

Effect of Neem Products on Seed Quality Parameters of Soybean during Storage

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ABSTRACT Seeds of three soybean cultivars namely, PK-472, PS-564 and PS-1024 were obtained from JNKVV, Jabalpur and multiplied at IARI, New Delhi. The freshly harvested seeds were sun-dried for 2-3 days to bring down their moisture content to the level of 8 per cent and used in the present studies to observe the relative efficacy of neem products on seed storability. These seed lots were separately treated with tetra methyl thiuram disulphide (TMTD @ 0.3%) and with 0.25 and 0.50 per cent of Hexane extract of seed kernel (HESK), Methanol extract of seed kernel (MESK), Chloroform extract of seed coat and Methanol extract of seed coat (MESK) of neem seed. Suitable controls were maintained in each case. The seed lots were kept in 700 gauge polythene bags and stored under ambient conditions. The samples were drawn at an interval of 30 days to observe the influence of neem products and TMTD on germination, seedling vigour and seed health of soybean seeds over 120 days of storage. Significant increase in seedling dry weight was observed in all the treatments after 30 days of storage, with a marginal increase for TMTD treatment, and a more pronounced increase for neem products treated seeds *vis-a-vis* associated decline in the vigour of untreated seeds. In all the cultivars, MESK, HESK and MESK at 0.25 concentrations proved effective in controlling the associated mycoflora.

Keywords: Soybean, neem products, seed quality, storage, fungicides

Soybean (*Glycine max* (L.) Merrill) has become an important oilseed crop in India in recent years, occupying the second place in production among the oilseed crops. It is an excellent source of both proteins (40%) and oil (20%) and holds a great promise in solving the malnutrition problems and therefore, over the years it is gaining popularity across the world. However, the availability of good quality seed remains one of the major constraints of soybean cultivation, as the seeds lose viability under warm and humid conditions of storage [1]. The loss of viability in storage differs among the varieties within a species [2, 3]. Seed moisture content and storage temperature are the two major factors, which influence the rate of loss of viability in storage. Moreover, the mechanical injuries caused during processing have a direct bearing as they form the basis for attack and/ or invasion of

microorganisms, thereby adversely affecting the seed viability during storage. Due to non-availability of proper storage facilities in most parts of the country, majority of stored seeds gets infected with different mycoflora, which leads to the deterioration in seed quality [4].

Chemical treatments are applied to control internally or externally seed borne pathogens. Consequently, it not only enhances the germination potential of the seed but also increases the longevity of the seeds. However, the adverse effect of fungicides on seed quality is a major concern over the years [5]. Therefore, use of environmental friendly products like botanicals for seed treatment is emerging as a novel approach against losses in seed quality during storage. The beneficial effects of neem seed (*Azadirachta indica* L.) kernel extracts and

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neem oil to improve germination percentage and seedling vigour is documented in some crops [6]. With these objectives, an attempt was made to examine the relative efficacy of neem products and fungicides on the storability of three popular cultivars of soybean.

MATERIALS AND METHODS

Seeds of three promising soybean cvs. PK-262, PK-327 and PK-416 were collected from JNKVV, Jabalpur and further multiplied by following normal cultivation practices at research farm of IARI, New Delhi. Four neem products viz. hexane and methanol extracts of seed kernel (HESK and MESK respectively), chloroform and methanol extracts (CESC and MESC respectively) of seed coat were obtained from Division of Agricultural Chemicals, IARI, New Delhi. The moisture content of seeds was estimated by standard hot air oven method after pre-drying the seeds under sun so as to reduce moisture content to 8 per cent. A factorial experiment with five treatments (four neem products at two concentrations each, and Tetramethyl thiuram disulphide @ 0.3%) and one control was carried out in a completely randomized design at Division of Seed Science and Technology, IARI, New Delhi. Germination test was conducted by BP method at 25°C in four replicates of fifty seeds each, following ISTA method [7]. Ten random normal seedlings from each replicate were dried overnight in the oven at 90°C and final dry weight of the seedlings taken. For seed health, standard blotter method was followed to determine the per cent incidence of the various fungi associated with the seed lot using hundred seeds per treatment. The neem products were dissolved in acetone and the solution of appropriate concentration was then applied uniformly on the seed surface and dried under shade. After drying, the seeds were packed in 700 gauge polythene bags with an electric sealer and stored under ambient conditions for four months and the samples were drawn at an interval of thirty days for germination (%), seedling dry weight and seed health. The data were subjected to analysis of variance by completely randomized design.

