



Effect of seed treatments and storage containers on seed quality of berseem (*Trifolium alexandrinum* L.)

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Abstract

The study was conducted to determine the effect of seed treatments and storage containers on seed quality of berseem. The seeds treated with five combinations were stored for 24 months in four different seed storage containers along with control. The study showed that seed treatments and storage containers had significant effect on berseem seed health. The moisture content, mycoflora incidence and insects' infestation of seeds were gradually increased with increase in storage period, whereas seed germination and seed vigour were gradually decreased. Highest seed germination (88.83%) and seed vigour (714) were recorded with the seeds stored in polylined bag followed by earthen pot and lowest was recorded in gunny bag. Among the treatments, carbendazim and malathion were found more effective than neem, *eucalyptus* leaves and cow dung ash up to 24 months. The study indicated that bamboo containers and earthen pots were suitable for short term (6-12 months) and polylined bags for medium term (12-24 months) storage. The combination of seed treatment with carbendazim or malathion, and polylined bag as storage container was found suitable to maintain berseem seed viability for more than 24 months.

Keywords: Berseem seeds, Seed mycoflora, Seed quality, Storage

Introduction

Berseem (*Trifolium alexandrinum* L.) is one of the most important *Rabi* fodder legumes (Yadav *et al.*, 2015). Among cultivated fodders, berseem is a suitable fodder crop for areas below 1700 m altitude with irrigation facilities. It occupies 2 mha in India alone and is a prominent fodder legume in western and northern parts of India (Salama and Ismail, 2005; Vijay *et al.* 2016). The intrinsic characters such as multi-cut nature, long duration of green fodder availability, high green fodder yield (85 t/ha), good fodder quality (20% crude protein),

digestibility (up to 65%) and high palatability made berseem as popular fodder crop among livestock farmers (Vijay *et al.*, 2016; Manjunatha *et al.*, 2017).

Several factors affect the longevity of seeds during storage and these include the crop variety, initial seed quality, moisture content, seed microflora and storage conditions. The temperature and seed moisture content are the most important non-biotic factors controlling seed longevity (Vijay *et al.*, 2009), whereas insects (Ramzan *et al.*, 1990) and seed mycoflora have been recognized as an important biotic factors responsible for seed deterioration during storage (Gupta and Aneja, 2001). Bruchids (*Callosobruchus* spp.) are important storage pests of grain legumes especially in pulses grown in the tropics and sub-tropics (Ramzan *et al.*, 1990). The poor storage of berseem seeds is a problem because of *Bruchidius trifoli* (Keals *et al.*, 1997; Ibrahim *et al.*, 2010). The infestation usually starts from field and eventually carried into store house leading to seed deterioration at ambient storage conditions. However, several reports claimed that seed treatment with fungicides, insecticides and plant products showed maximum seed vigour and germination after longer period in storage. Similarly to overcome adverse effect by biotic and abiotic factors storing the seeds in moisture proof containers like polythene bag, aluminum foils, tins or any sealed containers was found more useful in maintaining the desired quality of seeds for longer period (Singh and Singh, 1992), unlike those stored in moisture pervious containers like cloth bag and gunny bag (Quais *et al.*, 2013).

The quality seed production is an important area that needs to be strengthened for enhancing the availability of cultivated fodder (Vijay *et al.*, 2016; Manjunatha *et al.*, 2016). Thus identification of suitable combination of seed treatment and seed storage container is most important to maintain required seed viability. Numbers of reports

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are available on pre storage treatments of various crops but less attention was paid in fodder crops. Hence, the present study was undertaken to know the effects of pre storage seed treatments and interaction thereof with different storage containers on seed quality of berseem.

Materials and Methods

Seed storage and treatments: The study was conducted at Division of Seed Technology, ICAR-Indian Grassland and fodder Research Institute, Jhansi during 2015-16 to ascertain storage potential of berseem seeds under ambient storage conditions. The experiment consisting of five seed treatments (T1: Carbendazim (bavistin 75 % WP) @ 2.5g per kg seeds, T2: Malathion @ 1g per kg seeds, T3: Eucalyptus leaves @ 3kg per 100kg seeds, T4: Neem leaves @ 3kg per 100kg seeds and T5: Cow dung ash @ 3kg per 100kg of seeds) and four containers viz., C1: Bamboo container (plastered with cow urine), C2: Gunny bag, C3: Polylined bag and C4: Earthen pots (initially washed with cow urine). The treated and untreated seeds were stored up to 24 months under ambient conditions.

