irrespective of all oil treatments, polythene bag (0.98) was found most superior as compared to metal box (1.33) and cloth bag (1.64). Among the all treatments, carbendazim and neem oil treatments were found most effective against the four fungal genera *viz.Alternaria*, *Helminthosporium*, *Curvularia* and *Fusarium* of sorghum seed in all the three

containers. Among the containers less incidence of mycoflora was observed in seed stored in polythene bag followed by metal box in the all the treatments along with control. Similar, results were reported by Signaboubo *et al*. (2015) [18] in an experiment in which seed treated with leaf extracts of *Azadirachta indica* and bark of *Boswellia dalzielii* reduced significantly ($P \leq 0,05$) seed borne infection, improved seed germination and vigour index of cotton seeds when compared to those treated with *Cassia sieberiana* bark extract and distilled water. Pawar (2011) [15] also reported that leaf extract of *Azadiracta indica* (neem) showed maximum activity against the seed borne pathogenic fungi *viz*. *Alternaria alternata*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium moniliforme* and *Trichoderma viride*. These plant extracts can possibly be exploited in the management of seed-borne pathogenic fungi to prevent biodeterioration of seeds in an eco-friendly way. The results are also supported by studies of Reddy *et al.* (2017) [16], Chethana *et al.* (2012) [6] and Ahmed *et al*. (2013) [2].

The reason for maintaining better quality of seed after a long storage may be due to systemic and quick activity of carbendazim which is a systemic fungicide. Better quality was found in case of neem oil application among all other oil treatments. This may be due to presence of azadirachtin compound in it, which has antifungal properties. The better quality in polythene bag may be due to its moisture imperviousness. Water and Moisture preciousness of cloth bag may be the reason for more quality degradation of stored seed.

Conclusion

It can be concluded that seed treatment with carbendazim 2 g kg^{-1} or neem oil @ 2ml kg^{-1} seed are the most effective to control seed storage mycoflora of sorghum for a period of 15 months without deterioration in the seedling vigour.

Acknowledgement

Mr. Bittu Ram, the first Author is thankful to Professor and Head, Department of Seed Science & Technology, CCS HAU, Hisar for providing necessary facilities to carry out the experiment during his M. Sc. (Agriculture) degree programme. Thanks are also due to Forage Section, Department of Genetics & Plant Breeding CCS HAU, Hisar for providing seed of sorghum variety to carry out the research work.

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