RESULTS AND DISCUSSION

Initial germination of all the three cultivars was found to be in the range of 85-96 per cent (Table 1). The germination for all the cultivars was recorded at an interval of 30 days up to 120 days of storage.

Germination of untreated seeds

A gradual decline in germination percentage was observed in all the cultivars at the end of 120 days storage in case of untreated seeds. The cv. PS-1024 showed the minimum germination of 69 per cent while cv. PK-472 showed a maximum germination of 71 per cent after 120 days of storage amongst the three cultivars studied. The germination fell from 96 to 71, 90 to 70 and 85 to 69 per cent in case of cultivars PK-472, PS-564 and PS-1024, respectively after 120- days of storage. Gradual loss of seed viability was observed under ambient conditions of storage in earlier investigations [8].

Germination of treated seeds of soybean

The initial germination of soybean seeds observed before storage in PK-472 was 96 per cent, which fell to 70 per cent, a loss of 26 per cent in case of untreated seeds in a span of 120 days. However, the seed treatments with TMTD and other neem products gave boost to germination percentage; they showed up to 6 per cent increase in germination at the end of 120 days of storage as compared to the control (Table 1). The range of germination of soybean seeds varied for the three cultivars in response to TMTD. The drop in germination ranged from 96 to 70 per cent (PK-472), 90 to 76 per cent (PS-564) and 85 to 70 per cent (PS-1024) after 120 days of storage period. Amongst the cultivars, the highest germination was recorded in PS-564 at the end of storage period (76 per cent).

Among the four neem products used, HESK 0.25 and MESK 0.5 showed relatively higher germination percentage in all the cultivars. The seed treatment with HESK (0.25) and MESK (0.5) gave 72 and 76 per cent germination, respectively after 120 days of storage in cultivar PK-472. However, there was a gradual decline in the germination of all the cultivars treated with neem

products at both the concentrations i.e. 0.25 per cent and 0.50 per cent. It ranged from 70-74 per cent in PK-472, 70-76 per cent in PS-564 and 70-71 per cent in PS-1024 at the end of storage period, among the different neem treatments. The maximum germination percentage was recorded in seeds treated with MESK at 0.5 concentrations in cultivar PK-472, which was 76 per cent after 120 days of storage.

Though there was a general loss in germination from 16-26 per cent during the storage period, but there were no significant differences among different treatments over control irrespective of cultivars. These findings are in agreement with earlier reports [9], which show that the untreated seeds stored in polythene bags gave better germination as compared to treated seeds stored in cloth bags. Likewise, moisture impervious containers were preferred over the moisture pervious containers for good quality storage [10]. However, in cultivar PS-1024, the untreated seeds (control) recorded a germination of 69 per cent after 120 days but treated seeds were able to maintain the germination level at or above 70 per cent. Although it is statistically insignificant with other treatments but it is highly significant from the certification point of view as the minimum germination percentage prescribed under Indian Minimum Seed Certification Standards for soybean is 70 per cent. Our results suggest that the seed treatments with TMTD and neem products can give marginal boost to the germination percentage and this idea can be further investigated for precious nucleus stocks of vegetable seeds keeping in view the cost benefit ratio of commercial seed production.

