Range Mgmt. & Agroforestry 38 (2) : 227-233, 2017 ISSN 0971-2070



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), Accepted: 8th December, 2017

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

Seed quality of berseem

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 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 Xray 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 Xray 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

- have dealing in a set or which is all stands
sharp decline in seed germination in all storage
containers except polylined bags (77.83%). The
germination of treated seeds was not affected with
increasing storage period up to 12 months of storage.
Among the seed treatments, seeds treated with
carbendazim recorded highest germination (77.85%)
even after 24th months of storage period followed by
malathion treated seeds (74.5%). No significant
interactive effect was observed between containers
and treatments up to 12 months of storage. The
carbendazim treated seeds stored in polylined bags
recorded maximum germination compared to other
treated seeds stored in different storage containers.
These results were in accordance with findings of
Hanif et al. (2010) and Masum et al. (2010) recorded
on storage of jute seeds. After 24 months there was
sharp decline in germination of jute seeds in gunny
bags as well as in other containers. The possible
reasons for rapid decline in seed germination were
due to fluctuations in the relative humidity and
temperature, moisture content of seeds in different
containers. Higher seed moisture might lead to rapid
deterioration of seeds in gunny bag (Nahar et al.,
2009) and the rate of deterioration was strictly related
to the duration of storage of the seeds in high relative
humidity environment (Quais et al., 2013). Higher
temperature was reported to increase the seed
deterioration rates by affecting enzymes that are
involved in reactive oxygen species scavenging and
repair (Bernal-Lugo and Leopold, 1998).

Seed vigour: After twelve months of storage there was no distinct difference in seed vigour among seeds stored in different storage containers (Table 2). The highest seed vigour (714) was observed in polylined bag, whereas lowest seed vigour (691) was found in earthen pots. After eighteen months of storage, there was significant differences in seed vigour were observed. The interaction effects of containers and seed treatments also influenced the vigour of berseem during storage period (Table 2). Maximum seed vigour (854.6) was recorded even after 24 months in polylined bags compared to other containers. These results were in agreement with findings of Quais et al. (2013) reported that seed vigour of radish gives highest value in metal and poly bag containers than in gunny bags. Gunny bags are usually perforated structures, which might be the main reason that the seeds stored in gunny bags had maximum losses of viability and germination (Rahman and Rahman, 2006).

Malathion Eucalyptus Neem 12 18 24 12 18 24 12 18 24 12 8 91.67 86.00 73 90.67 85.67 64 90.67 87.33 66 90.44 86 1) (73.3) (69.2) (58.7) (72.4) (70.5) (53.1) (72.2) (67.7) (54.3) (72.1) (6 3 91.33 86.00 70 90.00 84.33 61 89.67 87.00 64 90.00 84 1) (73.3) (58.1) (71.3) (57.8) (73.7) (71.7) (6 1) (71.3) (58.1) (71.3) (67.8) (53.7) (71.0) (7 6 91.67 89.03 88.67 73 90.33 88.00 78 89.33 88 65 90.89 87 10 17 10 17 10 17 10 17 10 </th <th>Structure</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>)</th> <th></th> <th></th> <th></th> <th></th> <th>ממכ</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>Ē</th> <th></th> <th></th>	Structure)					ממכ						Ē			
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LSD (0.05) CV (%) SEm LSD (0.05) O 1.35 - 0.49 1.39* - 0.76 2.16* 1.35 - 0.60 1.70* - 0.93 2.65* 3.31 2.23 1.20 NS 2.37 1.86 5.30					12 m	onths						18 mor	nths					24	month	s			
1.35 - 0.49 1.39* - 0.76 2.16* 1.65 - 0.60 1.70* - 0.93 2.65* 3.31 2.23 1.20 NS 2.37 1.86 5.30	Effect		SEn	_	LSD	(0.05)	C	(%)		SEm		LSD (0.05)	0	:V (%)	SEn		LSD	(0.05)		CV (%)		
1.65 - 0.60 1.70* - 0.93 2.65* 3.31 2.33 1.20 NS 2.37 1.86 5.30	Structure		0.4	7	-	35				0.49		1.39	*		, I	0.76		ю,	16*		, '		
3.31 2.23 1.20 NS 2.37 1.86 5.30	Treatment		0.5	8	1.1	65				0.60		1.70	*		ı	0.93		2.6	35 *		•		
	Structure ×	< treatm	ent 1.6	-	с	31	2.2	e		1.20		ž	S	. 1	2.37	1.86		5	.30		5.70		

