AN ABSTRACT OF THE THESIS OF

Grace Fuchs for the degree of Master of Science in Crop Science presented on April 19, 2023.

Title: Effects of Different Storage Conditions on Seed Quality of Hemp (*Cannabis sativa* L.)

Abstract approved:

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Sabry Elias

Hemp (*Cannabis sativa* L.) is a versatile crop with a wide range of pharmaceutical, commercial, and industrial uses. There is a lack of research and published studies on the ideal storage conditions and their effects on seed quality for hemp. Understanding ideal hemp seed storage conditions would reduce unnecessary seed deterioration and economic loss. The objectives of this study were to 1) determine the effects of three storage conditions on the magnitude of deterioration of five cannabidiol (CBD) hemp seed varieties stored for 18 months; and 2) determine and recommend protocols for the most effective vigor test(s) to measure the rate of deterioration in hemp seeds stored for 18 months. Five CBD hemp cultivars (V1 – V5) were evaluated, all which were grown in Oregon's Willamette Valley in 2020. Initial seed quality of all samples was determined. Seeds were evenly divided into three groups and stored in one of three storage conditions: 1) 20°C and 30% relative humidity (RH); 2) 10°C and 75% RH; and 3) 5°C and 90% RH. Seeds were evaluated at 4, 8, 12, 16, and 18 months to measure the magnitude of deterioration over time for each storage condition. Viability and vigor tests were used to measure seed quality for each storage period, including the tetrazolium (TZ), standard germination (SGT), speed of germination index (SGI), electric conductivity (EC), field emergence (FE), cold test (CT), accelerated aging (AAT), and seed moisture content (SMC). Seeds of all varieties stored at 10°C/75% RH showed the greatest

deterioration after 18 months, and the lowest at 5°C/90% RH. Seeds stored at 20°C/30% RH had higher quality than the 10°C/75% RH, indicating that the low RH (30%) storage environment compensated for the higher temperature (20°C). This indicates that short-term storage at higher temperatures could be utilized to reduce electricity costs, if humidity is low and stable. The 5°C/90% RH conditions most effectively preserved seed quality after 18 months, indicating that the low temperature compensated for the high RH. However, a dehumidifier and moisture-safe containers would further preserve seed quality. None of the three storage conditions prevented deterioration of seeds with low in initial quality, although the 5°C/90% RH slightly slowed their deterioration. Storage of seed with low initial quality is not recommended, but if necessary, should not exceed 4 months. If longer-term storage of low-quality seeds is required, low temperature and RH is recommended, as well as additional precautions such as a dehumidifier and moisture-safe containers. The AAT was the most effective vigor test to differentiate between seed quality of different storage periods. The CT and EC tests did not differentiate between the qualities of seed lots, therefore they are not recommended. Two of the five varieties, V4 and V5, had low vigor results (14% and 18%) after 18 months across all storage conditions, but their germination results remained high (93% and 96%). This indicates that high viability does not necessarily indicate high overall quality, and that vigor tests should be considered for a full picture of seed quality.

Effects of Different Storage Conditions on Seed Quality of Hemp (*Cannabis sativa* L.)

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Grace Fuchs, Author

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Introduction

Hemp (*Cannabis sativa* L.) is an herbaceous annual crop in the Cannabaceae family that is valued worldwide for its versatile applications. It is one of the oldest cultivated plants, with evidence of farming as far back as 2000 BC (Crocq 2020). Today, it is produced by over 30 countries for food, fiber, medicine, and industrial products (Johnson 2018). Despite sharing the same genus and species, *Cannabis sativa*, hemp and marijuana are genetically distinct. They differ in their applications, biological effects, and chemical composition (FDA 2020). Hemp contains less than 0.3% of the psychoactive compound tetrahydrocannabinol (THC), a distinction that only recently became recognized by the US government. In 1937, U.S. Congress passed legislation prohibiting the production, research, and sales of hemp (Tancig, 2021). Decades later, the 2018 Farm Bill removed hemp from the definition of marijuana under the Controlled Substances Act. Opportunities for research and production expanded in the following years. By 2021, over 54,000 acres of hemp were planted in the US with a total harvested value of $812 million (USDA, 2022).