Seedling vigour of soybean seeds during storage

Seedling vigour was measured as the dry weight of ten randomly selected normal seedlings after the final count of germination from each replicate. It was expressed as the dry weight (mg) per ten seedlings. During the course of storage, all the cultivars exhibited gradual decline in seedling dry weight (Table 2) for untreated seeds.

The initial seed vigour measured in terms of seedling dry weight ranged from 372mg

(PS-564) to 402 mg (PS-1024). The treated seeds showed a significant increase in seedling dry weight in comparison to control. There was an increase in seedling dry weight upto 30 days of storage in case of seeds treated with TMTD and neem products, which declined gradually as the storage period progressed. This initial increase was observed in all the cultivars. Amongst all the treatments, MESK at 0.25 concentrations proved to be very effective in increasing the dry weight at the end of 30 days period, after which the dry weight declined gradually. In cultivar PK-472, it increased from 377 mg to 403 mg after 30 days of storage and then fell to 372 mg at 120 days of storage. Similarly, the seedling dry weight rose from 372 mg to a maximum of 415 mg after 30 days of storage and then declined to 380 mg in PS-564 while it increased from 431 mg to 449 mg and then declined to 372 mg at the end of storage period in PS-1024. Though the decline in seedling dry weight in all the treatments was evident throughout the storage period, the decline was rather slow as compared to control. There was significant interaction between treatments and concentrations for two cultivars only, namely PK-472 and PS-564. On the contrary, none of the cultivars had significant interaction among all other parameters. Earlier reports suggest that loss in seedling vigour precedes loss in seed viability [11, 12]. The results of this study are in agreement with another interesting phenomenon of significant increase in the seedling dry weight after 30 days of storage in all the treated seeds. However, this increase was more pronounced in seeds treated with neem products as compared to TMTD. It can be attributed to the fact that in treated seeds the pathogen infection was less compared to that of control. Seedlings from seeds treated with neem products were found to be more vigorous, with higher root and shoot growth indexes and dry weights over control [13]. Total seedling length was found to be greater when the wheat seeds were treated with neem products as compared to untreated [14].

Seed health

A large number of field and storage fungi including *Cercospora*, *Fusarium*, *Alternaria*,

Table 1. Per cent germination of treated seeds of three varieties of soybean at different periods of storage

Treatment	Dose (%)	Cultivar	Period of storage (days)					Mean
			0	30	60	90	120	
Control	-	PK-472	96	88	79	73	71	77.75
	-	PS-564	90	86	80	76	70	78.00
	-	PS-1024	85	80	77	72	69	74.50
Thiram	0.3	PK-472		91	84	76	70	80.25
		PS-564		87	81	79	76	80.75
		PS-1024		81	77	72	70	75.00
HESK	0.25	PK-472		90	85	78	72	81.25
		PS-564		85	80	76	74	78.75
		PS-1024		82	79	73	70	76.00
	0.50	PK-472		89	84	80	71	81.00
		PS-564		87	83	75	70	78.75
		PS-1024		81	76	71	71	74.75
MESK	0.25	PK-472		92	87	81	75	83.75
		PS-564		88	81	77	71	79.25
		PS-1024		83	78	71	70	75.50
	0.50	PK-472		91	86	80	76	83.75
		PS-564		86	80	75	73	78.50
		PS-1024		78	76	73	71	74.50
CESC	0.25	PK-472		88	84	78	72	80.50
		PS-564		85	81	74	70	77.50
		PS-1024		80	77	74	71	75.50
	0.50	PK-472		90	82	76	72	80.00
		PS-564		88	81	76	70	78.75
		PS-1024		79	74	72	70	73.75
MESK	0.25	PK-472		92	87	79	71	82.25
		PS-564		86	81	73	71	77.25
		PS-1024		81	78	73	70	75.50
	0.50	PK-472		90	81	74	70	78.75
		PS-564		87	79	74	72	78.75
		PS-1024		80	76	73	71	75.00
CD(P=0.5)		PK-472		PS-564		PS-1024		
Storage period(S)		0.9361		0.9393		0.8300		
Treatments								
Concentration(C)		1.0466		1.0502		0.9280		
S x T		0.6619		0.6642		0.5869		
T x C		NS		NS		NS		
S x C		1.4800		1.4851		1.3123		
S x T x C		NS		NS		NS		
		NS		NS		NS		

