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Effects of ageing on seed viability and oil quality of black cumin cultivars in Ethiopia

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Abstract

Seed quality is crucial for the success of crop production, as it affects germination capacity, emergence potential, and seedling growth. This study investigated the impact of extended storage on the germination capacity, oleoresin and essential oil contents of black cumin seeds from three black cumin cultivars in Ethiopia *viz.* Aden, Dershaye, and Darbera. Seeds were stored for up to three years, and germination studies were conducted using a completely randomized design. Results showed that storage period significantly affected various germination parameters, with a decrease in germination percentage observed with increasing storage period. However, cultivar and the interaction between storage period and cultivar had no statistically significant effect on germination percentage. The study also provides valuable insights into the correlations between different germination indices. Additionally, as black cumin seeds aged, their oleoresin and essential oil contents decreased in all three cultivars. Proper seed storage practices are essential in maintaining the germination potential of black cumin seeds, particularly over extended storage periods. Overall, this study provides insights into the impact of extended seed storage on black cumin seed quality and germination potential, highlighting the importance of proper seed management practices to support sustainable crop production.

Keywords: Germination, seed ageing, seedling emergence, seed storage, seed viability

Introduction

Black cumin (*Nigella sativa* L.) is a vital medicinal plant used for centuries in traditional medicine to address various ailments (Ahmad *et al.*, 2013). It is cultivated globally, particularly in Ethiopia, and is valued for its numerous biological activities, including antibacterial and anti-inflammatory properties (Kahaliw *et al.*, 2021). The seeds are also a rich source of essential oil, which is widely applied in the food, cosmetics, and pharmaceutical industries (Kahaliw *et al.*, 2021). The quality of black cumin seeds is crucial for successful crop production, as it directly impacts germination capacity, seedling vigor, and overall crop yield (Kibinza *et al.*, 2011). However, the fluctuating demand for black cumin can lead to extended storage periods, which significantly affects seed quality. Seed ageing, a natural process that occurs during storage, can result in a decline in germination capacity and seed vigor, ultimately compromising crop productivity (Kibinza *et al.*, 2011; Khan *et al.*, 2016). Research indicates that prolonged storage can lead to lipid autoxidation and increased fungal activity, particularly in oil seeds like black cumin, which are high in polyunsaturated fatty acids. This deterioration not only affects germination rates but also the oil yield and quality attributes of the seeds (Balešević *et al.*, 2007; Genes & Nyomora, 2018). Understanding the importance of black cumin and the effects of seed ageing on its quality parameters is essential for enhancing agricultural productivity and ensuring the effective use of this valuable medicinal crop. Currently, there is limited information on the effect of extended storage on seed viability and oil quality of black cumin cultivars in Ethiopia. The study therefore is aimed to investigate the effect of extended storage on the germination, oleoresin, and essential oil content of black cumin seeds under ambient conditions and to determine the optimal storage duration for maximum retention of seed viability and oil content as this information may be useful to plan for any production, storage, or marketing activities after harvest.

Materials and methods

Experimental site: The study was conducted at Kulumsa Agricultural Research Center (KARC) in Tiyo district, located in the Arsi Administrative Zone of the Oromia Regional State, Ethiopia.

Experimental material: The study consisted of two experiments: Seed germination test and oleoresin content quantification. Seed lots from three black cumin cultivars in Ethiopia - Aden, Dershaye, and Darbera (Table 1) - from four consecutive years of production (2016, 2017, 2018, and 2019) were used for the study. Seeds were selected from seed lots in a sack stored for four years, three years, two years, and one year within a seed store that maintained ambient storage conditions (with an average temperature of 18.3°C and relative humidity of 65.4%). The sampled seeds possessed a moisture content of 11-12% at the time of sampling and were obtained from the upper, middle, and bottom sections of the sack. The seed samples were subsequently combined for the germination test. The three black cumin cultivars and the four storage periods which made up twelve treatment combinations were arranged in a 3x4 factorial experiment with Completely Randomized Design (CRD).