Observations recorded: The seeds were tested for their moisture content and germination before and after storage (ISTA, 2012). The observations were taken 12 months after storage up to 24 months at 6 months interval on seed germination, seed vigour, insect infestation and seed mycoflora incidence on four replications following standard methods (Perry, 1978; Gupta, 1993; ISTA, 2012). Each sample after each storage period (12, 18 and 24 months) was inspected, but four replications of 100 seeds from each sample were used to estimate insect infestation. Initially the seeds were carefully visualized with naked eye for infestation/damage due to insect pests. For X-ray radiography, the seeds were placed on glass plates (90mm in diameter) in X-ray machine and exposed to X-ray beam for 11.15 seconds at MX-20 X-ray machine at National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The X-ray images of the seeds were analyzed for insect infestation (%). A blotter test was also used to identify and enumerate the fungal load during storage (ISTA, 2012). Two sterilized blotter sheets were saturated with distilled water. Twenty five seeds were placed on the blotter in 4 different petriplates and they were evenly spaced using forceps. Seeds were incubated for 10 days at 25 °C. Seeds were then examined for fungal growth on 5, 7, and 10 days after plating and their incidence (%) was recorded.

Data analysis: The data were analysed using standard method of analysis for variance (ANOVA) for two factor completely randomized design (CRD) factorial concept for laboratory experiment as per Snedecor and Cochran (1980). The least significant difference was noted at probability of less than 0.05.

Results and Discussion

The berseem seeds were subjected to storage treatment and the observations for moisture content, seed germination, seed vigour, incidence of seed mycoflora and insect infestation were taken at 12, 18 and 24 months of storage.

Seed moisture: There was no change in initial moisture content (8.9%) of stored seeds up to 6 months. But moisture content of the seeds was increased significantly after 12, 18 and 24 months. The moisture content of berseem seeds ranged from 9.7-10.6%, 10.3-11.2% and 10.8-11.7% after 12, 18 and 24 months, respectively (data not shown). As the storage period advances, moisture content in the surrounding area increased particularly in rainy season (June to September). Similarly in hot weather (May to August) the temperature of the storage seeds increased due to rise in outside temperature up to 48 °C. The relative humidity of the storage containers varied significantly within wide amplitude; until the moisture content of the seed attained equilibrium with surrounding environment they used to adsorbed or desorbed moisture. Thus the moisture content of berseem seeds showed constant fluctuation during the length of our storage study. As Vieira *et al.* (2001) reported that moisture content of seed was strictly linked to its viability, which depends on relative humidity of storage environment. Mbofung *et al.* (2013) reported similar findings, in which seeds of soybean stored in different environments with fluctuating relative humidity and temperature for periods longer than 12 months rapidly lost their viability. The declined seed viability of medicinal plant seeds was also observed with increased temperature and higher relative humidity in the storage containers (Bhardwaj *et al.*, 2014).

Seed germination: The storage containers did not affect germination up to 12 months. Later germination was gradually decreased. After 18 months, the lowest seed germination was recorded from bamboo (86.61%) and gunny bag (86.28 %) storage containers (Table 1), whereas highest seed germination was recorded (88.83%) in polylined bags. After 24 months, there was

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Table 2. Influence of storage containers and seed treatments and their interaction on seed vigour of berseem

Structure	Seed vigour after months of storage												Mean								
	Control			Ash			Carbendazim			Malathion						Eucalyptus			Neem		
	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24			
Bamboo	641.8	567.5	607.6	706.6	635.5	754.8	742.2	762.5	975.0	714.6	634.3	839.5	710.2	594.0	673.9	661.8	651.7	693.0	696.2	640.9	757.3
Gunny bag	598.7	510.3	570.0	704.7	622.3	695.5	767.2	756.3	861.4	739.3	626.0	805.0	653.9	674.4	628.3	645.3	621.7	672.0	684.8	635.2	705.3
Polylined bags	657.9	666.2	707.0	672.0	783.3	845.3	755.2	764.5	1050.0	768.2	822.9	954.5	708.6	822.1	751.9	722.2	697.9	819.0	714.0	759.5	854.6
Earthen pots	629.0	690.0	558.6	685.9	710.6	676.7	775.5	795.0	950.0	686.8	731.5	828.0	697.3	720.7	621.9	671.8	649.4	682.5	691.1	716.2	719.6
Mean	631.9	608.5	610.8	692.3	687.9	743.0	760.0	769.6	959.1	727.2	703.7	856.7	692.5	702.8	669.0	675.3	655.2	716.6	696.5	687.9	759.2
Effect	SEM			LSD(0.05)			CV (%)			SEM			LSD(0.05)			CV (%)					
Structure	10.67			30.33			-			19.54			55.56*			11.27*					
Treatment	13.07			37.15**			-			23.93			68.05*			13.80*					
Structure x treatment	26.13			74.30			6.49			47.86			NS			27.61*					