Table 2. Dry weight (mg) of ten normal seedlings of treated seeds of three varieties of soybean at different periods of storage

Treatment	Dose (%)	Cultivar	Period of storage (days)					Mean
			0	30	60	90	120	
Control	-	PK-472	377	354	336	321	309	330
	-	PS-564	372	378	361	342	327	352
	-	PS-1024	402	389	371	362	363	371.25
Thiram	0.3	PK-472		389	374	358	341	365.5
		PS-564		402	389	371	362	381
		PS-1024		412	401	387	398	399.5
HESK	0.25	PK-472		399	385	372	364	380
		PS-564		411	401	387	378	394.25
		PS-1024		426	411	392	387	404
	0.50	PK-472		401	388	379	366	383.5
		PS-564		408	399	384	371	390.5
		PS-1024		423	416	405	379	405.75
MESK	0.25	PK-472		403	390	381	372	386.5
		PS-564		415	402	389	380	396.5
		PS-1024		428	414	399	371	403
	0.50	PK-472		398	387	374	360	379.75
		PS-564		412	396	381	373	390.5
		PS-1024		418	400	386	361	391.25
CESC	0.25	PK-472		405	391	376	363	383.75
		PS-564		409	397	383	372	390.25
		PS-1024		416	398	379	376	392.25
	0.50	PK-472		401	389	379	359	382
		PS-564		413	404	388	375	395
		PS-1024		420	410	393	402	406.25
MESK	0.25	PK-472		394	381	370	358	375.75
		PS-564		405	391	381	369	386.5
		PS-1024		419	397	382	369	391.75
	0.50	PK-472		392	379	364	352	371.75
		PS-564		410	397	384	368	389.75
		PS-1024		421	403	386	371	395.25
CD(P=0.5)		PK-472		PS-564		PS-1024		
Storage period(S)		3.9147		12.0322		18.0085		
Treatments		4.3768		13.4524		20.1341		
Concentration(C)								
S x T		2.7681		8.5080		0.5869		
T x C ¹⁾		NS		NS		NS		
S x C		6.1897		19.0246		NS		
S x T x C		NS		NS		NS		
		NS		NS		NS		

Cladosporium and *Penicillium* etc., have been found to be associated with soybean seeds during storage [15]. Seeds of all the three cultivars were found to be associated with *Cladosporium* spp., *Alternaria alternata* (Fr.) Keissler, *Aspergillus* spp. and *Penicillium* spp., which is perhaps due to the fact that all the seeds were collected from same natural populations. The incidence of *Cladosporium* spp. was initially higher in both the untreated as well as treated seeds in all the cultivars. This could be attributed to the fact that *Cladosporium* got associated with the seeds at the time of harvest in the field itself and continued its association during the storage also. It decreased gradually to a lesser extent in the untreated seeds but was completely eliminated in case of treated seeds of many cultivars as the storage period progressed. However, the incidence of *Cladosporium* was found negligible at the end of 120 days of storage in all the cultivars.

As stated above, the incidence of *Cladosporium* was quite high in all the cultivars. It ranged from 12-22 in PK-472, 12-25 in PS-564, and 15-30 in PS-1024 in treated seeds and followed by *Alternaria alternata*, which declined gradually in all the cultivars during storage (Table 3). The incidence of *Aspergillus* spp. started only after 30 days of storage in all the cultivars, except PS-1024 where it started after 60 days. On the other hand, the presence of *Penicillium* spp. was observed at 30 days of storage in all the three cultivars (Table 4). Although *Cladosporium* and *Alternaria* tend to decline during the course of storage period, including untreated control but the incidence of *Aspergillus* and *Penicillium* showed an increasing trend as the storage period progressed in all the cultivars. The incidence of *Aspergillus* in untreated controls increased from 5 to 16, 6 to 24 and 0 to 24 per cent in cultivars PK-262, PK-327 and PK-416, respectively during the storage period. Similarly, the incidence of *Penicillium* was observed from 0 to 10, 0 to 18 and 0 to 16 per cent for the cultivars in the same order.