For the seed germination experiment, 100 seeds were sown on sterilized petri dishes lined with moistened filter paper to maintain moisture for germination. The petri dishes were kept in a room with access to partial light and kept at room temperature (27°C) and Relative humidity of 70%. Seeds of each treatment combination were sown in triplicates and irrigated uniformly until germination count was completed.

Determination of oleoresin and essential oil content: Oleoresin was extracted from the seeds using a Soxhlet extractor. The seeds of the three cultivars were coarsely ground to 30 g each with 300 ml of hexane for 12 h and stored in an amber glass screw cap bottle at room temperature until use (Singh *et al*., 2014; Dinagaran *et al*., 2017). The ground seeds were then placed in a distillation chamber and steam was passed through them. The steam causes the essential oil to vaporize and rise up into a condenser, where it is cooled and condensed back into a liquid form (Salem *et al*., 2013). The resulting liquid is a concentrated form of essential oil, which can be further processed to remove any remaining water or impurities. Each sample was replicated three times. The solvent was removed using a rotary evaporator operated at 45°C, and the final traces of solvent were removed under a stream of nitrogen (Rao *et al*., 2007).

Germination count and measurement: The study evaluated seed germination using various indices, including final germination percentage (GRP) (Scott *et al*., 1984; ISTA, 2015), mean germination time (MGT) (Ranal & Santana, 2006), first day germination (FDG), last day germination (LDG), time spread of germination (TSG) (Kader, 2005), germination rate index (GRI) (Wardle *et al*., 1991; Esechie, 1994), germination index (GI) (Bench *et al*., 1991), MGR (mean germination rate) (ISTA, 2019); germination speed (GSP) (ISTA, 2019); variance of germination time (VGT); and standard deviation of the germination time (SDG). Cumulative germination was used to develop time-germination curves, as described by Ellis & Roberts (1981) and Ruan & Xue (2002).

GRP measured the germination capacity of seeds in percentage, calculated as Ng/Nt X100, where Ng is the number of final seeds germinated and Nt is the total number of seeds sown. MGT estimated the average time required for maximum germination, calculated as MGT = (∑(NiTi))/(∑Ni), where Ni is the number of seeds germinated at the ith time, Ti is the time from the start of the experiment to the i^{th} observation, and k is the last time of germination. FDG and LDG determined the first and last day of germination, respectively. TSG estimated the time between the first and last germination events, calculated as the difference between LDG and FDG. GRI estimated the average percentage of germination on each day, calculated as G1/T1 + G2/T2 + ... + Gi/Ti, where G1, G2, G3, ..., Gi are the number of germinated seeds observed at time (days) T1, T2, T3, ..., Ti after sowing. GI estimated both the percentage and speed of germination, calculated as GI =

$$GI = (t_f * n1) + (t_{f-1} * n2) + (t_{f-2} * n3) + (t_{f-3} * n4) + \cdots (t_{f-n} * nf),$$

where t is the time in days from sowing to the ith germination event and n is the cumulative number of seeds germinated up to that time. Mean germination rate (MGR) is related to the mean germination percentage (MGP) but different measures of seed viability. The germination percentage is simply the percentage of seeds that have germinated in a particular test, whereas the mean germination rate is the average rate at which the seeds in a particular lot germinate over multiple tests. Germination speed (GSP) is the rate of germination in terms of the total number of seeds that germinate in a time interval. Higher values indicate greater and faster germination. GSP can be used interchangeably with MGR and both are the reciprocal of germination time (MGT).

Besides, VGT (variance of germination time) was used as measure of the variability of the germination times. It is calculated by taking the average of the squared differences between each germination time and the mean germination time. SDG (standard deviation of germination time) is another measure of the spread of the germination time. It is calculated as the square root of the variance. The standard deviation is often used instead of the variance because it has the same units as the data (in this case, time) and is therefore more interpretable (Bewley & Black, 1994; McDonald, 1999).

Correlation analysis

The relationships between germination indices, including MGT, GRP, MGR, GSP, VGT and SDG were estimated using Pearson correlation (Pearson, 1895).