Table 3. Influence of storage containers and seed treatments and their interaction on insect infestation (%) of berseem

Structure	Insect infestation (%) after months of storage												Mean								
	Control			Ash			Carbendazim			Malathion						Eucalyptus			Neem		
	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24			
Bamboo	8.00	10.67	13.2	3.67	7.33	10.6	2.67	5.67	9.7	2.67	6.00	8.9	3.33	7.00	10.0	2.67	7.00	9.2	3.83	7.28	10.3
Gunny bag	10.33	12.67	14.7	7.00	9.67	12.7	5.00	7.00	10.5	5.00	6.67	10.0	6.00	9.00	14.4	6.33	9.33	11.3	6.61	9.06	12.3
Polylined bags	8.67	10.67	9.8	5.33	5.67	8.3	4.33	4.67	7.9	4.00	4.33	7.4	5.00	4.00	8.9	4.33	4.00	7.9	5.28	5.56	8.4
Earthen pots	8.67	10.00	12.8	3.67	5.00	11.6	1.33	4.00	10.6	2.67	4.00	9.8	4.00	5.67	12.0	3.33	6.00	11.2	3.94	5.78	11.3
Mean	8.92	11.00	12.6	4.92	6.92	10.8	3.33	5.33	9.7	3.58	5.25	9.0	4.58	6.42	11.3	4.17	6.58	9.9	4.92	6.92	10.6
Effect	SEM			LSD(0.05)			CV (%)			SEM			LSD(0.05)			CV (%)					
Structure	0.27			0.78**			-			0.29			0.83*			2.39*					
Treatment	0.34			0.96**			-			0.36			1.02*			2.93					
Structure x treatment	0.67			1.91			23.6			0.72			NS			17.9					

Insect infestation and incidence of seed mycoflora:

The storage containers and treatments had impact on insect infestation and seed mycoflora during storage (Table 3-4). In the present study it was observed that among the storage containers polylined bag was found best for storage of berseem seeds than gunny bags, bamboo container and earthen pots. Among the different treatments carbendazim and malathion treated seeds had less incidences of seed mycoflora at end of storage period. The X-ray radiography observation after 12 months of storage showed highly significant difference in insect infestation among different containers. The highest seed damage was observed in gunny bags (6.61 %), whereas lowest in bamboo structures (3.83 %). Similarly, highly significant difference in insect damage was observed among different seed treatments. The carbendazim treated seeds infested less due to insects (3.33 %) compared to other treatments and more insect damaged seeds were observed in untreated control (8.92 %). The insect infestation was increased both in different storage containers and treatments after 18 and 24 months of storage. The high values of 9.06% and 12.3% insect damaged seeds were observed in gunny bags after 18 and 24 months of storage respectively. Whereas less seed damage was noticed in malathion treated seeds even after 18 and 24 months of storage followed by carbendazim. The botanicals *i.e.* neem, *eucalyptus* leaves and organics *i.e.* cow dung ash as used as seed protectants were most effective up to 12 months. There was no significant interactive effect between storage containers and treatments over a time in reducing the insect infestation.

Several mycoflora were recorded from seeds of berseem viz., *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium moniliformae*, *F. oxysporum* and *Rhizopus nigricans*. These mycoflora were seen growing with almost all the untreated and treated seeds stored in all containers during storage. But the growth of seed mycoflora increased with the increase in storage period (Table 4). No significant difference in seed mycoflora load was observed after 12 months among storage containers. But highly significant difference in seed mycoflora load was observed in different seed treatments. The carbendazim treated seeds recorded less mycoflora load (0.17%) compared to untreated control (3.42%). The seed mycoflora load was increased both in different storage containers and seed treatments after 18 and 24 months of storage. The more seed mycoflora load of 4.2% was recorded in gunny bags after 24 months of storage, whereas it was less in polylined

Table 4. Influence of storage containers and seed treatments and their interaction on incidence of seed mycoflora (%) of berseem