Botanically, the seed is a dry fruit known as an achene, which possesses varying levels of dormancy and oil content depending on the characteristics of the genotype (Elias et al., 2020). Hemp seed is a valuable commodity in the worldwide market, being used for a variety of products, including nutritional supplements, animal feed, pharmaceuticals, cosmetics, shampoo, and more (Johnson, 2018).

Hemp seed end-uses fall into one of three categories: oil, food, and cannabidiol (CBD). As food, hemp seeds contain approximately 30% protein, 25% starch, and 30% oil. Fibers are primarily used to make high quality papers, ropes, nets, oil absorbent materials, alternative construction concrete and other industrial products. Finally, hemp is used for medicinal purposes, as it produces over 100 known cannabinoids, most notably CBD. This study focused on CBD hemp, which is grown for its medicinal flowers. These flowers contain potent, extractable cannabinoids with topical and internal applications. Following the 2018 Farm Bill, the first CBD-derived medication, Epidoliex, was approved by the FDA for treatment of rare seizure disorders (FDA, 2018). There is limited, yet promising, data on the efficacy of ingested CBD as treatment for anxiety and depression (Rapin et al., 2021; Wieckiewicz et al., 2022; Wright et al., 2020). Additionally, topical CBD products have shown to reduce symptoms of arthritis (Frane et al., 2022; Hammell, 2016).

Hemp seeds are marketed in two ways. First are direct sales, where seed producers rely on the immediate availability of a robust market in which they can efficiently sell their seeds. If this market is not available, the seed will inevitably need to be stored. The second is carry-over seed, where seed is stored for a period and sold later. The duration of storage depends on the state of the market and consumer demand, which could range from a few months to a few years. Therefore, it is crucial to understand the processes that lead to seed deterioration and the conditions in which seed should be stored that will preserve its quality.

Seed deterioration (SD) presents agriculturalists worldwide with a significant obstacle. Almost 25% of the annual value of harvested crops is lost due to SD. This loss amounts to billions of U.S. dollars each year (McDonald & Nelson, 1986). In China, 15 billion kg of viable rice seed is lost annually due to SD in storage (Xu et al. 2015). Understanding and reducing SD is imperative to avoid such economic losses. Much is still unknown about the mechanisms that cause SD due to a multitude of interdependent factors (Benech-Arnold & Sanchez, 2004). Not only is SD inevitable and irreversible, but it also varies among crops, varieties, and even within seed lots of the same variety (Delouche & Baskin, 1973). Additionally, low quality seeds deteriorate at a faster rate than high quality seeds (Elias et al., 2006, Justice and Bass, 1978).

Environmental stress conditions during seed maturation, such as extreme temperatures, nutrient deficiency, and drought, reduce initial quality and increase seed susceptibility to deterioration (Elias et al., 2006). Once the seed reaches physiological maturity, it is essentially being stored on the plant before it is harvested and stored by the farmer. Although the pre-harvest storage conditions (precipitation, temperature, and humidity) are out of the farmer's control, certain harvesting practices can be implemented to optimize seed quality. Timing of harvest is crucial and should occur when most of the seed lot has reached ideal harvest maturity for the individual crop. The indeterminate growth pattern of the hemp inflorescences causes varying degrees of ripeness based on the location of the seed. Elias et al. (2020) determined that the top 2/3 of the inflorescence ripen about 8 days earlier than the bottom 1/3. If the entire inflorescence is harvested at once, this will decrease the overall viability of the seed lot, thus increasing the rate of deterioration over time. Additionally, seeds that are mechanically damaged during harvest or cleaning are more prone to rapid deterioration than undamaged seeds (Copeland and McDonald, 2001).

Harrington (1973) proposed that each 1°C reduction in temperature or each 1% reduction

in relative humidity doubles the life of the seed. Higher moisture levels (≥ 14%) increase seed respiration and susceptibility to fungal invasion (Harrington 1973). Due to the hygroscopic nature of seeds, their moisture content is in a constant state of flux (Stanwood and McDonald, 1989). Their hygroscopicity allows them to absorb and release water based on the humidity of the surrounding environment. If seed is stored in a fully sealed container, moisture content will only be affected by the air in the surrounding container. If seed is stored in a container that is not fully moisture proof, seeds will equilibrate with the conditions in the warehouse or cooler.