Data analysis

The data for the aforementioned parameters were subjected to analysis of variance (ANOVA) using R statistical software (http://www.R-project.org/; accessed online on June 5, 2020), and means were separated using the least significant difference (LSD) test at the 5% probability level. Curves were developed to show the cumulative germination over time intervals (days) for storage periods and cultivars. Bar graphs were also produced to show the differences in germination percentages, germination speed, and mean germination time among the storage periods and cultivars.

Results and discussion

Germination indices

The results showed that seed ageing had a significant effect on the final germination percentage of black cumin cultivars ($p < 0.001$) (Table 2). Specifically, there was a decrease in germination percentage as the storage period increased. The cultivars exhibited significant differences along with significant interaction with storage period for mean germination time, last day of germination, time spread of germination, germination rate index, and germination index, but not for final germination percentage. ($p < 0.05$) (Table 2).

The mean separation tests showed that storage period had a significant effect on the final germination percentages, but no significant differences between cultivars (Table 3) was detected. The highest final germination percentage (96.22%) was obtained from fresh seed (2019 harvest), and this percentage decreased linearly with the storage period. Thus, it can be stated that the longer the seeds stored, higher is the damage, although the reduction in germination is less in the shorter storage period of one and two years. These findings suggest that proper storage conditions and appropriate storage periods are crucial for maintaining the germination potential of black cumin seeds, and that long-term storage can significantly reduce their germination percentage.

The results of the germination percentage were also presented in bar graphs, which illustrated significant differences between storage periods as well as non-significant differences among the cultivars (Fig. 1). The graphs showed significant differences in germination percentage among the four storage periods for each cultivar. Specifically, significant differences were observed in germination percentage between fresh seed (2019) and three-year-old seed (2016), indicating that black cumin seed could be stored for up to two years without significant loss of germinability under ambient storage conditions. The bar graphs also depicted non-significant differences in germination percentage among the three cultivars, regardless of the storage periods (Fig. 1). These findings suggest that the differences in germination percentage observed in the present study were mainly due to seed ageing rather than to varietal differences.

Table 1. Description of the three black cumin cultivars used in the present study

Cultivar name	Release		Altitude (m.a.s.l)	Maturity (days)	Productivity ($t\ ha^{-1}$)		Oleoresin (%)	Essential oil (%)
	Year	*Breeder			Research	Farmer		
Aden	2009	MARC/EIAR	1500-2400	134-150	0.9-1.6	0.8-1.2	27.2-32.4	0.6-1.2
Dershaye	2009	MARC/EIAR	1500-2400	134-150	0.9-1.6	0.8-1.1	30.8	0.7-1.3
Darbera	2006	SARC/OARI	1650-2004	155-173	1.5-1.9	0.7-1.1	28.31	0.56

*MARC: Melkasa Agricultural Research Center, EIAR: Ethiopian Institute of Agricultural Research, SARI: Sinana Agricultural Research Institute, OARI: Oromia Agricultural Research Institute. Source: (Alemaw *et al*., 2010).

Besides, final germination percentage, the storage period affected other germination indices, viz., mean germination time, last day of germination, time spread of germination, germination rate index, and germination index (Table 3). Mean germination time was significantly affected by the storage period, and it increased significantly as the storage period was extended from 0 to three years (Table 3). Mean germination time represents the average time (in days) needed for the seed lot to reach maximum germination percentage; it took 7.01 days for the three year old seeds to germinate while fresh seed germinated in 5.5 days (Table 3 and Fig. 2). These findings suggest that seed ageing can significantly increase the mean germination time, and that fresh seed attain maximum germination percentage in relatively shorter time compared to aged seed.