In all the cultivars, neem products were found to decrease the incidence of all the fungi investigated at different periods of storage. Moreover, the neem products viz. MESK, HESK and MESC were found to be on par with TMTD

in controlling the associated mycoflora. It was also noticed that these neem products were more effective at 0.25 per cent concentration than at 0.5 per cent concentration. Therefore, it is advisable to use neem products for pre-storage treatment of seeds in view of the associated adverse effect of synthetic fungicides.

The efficacy of neem oil against *Aspergillus niger* and *Alternaria alternata* has been documented in earlier investigations [16]. The antifungal activity of leaf extract of neem on seed mycoflora of wheat was investigated and a marked reduction in seed mycoflora as well as an enhancement of seed germination was noted with the above treatments. The studies on the seed mycoflora of lentil and their control in Pakistan revealed a total of 21 fungal species isolated from 4 lentil cultivars using the blotter technique and of these 8 were pathogenic to various crops. The main species isolated were *F. moniliforme* [*Gibberella zae*], *F. oxysporum* and *F. semitectum* [*F. pallidoroseum*] and seed treatment with 80 ppm neem bitter extracts controlled the seedborne mycoflora [18]. Further, the effect of neem products as mould inhibitors against post harvest fruit rotting fungi (namely *Penicillium italicum*, *Alternaria alternata* and *Aspergillus niger*) of tomato *in vitro* was reported and neem oil was found as effective as thiabendazole in checking the growth of these fungi [19]. The antifungal activity of hexane extracts of *Azadirachta indica* seed against *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, fungi commonly causing spoilage of stored grains has been reported [20]. The effect of plant products on seedborne mycoflora of sorghum was tested *in vitro* and it was found that the leaf extract of *Azadirachta indica* inhibited mycelial growth and spore germination of seed borne mycoflora of sorghum including *Alternaria tenuis* [*A. alternata*], *A. flavus*, *Curvularia lunata* [*Cochliobolus lunatus*], *F. moniliforme* and *Rhizopus stolonifer*. The neem extracts and those of *Catharantus roseus* and *Lantana camara* were more effective than the other plant extracts tested [21]. The seed borne fungi of mustard was tested with indigenous plant extracts for their control and four of them were found effective in reducing the prevalence of seed borne fungi. However, neem and garlic extracts were superior, followed by ginger and onion bulb [22].

Table 3. Per cent incidence of field fungi associated with the treated seeds of three varieties of soybean at different periods of storage