Different types of seeds have varying levels of storability. Oilseeds, such as hemp, are prone to more rapid deterioration than starchy seeds, such as corn (Elias and Copeland, 1994). This is due to lipid peroxidation, a process which creates free radicals that damage the seed membrane (Benech-Arnold et al., 2004). Autoxidation occurs at seed moisture contents below 6%, whereas peroxidation via the lipoxygenase enzyme occurs above 14% moisture content (Copeland and McDonald, 2001). Both methods of lipid peroxidation cause irreversible damage to the seed, beginning with the seed membrane. Seed membrane degradation initiates the deterioration process, which eventually leads to the loss of germinability of the seed (Delouche and Baskin, 1973). Hemp seeds have been shown to retain initial viability after 15 years, when stored at 5.7% moisture at -10 and 10°C in sealed containers (Justice and Bass 1978).

A lack of available research about ideal storage conditions for hemp has caused uncertainty among hemp seed growers (Sabry Elias, personal communication, October 1 2020). It is widely understood that storage temperature and relative humidity are the two most important factors that influence seed quality in storage (Copeland & McDonald, 2001; Harrington, 1973). However, the effects of these two storage factors on the seed quality of different hemp genotypes over time are not understood or documented - and both are urgently needed.

Both viability and vigor are important parameters used to evaluate seed quality. Vigor provides a more complete picture of a seed's quality than only viability, because it examines the seed's ability to: 1) germinate rapidly and uniformly, 2) grow into a vigorous seedling with essential structures, and 3) produce these results under a wide range of environmental conditions (AOSA Seed Vigor Handbook, 2009). Germination, an expression of viability, is the seed's ability to germinate under optimal conditions. Environmental conditions are not always optimal, which can lead to unrealistic expectations among growers who base farming decisions solely on viability test results from the standard germination or tetrazolium tests. There currently are no

written protocols in the AOSA Seed Vigor Testing Handbook for the electric conductivity (EC), the accelerated aging test (AAT), or the cold test (CT) for hemp. To our knowledge, no published tests are available which examine the magnitude of deterioration in seed vigor over time of different hemp varieties under different storage conditions.

Tests used to evaluate seed viability of hemp

Currently, there are two tests used to evaluate seed viability of hemp, the tetrazolium test (AOSA, 2010), and the standard germination test (AOSA, 2022).

Tetrazolium Test (TZ): This is a biochemical test that measures the activity level of dehydrogenase enzymes as an indication of seed viability. Viable tissues stain red, and dead tissues remain unstained. The intensity and the pattern of staining are important factors in interpreting the results. Among the advantages of the TZ test are: 1) it is rapid, and can be completed in 48 hours, and 2) it can determine viability despite the presence of seed dormancy, which makes the test valuable for many crops, including hemp, which have different levels of dormancy after harvest. This test cannot determine the physiological development of the embryo, which differentiates it from the standard germination test.

Standard Germination Test (SGT): This is a physiological test and is the most commonly used test for determining seed viability. Seeds are planted under optimal conditions of temperature, moisture, light, and air. Protocols for germinating various crops are listed in the AOSA Seed Testing Rules (2022) and in the International Rules for Seed Testing (ISTA, 2022). After the designated test period (typically 7 days), germination of seedlings with essential structures is recorded.

Tests used to evaluate the seed vigor of hemp

The following tests will be evaluated in this research to determine their effectiveness in measuring seed vigor of hemp:

Accelerated Aging Test (AAT): This is a stress test where seeds are exposed to stressful conditions of high temperature and relative humidity (RH) for a period of time, which causes rapid deterioration. Afterwards, seeds are germinated. Vigorous seeds germinate more rapidly and produce healthier seedlings than poor quality seeds after the stress exposure period (AOSA,

2009). This test mimics stressful storage or environmental conditions, but also produces changes in the seed that are similar to natural aging (Likhatchev et al., 1984).

Speed of Germination Index (SGI): The SGI can be performed in conjunction with the SGT to measure the speed at which the seeds show first signs of germination. 48 h after planting the SGT, germination rates are counted daily. Faster germination indicates higher quality and is an indicator of seed vigor (AOSA, 2009).

Cold Test (CT): The CT measures the seed tolerance to cold stress. Seeds are exposed to cold conditions, usually 5-10°C for 5-7 days before planting in optimal conditions (AOSA, 2009). This test can be conducted with or without soil. It is useful when crops will be planted in cold soil in late fall or early spring. Some varieties have more tolerance to cold temperatures than others. The ability of seeds to germinate and grow in cold, wet soil is affected by genotype, maturity, aging, mechanical damage, as well as environmental conditions (Fiala, 1987).