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seed treatments and their interaction on seed germination (%) of berseem

and

Table 1. Influence of storage containers

Table 2. Ir	ofluence o	Table 2. Influence of storage containers and seed treatments and their interaction on seed vigour of berseem	containe	rs and s	seed tre	atment	s and t	their inte	sraction	on see	d vigc	our of b	erseer	۶						
Structure						Seed	vigour	after n	Seed vigour after months of storage	of stor	age							ž	Mean	
	Ũ	Control		Ash		Carb	Carbendazim	<u> </u>	Mala	Malathion		Euca	Eucalyptus			Neem				
	12	18 24	12	18	24	12	18	24	12		24	12	18	24	12	18	24	12	18	24
Bamboo	641.8 5	567.5 607.6	706.6	635.5	754.8	742.2	762.5	975.0	714.6 6	634.3 8	839.5 7	710.2	594.0	673.9	661.8	651.7	693.0	696.2	640.9	757.3
Gunny	598.7 5	510.3 570.0 704.7	704.7	622.3	695.5	767.2 756.3	756.3	861.4	739.3 6	626.0 805.0 653.9	05.0 6		674.4 628.3		645.3	621.7	672.0	684.8	635.2 705.3	705.3
bag					1															
Polylined	657.9 6	657.9 666.2 707.0 672.0 783.3	672.0		845.3	755.2	764.51	755.2 764.51050.0 768.2		822.9 954.5 708.6	54.5		822.1	822.1 751.9 722.2	122.2	697.9		714.0	819.0 714.0 759.5 854.6	854.6
bags Earthen	629.0 6	629.0 690.0 558.6 685.9	685.9	710.6	676.7	775.5 795.0	795.0	950.0	686.8 731.5 828.0 697.3	31.5 8	28.0 6		720.7 621.9		671.8	649.4		682.5 691.1	716.2 719.6	719.6
pots Mean	631 9 6	608.5, 610.8	692.3	687.9	743.0	760.0.769.6	769.6	959 1	727 2 703.7 856 7	03.7 8	56.7.6	692 5	702 8 669 0		675.3	655.2	716.6	655 2 716.6 696 5	687 9 759.2	759.2
-			'l						18	month	JS					24	24 months	hs b		
Effect .		SEm	LSD	LSD (0.05)	CV (%)	(%)		SEm		LSD (0.05))5)	∂ 	CV (%)	SEm			SD (0.05)		CV (%)	
Structure .		10.67	ς Γ	30.33	5			19.54		55.56*	*	5		3.96			11.27*			
Treatment		13.07	37.	37.15**		ı	2	23.93		68.05*	*		,	4.85	10	1.0	13.80*		ı	
Structure × treatment 26.13	treatmer	ıt 26.13	7	74.30	6.49	6	4	47.86		SN	~	12.05	05	9.71	_	27	27.61*		2.21	
											•								:	
Structure						Insect	intest	ation ('	Insect infestation (%) after months	month	s of s	of storage							Mean	
		Control		Ash		Carb	Carbendazim	<u>n</u>	Malathion	thion		Euca	Eucaly ptus		2	Neem				
			12	18	24	12	18	24		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	\sim	9.7		6.00	8.9	~	7.00	10.0	2.67	7.00	9.2	3.83	7.28	10.3
Gunny	10.33 1	12.67 14.7	7.00	9.67	12.7	5.00	7.00	10.5	5.00	6.67 1	10.0	6.00	00.6	14.4	6.33	9.33	11.3	6.61	9.06	12.3
bag					c	00		0 1						0			0 1			
Polylined	8.67	10.67 9.8	5.33	10.0	<u></u> а.3	4.33	4.67	P. /	4.00	4.33	4.	00.c	4.00	0.0 0.0	4.33	4.00	P. /	87.C	00.0	ά. 4
bags Earthen	8.67 1	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
pots																				
Mean	8.92	11.00 12.6	4.92	6.92	10.8	3.33	5.33	9.7	3.58	5.25	0	4.58	6.42	11.3	4.17	6.58	9.9	4.92	6.92	10.6
i			12 m	12 months					18	18 months	JS					24	24 months	hs		
Effect		SEm	LSD	LSD (0.05)	CV (%)	(%)		SEm		SD (0.05)	<u>)5)</u>	ວ ວ	CV (%)	SEm	_	LSD	SD (0.05)		CV (%)	
Structure		0.27	o.	0.78**				0.29		0.83*			ı	0.84		0	2.39*		'	
Treatment		0.34	o.	0.96**				0.36		1.02*			ı	1.03	m	N	2.93		'	
Structure × treatment	treatmer	nt 0.67		1.91	23.6	9 Q		0.72		NS		17	17.9	2.06	0	5	5.87		34.04	