To complement the mean germination time, germination peaks were developed using a germination-time graph to show the maximum number of seeds germinated during the germination process for each storage period (Fig. 3). The fresh seed lot (2019) had the highest maximum number of seeds germinated (49.78), followed by the one-year-old (43.33), two-year-old (40.78) and three-year-old seeds (41.33). It is important to note that this comparison is not meant to show differences in the maximum number of seeds germinated between storage periods, but rather to graphically illustrate the differences in time (in days) required to reach the maximum germination peak for black cumin seeds of different ages. Regardless of the number of seeds germinated, the fresh seed (2019) reached its peak germination percentage faster than the older seeds (2018, 2017, and 2016). The cumulative germination data, presented in an in-time germination graph (Fig. 4), clearly showed differences between the storage periods in the amount of seeds germinated over time. No significant differences were observed in the cumulative seed germination percentage between the storage periods until the fifth day after sowing. On the fifth day of observation, over 50% of the seeds from the 2019 seed lot had germinated while it was between 0-10% for the 2016 seed lot. This trend was maintained for the subsequent days of observation until the final germination count on day 10 (Fig. 4). These findings suggest that seed ageing can significantly affect the rate of cumulative germination over time, and that fresh seed has a higher rate of germination compared to aged seed.

Table 2. Analysis of variance for germination percentage, mean germination time, germination speed and mean germination rate. The numbers with asterisk are mean squares

Source of variation	Df	GRP (%)	MGT (days)	LDG (days)	TSG (days)	GRI (%/day)	GI
Storage period (Y)	3	828.3***	3.448***	5.361***	5.361***	47.62***	83627***
Cultivar (C)	2	47.6ns	0.465***	2.111*	2.111*	3.75**	7506***
C x Y	6	25.5ns	0.134***	1.222ns	1.222ns	0.55ns	1149ns
Error	24	23.1	0.022	0.556	0.556	0.58	781ns
CV (%)		5.40	2.40	7.82	21.12	5.99	6.44

***Very highly significant at $p<0.001$, **highly significant at $p<0.01$, *significant at $p<0.05$, ns: non-significant. FGP= Final germination percent, MGT=Mean germination time, LDG= Last day of germination, TSG=Time spread of germination, GRI=Germination rate index, GI= Germination index.