Structure	Mycoflora infection (%) after months of storage												Mean								
	Control			Ash			Carbendazim			Malathion			Eucalyptus			Neem					
	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24			
Bamboo	3.67	5.33	5.6	2.33	3.67	3.8	0.00	1.33	1.5	1.67	1.00	1.7	1.67	3.67	4.0	1.00	3.33	3.7	1.72	3.06	3.4
Gunny bag	3.33	4.33	6.4	1.67	2.33	4.4	0.00	0.00	2.0	0.00	1.00	2.3	1.33	2.67	5.7	1.33	2.00	4.2	1.28	2.06	4.2
Polylined bags	3.67	5.00	4.1	1.33	3.00	3.0	0.00	0.67	1.0	0.00	0.00	1.5	1.00	2.67	3.5	1.00	2.33	3.1	1.17	2.28	2.7
Earthen pots	3.00	5.00	5.5	1.00	3.00	3.5	0.67	0.67	1.7	0.00	1.33	1.7	1.33	4.00	4.1	2.00	3.00	3.6	1.33	2.83	3.4
Mean	3.42	4.92	5.4	1.58	3.00	3.7	0.17	0.67	1.6	0.42	0.83	1.8	1.33	3.25	4.3	1.33	2.67	3.7	1.38	2.56	3.4
Effect	12 months			18 months			24 months			18 months			24 months			CV (%)					
Structure	SEM	LSD(0.05)	CV (%)	SEM	LSD(0.05)	CV (%)	SEM	LSD(0.05)	CV (%)	SEM	LSD(0.05)	CV (%)	SEM	LSD(0.05)	CV (%)	SEM	LSD(0.05)	CV (%)	SEM	LSD(0.05)	CV (%)
Structure	0.48	1.35	-	0.59	NS	-	0.25	0.71*	-	0.25	0.71*	-	0.25	0.71*	-	0.25	0.71*	-	0.25	0.71*	-
Treatment	0.58	1.66**	-	0.72	2.04*	-	0.30	0.87*	-	0.30	0.87*	-	0.30	0.87*	-	0.30	0.87*	-	0.30	0.87*	-
Structure x treatment	1.17	3.32	14.9	1.44	NS	9.72	0.61	1.75	31.04	1.44	NS	9.72	0.61	1.75	31.04	1.44	NS	9.72	0.61	1.75	31.04

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bags. The seed treatments showed significant difference in mycoflora load even after 18 and 24 months of storage. Among the treatments, carbendazim and malathion showed their effects in reducing mycoflora load even after 24 months of storage when compared to leaves (neem and eucalyptus) and cow dung ash. Gunny bags and bamboo structures were reported as inferior and also raised storage temperature which was most favourable for insect infestation and seed mycoflora growth (Gupta *et al.*, 2006). Simultaneously the insect eggs lying inert on seed surface become active, as the storage period increases, their population increased and they started to damage the seeds (George and Patel, 1992; Kamara *et al.*, 2014). Further by combined metabolic activities of seed mycoflora and insects, the temperature and humidity were increased in storage containers and accordingly the damage was also increased considerably as the storage period increases (Mersal *et al.*, 2006; Mbofung *et al.*, 2013). Storage containers and locally available plant products were used to store the seeds and to ward off seed pests and pathogens on seed storage as common practice in India and elsewhere as a part of successful short term protection measures (Meshram, 2000; Das *et al.*, 1998). In general, prevalence of seed microflora was found lower in the seed stored in polythene bag due to lower seed moisture of the seed. Irrespective of storage containers, load of seed microflora increased with the increase of storage period. Rahman and Rahman (2006) observed that sealed container was the best to prevent the attack of pathogen (fungus) among all the containers under study. According to Justice and Bass (1978) when in storage the moisture content of seed rises above 8-9% then the risk of fungal and insect infestation increases.

Conclusion

The study indicated that storage containers and seed treatments had significant impact on berseem seed health. The seed germination and seed vigour was found maximum in polylined bags among the different storage containers after 24 months of storage. Carbendazim and malathion were found suitable to maintain less load of seed mycoflora and insect infestation, respectively during storage even after 24 months. Hence, the combination of polylined bag with either carbendazim or malathion treatment can be recommended for medium term storage (12-24 months) of berseem seeds. Whereas the perforated/pervious storage containers (gunny bags, bamboo structures and earthen pots); and plant products (neem and eucalyptus leaves) and organics (cow dung ash) are suitable for short term (6-12 months) storage

only.

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