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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	uenc	e of sto	rage ci	ontainer	s and s	eed tre	atments	s and th∈	eir inter	action oi	n incide	nce of	seed m	ycoflor	a (%) c	of bers	eem			
Structure	_						Myco	flora int	fection	Mycoflora infection (%) after months of storage	sr mont	hs of s	torage						ž	Mean
		Control			Ash		Carb	Carbendazim	٤	Malathion	hion	Ú	Eucaly ptus	sn		Neem	٤	1		
	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24	12 18 24	8 24
Bamboo 3.67 5.33 5.6	3.67	5.33	5.6	2.33	2.33 3.67 3.8	3.8	0.00 1.33	1.33	1.5	1.67	1.67 1.00 1.7	1.7	1.67	1.67 3.67 4.0	4.0	1.00	3.33	3.7	1.00 3.33 3.7 1.72 3.06 3.4)6 3. ^z
Gunny	3.33	3.33 4.33 6.4	6.4	1.67	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	2.00 4.2	1.28 2.06 4.2	06 4.2
bag																				
Polylined 3.67 5.00 4.1	3.67	5.00	4.1	1.33	1.33 3.00	3.0	0.00	0.67	1.0	00.00	00.0	1.5	1.00	2.67	3.5	1.00	2.33	3.1	1.17 2.28 2.7	28 2.7
bags																				
Earthen 3.00 5.00 5.5	3.00	5.00	5.5	1.00	1.00 3.00 3.	3.5	0.67	0.67	1.7	0.00	1.33	1.7	1.33	4.00	4.1	2.00	2.00 3.00 3.6	3.6	1.33 2.83 3.4	33 3.4
pots																				
Mean	3.42	4.92	5.4	3.42 4.92 5.4 1.58 3.00 3.7	3.00	3.7	0.17	0.17 0.67 1.6	1.6	0.42	0.83	1.8	1.33	3.25	4.3	1.33	2.67	3.7	0.42 0.83 1.8 1.33 3.25 4.3 1.33 2.67 3.7 1.38 2.56 3.4	56 3.4
				12 m	12 months					18 I	18 months						24 months	nths		
Effect		SI	SEm	LSD	LSD (0.05)	C C	CV (%)	SI	SEm	ΓS	LSD (0.05)		CV (%)	S	SEm		LSD (0.05)	05)	CV (%)	(9
Structure		0	0.48		1.35		, , 1	0	0.59		NS,		, I	J	0.25		0.71*	*	,	
Treatment		0.	0.58	,	1.66**		ı	0	0.72		2.04*		•	J	0.30		0.87*	*		
Structure × treatment 1.17	< treatr	nent 1.	17		3.32	14.9	6	-	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.

Acknowledgement

The authors are grateful to the Director, ICAR-Indian Grassland and Fodder Research Institute, Jhansi for providing facilities for conducting this study.

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