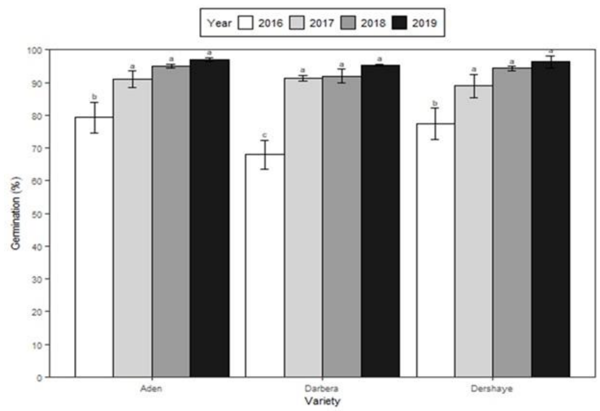

Fig. 1. Germination percentage of black cumin cultivars during storage

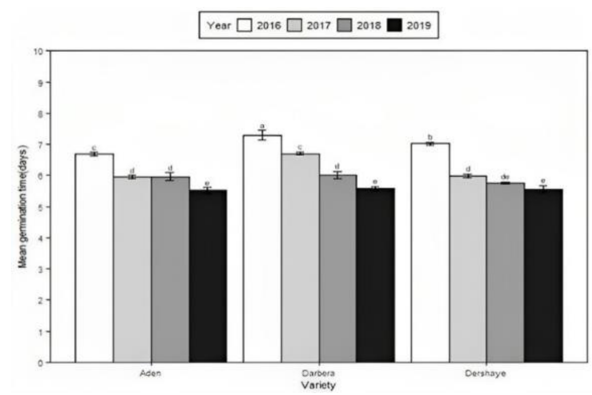

Fig. 2. Mean germination time (days) of black cumin cultivars during storage

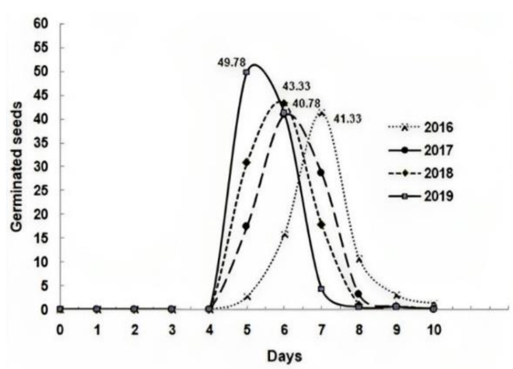

Fig. 3. Number of seeds germinated along average time (days) of germination

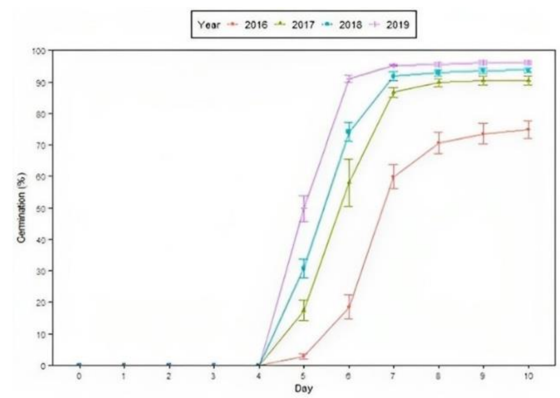

Fig. 4. In-time cumulative germination curves for the four storage periods

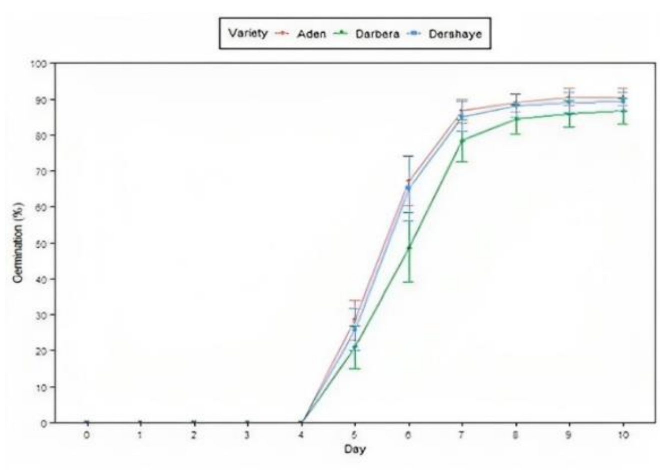

Fig. 5. In-time cumulative germination curves for the four cultivars

Correlations between germination indices

Table 4 presents correlations among various germination indices, revealing that mean germination time (MGT) has a strong negative correlation with final germination percentage (GRP), germination speed (GSP), mean germination rate (MGR), variance of germination time (VGT), and standard deviation of germination time (SDG). This indicates that as MGT increases, GRP and other indices decrease. Notably, GSP and MGR are perfectly positively correlated, suggesting they can be used interchangeably to measure germination rate; both are negatively correlated with MGT, serving as its reciprocal. Additionally, VGT and SDG show a strong positive correlation, indicating that as variance increases, standard deviation also rises, reflecting the spread of germination times. High standard deviation signifies a wider spread of germination times, while low indicates that they are closely clustered around the mean.

Oleoresin and essential oil content

Table 4 reveals that as black cumin seeds age, their oleoresin and essential oil contents decrease across all three cultivars, suggesting that seed ageing can significantly impact the chemical composition and quality of seeds, including their essential oil and oleoresin content. Such changes are known to occur as seeds lose moisture and undergo biochemical alterations over time.

Table 3. Mean separation for germination indices as affected by storage period and cultivar

Treatments*	GRP	MGT	LDG	TSG	GRI	GI	PGL
Storage period							
2019 (fresh seed))	96.22 a	5.55 d	9.00b	3.00b	4.81a	524.11a	-
2018 (1 year)	93.77 ab	5.91 c	9.33b	3.33b	13.74b	477.11b	2.45
2017 (2 years)	90.44 b	6.21 b	9.11b	3.11b	12.70c	432.77c	5.78
2016 (3 years)	74.88 c	7.01 a	10.66a	4.66a	9.49d	300.22d	21.34
LSD (5%)	4.67	0.14	0.725	0.628	0.641	27.182	
Cultivar							
Aden	90.58	6.03b	9.58ab	3.58ab	13.13a	452.66a	
Dershaye	89.25	6.08b	9.08b	3.08b	12.86a	442.75a	
Darbera	86.66	6.39a	9.91a	3.91a	12.06b	405.25b	
LSD (5%)	4.05	0.12	0.63	0.63	0.64	23.54	