Treatment	Dose(%)	Cultivar	Period of storage (days)									
			0	30	60	90	120	0	30	60	90	120
			<i>Alternaria alternata</i>					<i>Cladosporium cladosporioides</i>				
Control	-	PK-472	-	-	5	4	-	48	42	25	8	-
	-	PS-564	18	12	10	-	-	54	50	32	12	-
	-	PS-1024	-	-	2	6	-	50	46	28	12	-
Thiram	0.3	PK-472	-	-	-	-	5	-	20	10	-	-
		PS-564	-	10	-	-	-	-	12	8	-	-
		PS-1024	-	2	-	-	-	-	22	15	-	-
HESK	0.25	PK-472	-	-	-	-	-	-	16	-	8	-
		PS-564	-	-	-	-	-	-	22	-	10	-
		PS-1024	-	-	-	-	5	-	15	-	6	-
	0.50	PK-472	-	-	-	3	-	-	12	8	-	-
		PS-564	-	-	2	-	3	-	15	8	-	-
		PS-1024	-	-	-	3	-	-	25	12	-	-
MESK	0.25	PK-472	-	-	-	-	-	-	15	-	6	-
		PS-564	-	-	-	-	-	-	18	12	-	-
		PS-1024	-	-	5	-	-	-	18	-	-	-
	0.50	PK-472	-	-	5	-	-	-	22	12	-	-
		PS-564	-	5	-	-	8	-	20	-	8	-
		PS-1024	-	-	6	8	-	-	20	-	8	-
CESC	0.25	PK-472	-	3	-	-	-	-	18	10	-	-
		PS-564	-	-	-	-	-	-	25	15	-	-
		PS-1024	-	-	-	-	-	-	30	18	-	-
	0.50	PK-472	-	-	-	-	6	-	12	5	-	-
		PS-564	-	-	8	-	6	3	22	12	-	-
		PS-1024	-	-	-	3	-	-	24	12	-	-
MESK	0.25	PK-472	-	-	-	-	-	-	18	-	6	-
		PS-564	-	-	-	-	-	-	14	-	-	-
		PS-1024	-	-	-	-	-	-	18	-	-	-
	0.50	PK-472	-	-	6	2	-	-	14	6	-	-
		PS-564	-	-	-	6	-	-	18	10	-	-
		PS-1024	-	-	-	-	10	-	20	-	6	-

Table 4. Per cent incidence of storage fungi associated with the treated seeds of three varieties of soybean at different periods of storage

Treatment	Dose(%)	Cultivar	Period of storage (days)									
			0	30	60	90	120	0	30	60	90	120
			<i>Aspergillus flavus</i>					<i>Penicillium spp.</i>				
Control	-	PK-472	-	5	8	12	16	-	-	6	8	10
	-	PS-564	-	6	12	20	24	-	-	10	10	18
	-	PS-1024	-	-	10	22	24	-	-	-	8	16
Thiram	0.3	PK-472	-	-	-	-	-	-	-	-	-	5
		PS-564	-	-	-	-	5	-	-	-	5	-
		PS-1024	-	-	-	-	10	-	-	-	3	-
HESK	0.25	PK-472	-	-	3	-	-	-	-	-	-	-
		PS-564	-	-	-	-	-	-	-	-	4	-
		PS-1024	-	-	-	-	-	-	-	-	-	-
	0.50	PK-472	-	-	-	6	10	-	-	-	-	8
		PS-564	-	-	-	-	-	-	-	-	-	5
		PS-1024	-	-	4	6	-	-	-	-	-	10
MESK	0.25	PK-472	-	-	-	-	-	-	-	-	-	-
		PS-564	-	-	-	4	-	-	-	-	-	-
		PS-1024	-	-	-	-	-	-	-	-	-	-
	0.50	PK-472	-	-	-	8	-	-	-	-	-	3
		PS-564	-	-	-	8	-	-	-	-	-	-
		PS-1024	-	-	10	-	10	-	-	-	-	-
CESC	0.25	PK-472	-	-	-	-	2	-	-	-	-	-
		PS-564	-	-	-	-	3	-	-	-	-	-
		PS-1024	-	-	12	2	-	-	-	2	-	-
	0.50	PK-472	-	-	-	-	10	-	-	5	5	-
		PS-564	-	-	5	2.5	5	3	-	3	-	10
		PS-1024	-	-	-	-	8	-	-	-	-	12
MESC	0.25	PK-472	-	-	-	-	-	-	-	-	-	-
		PS-564	-	-	-	-	-	-	-	-	-	-
		PS-1024	-	-	-	-	-	-	-	-	-	-
	0.50	PK-472	-	-	-	4	-	-	-	-	2	4
		PS-564	-	-	-	10	10	-	-	-	-	-
		PS-1024	-	-	-	8	-	-	-	12	12	-

In conclusion, our results suggest that seed treatment of soybean seeds with neem products is beneficial in maintaining germination and vigour of seeds and also in reducing the associated mycoflora.

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