Field Emergence (FE): The purpose of using FE in our study was to determine whether the results from the vigor laboratory tests correlated with field performance as seeds were exposed to natural environmental conditions. Typically, field emergence correlates with germination test results when environmental conditions are favorable (Hampton, 1981). Hall (1987) reported significant correlation between some vigor tests and field performance of meadow bromegrass.

Electrical Conductivity Test (EC): This vigor test measures the leakage of solutes, such as amino acids and inorganic ions, through cell membranes. As seeds deteriorate, the membrane structure of low-quality seeds becomes more permeable and the leakage through membranes increases. Seed samples with low electrolyte leakage are considered of high vigor. Therefore, the higher the electrical conductivity reading, the lower the quality of the seeds.

The objectives of this study were to: 1) Determine the effects of three storage conditions on the deterioration rate of five CBD hemp seed varieties stored for 18 months; and 2) Determine and recommend protocols for the most effective vigor test(s) to measure the magnitude of deterioration in hemp seeds stored under different conditions for 18 months.

Materials and Methods

Seed Materials

Five hemp varieties (V1 - V5) grown in Oregon were used in the study. Seed materials were donated by two Willamette Valley hemp seed producers. All varieties were the same species of CBD hemp (*Cannabis sativa* L.) V1 and V2 were harvested July, 2020, and V3 - V5 were harvested in June, 2010. After harvest, the seeds were stored in the 5°C/90% RH cooler at the Oregon State University Seed Laboratory until the project began in October, 2020.

Methods

Seed Moisture Content (SMC) Determination

Moisture content is a crucial factor that influences the seed's ability to maintain quality in storage. SMC was measured by placing two replicates of 1.25 g of seed in glass containers. The container and the seeds were weighed to determine the "wet" (fresh) weight. Then, the containers were placed in a drying oven (Sheldon Manufacturing SMO5) for 1 h at 130°C (AOSA seed Moisture Determination Handbook, 2018). The containers were weighed after drying to determine the "dry" weight. The moisture content was calculated using the following formula:

% seed moisture content = [(fresh seed – dry seed)/fresh seed] x 100

Tests used to measure seed viability and vigor of stored seeds

The extent and progress of deterioration were evaluated for each sample using the following laboratory tests:

Tetrazolium (TZ) Test

Seeds were imbibed in water for 24 h to hydrate the seed tissues and to provide ease in cutting the seeds, then were cut longitudinally with a blade. The larger half of each seed was soaked in 0.5% 2,3, 5-triphenyl tetrazolium chloride solution for 24 h at 30°C. This solution is colorless, but stains viable tissue red when reduced to formazan by the hydrogen ions within the seed. Dehydrogenase enzymes catalyze this reaction and are used as an index of viability and respiration rate (Copeland and McDonald, 2001). After 24 h, seeds were classified as either

viable or nonviable based on the staining pattern. To be classified as viable, the cotyledons and embryonic axis must be uniformly stained red, without any spots of unstained tissue.

Standard Germination Test (SGT)

Two replications of 100 seeds of each treatment were placed on germination paper that was pre-moistened with water. The paper was rolled, placed in a plastic bag to prevent evaporation, and then placed in the 20-30°C germination chamber. This room simulates day and night conditions, maintaining a temperature of 20°C for 16 h in the dark, followed by 30°C for 8 h in the light. After seven days, seedlings were evaluated per AOSA guidelines. Based on the development of essential root and shoot structures (primary root, hypocotyl, epicotyl, and cotyledons), seedlings were categorized as either normal, abnormal, or dead (AOSA, 2021).