Means with the same letter are not statistically different; LSD=Least significant difference at 5% probability. FGP= Final germination percent, MGT=Mean germination time, LDG= Last day of germination, TSG=Time spread of germination, GRI=Germination rate index, GI= Germination index, PGL=Percent germination loss from the control, Significance=statistical significance at $p<0.05$. Under treatment column, storage period the years (2019-2016) signify season (year) of production, while numbers in brackets are number of years stored after harvest.

Table 4. Pearson correlation matrix showing correlation coefficients between germination indices

	MGT	GRP	MGR	GSP	VGT	SDG
MGT						
GRP	-0.84****					
MGR	-1.00****	0.82****				
GSP	-1.00****	0.82****	1.00****			
VGT	0.63****	-0.51**	-0.64****	-0.64****		
SDG	0.64****	-0.51**	-0.65****	-0.65****	1.00****	

Table 5. Seed oleoresin and essential oil content of the three black cumin cultivars under different storage periods

Cultivar	Year (storage period)	Oleoresin (%)	Essential oil (%)
Aden	2016 (3 years)	31.14	3.5
	2017 (2 years)	35.64	4.0
	2018 (1 year)	37.50	4.3
	2019 (fresh seed)	38.76	4.8
Darbera	2016 (3 years)	30.04	3.1
	2017 (2 years)	31.65	3.5
	2018 (1 year)	39.00	3.9
	2019 (fresh seed)	40.93	4.3
Dershaye	2016 (3 years)	35.83	3.5
	2017 (2 years)	36.95	4.4
	2018 (1 year)	38.15	4.4
	2019 (fresh seed)	39.13	4.7

The present study highlights the significant impact of seed ageing on the final germination percentage of black cumin seeds, aligning with previous findings that demonstrate decreased germination rates with extended storage (Chauhan and Singh, 2014; Kumar *et al.*, 2016). While no statistically significant effects of seed cultivar or their interaction with storage duration were observed, literature indicates varying responses among cultivars, as seen in tomato and mung bean studies (Rao and Venkateswarlu, 2008; Oyedeji *et al.*, 2012). Additionally, both seed cultivar and storage period influenced key germination indices, such as mean germination time and germination rate index, corroborating earlier research on seed ageing in wheat and soybean (Baskin and Baskin, 2014; Wang *et al.*, 2019). The correlation analysis revealed strong negative relationships between mean germination time and other indices, consistent with findings that longer germination times correlate with reduced rates (Bewley and Black, 1994; Kucera *et al.*, 2005). The perfect positive correlation between germination speed and mean germination rate indicates these metrics can be used interchangeably (Ellis and Roberts, 1981). Additionally, the strong correlation between variance and standard deviation of germination time suggests consistent distribution patterns (Bewley and Black, 1994; McDonald, 1999). Importantly, seed ageing also affects the quality and chemical composition of black cumin seeds, particularly their essential oil and oleoresin content, which are vital for their medicinal properties. Previous studies reported significant decline in essential oil content with ageing, while oleoresin levels varied (Akhila *et al.*, 2012; Kumar *et al.*, 2015). These findings underscore the need for further research to explore the biochemical mechanisms behind these changes and to establish optimal storage practices for maintaining seed quality.

Conclusion and recommendation

This study highlights the critical importance of proper storage practices for maintaining the germination potential and chemical quality of black cumin seeds. Seed ageing significantly impacts germination rates and essential oil content, underscoring the need for effective seed management strategies. Although no significant differences were found among cultivars regarding their response to ageing, further investigation is warranted. Future research should focus on determining optimal storage conditions for various black cumin cultivars and exploring the effects of storage duration on other quality parameters. Additionally, studies should assess the impact of environmental factors like temperature and humidity on seed viability and quality. These efforts will provide essential insights for improving seed storage practices in the industry.

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