Speed of Germination Index (SGI)

SGI is performed in conjunction with the SGT to measure the speed at which the seeds show first signs of germination. Faster germination speeds indicate higher quality and vigor (AOSA, 2009). 48 h after initial planting of the SGT, seeds were removed from the germination chamber and evaluated. Germination was confirmed when the following conditions were met: 1) the radicle reached 1.5 cm in length, and 2) the radicle showed normal development, with a tapered, pointed tip and visible root hairs. Seeds were then returned to the germination chamber and evaluated each day until the final count on day 7. SGI was calculated using the following formula, where t = days in storage, and N = number of newly germinated seeds.

$$S = \frac{N_1}{T_1} + \frac{N_2}{T_2} + \frac{N_3}{T_3} + \ldots + \frac{N_k}{T_k}$$

Accelerated Aging test (AAT)

This vigor test exposes seeds to stressful conditions, at 43°C and 100% relative humidity (RH) for 48 h before planting, to differentiate between the seeds with high and low vigor. Seeds were placed on screen trays inside plastic boxes, then 50 ml of water was added to each box. The lid was sealed shut with a tape to prevent condensation and create an environment of 100% relative humidity. The boxes were placed in an oven to dry (SMO5) at 43°C for 48 h. The seeds were removed from the oven and allowed to dry for 24 h. Seeds were then planted using the same protocol as the standard germination test. After seven days, normal seedlings were counted and recorded.

Electric Conductivity Test (EC)

This is a biochemical test that measures leakage of solutes through the seed membrane. As seeds deteriorate, their membrane becomes more permeable and the leakage through this membrane can be detected in water. Four replications of 50 seeds were weighed for each treatment. A *Thermo Scientific* Conductivity Meter was used to obtain a baseline reading of 50 ml of water prior to submersion of the seeds. Initial conductivity was recorded as (μs/cm). Each replication was placed in a beaker with 50 ml of distilled water for 24 h. The conductivity of the water was recorded again, and the difference between the two conductivity readings was calculated using the following formula:

Leakage = [(Conductivity reading - baseline) / seed wt.

Field emergence test

The purpose of this test was to determine whether the results from the various lab tests correlated with field performance. Seeds were planted in a raised bed using sandy loam soi provided by OSU. A completely randomized block design was used for planting. The seedbed was kept evenly moist during months with low rainfall. Normal seedlings were counted one, two, and three weeks after planting.

Table 1. Plot layout of the field emergence trial, created by MSTAT statistical package.

Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1	Rep 1
Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115
A= 1	A= 1	A= 1	A= 2	A= 2	A= 2	A= 3	A= 3	A= 3	A= 4	A= 4	A= 4	A= 5	A= 5	A= 5
B= 1	B= 2	B= 3	B= 1	B= 2	B= 3	B= 1	B= 2	B= 3	B= 1	B= 2	B= 3	B= 1	B= 2	B= 3

Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2	Rep 2
Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot	Plot
201	202	203	204	205	206	207	208	209	210	211	212	213	214	215
A= 4	A= 1	A= 5	A= 2	A= 1	A= 2	A= 5	A= 2	A= 1	A= 3	A= 4	A= 5	A= 3	A= 4	A= 3
B= 3	B= 3	B= 2	B= 1	B= 1	B= 3	B= 1	B= 2	B= 2	B= 2	B= 2	B= 3	B= 3	B= 1	B= 1

Factor A – Varieties: V1, V2, V3, V4, and V5
Factor B – Storage conditions: B1, 5°C /90% RH; B2, 10°C /75% RH; and B3, 20°C /30 RH%.

Cold Test (CT)

This vigor test exposes seeds to cold stress temperature (5°C for 5 days). Two replications of 100 seeds each were planted in sterile soil (growing mix) within a plastic box, then were moistened with water. The boxes were placed in the 5°C cooler for 5 days, then transferred to the 20°C/30°C germination chamber for 7 days. Normal, abnormal, and non-germinated seeds were evaluated following the AOSA guidelines.

Results and Discussion

The ANOVA results (Table 2) showed that variety, storage conditions, storage periods, and the interactions among them (V x C), (V x P), (C x P), and (V x C x P) affected the quality of the stored seeds with different rates. Following are the specific effect of each variable as measured by the various viability and vigor tests.

Tetrazolium Test (TZ)

The ANOVA results showed that variety, storage conditions, and storage periods, had significant effect on the quality of the stored seeds as measured by the TZ test at $p \leq 0.001$ and 0.0001. The interactions among variables (V x C), (V x P), and (V x C x P) were also significant, indicating that varieties responded differently to storage periods and conditions (Table 2).

Varieties, storage condition and duration of storage affected the percentage of viable seeds within seed samples. The two-way interaction between storage condition and storage period was significant. However, TZ means were different among varieties only at 10°C in period three (8 months). At 5°C and 20°C, there was no significant difference between means across varieties. These results signify that the 10°C storage environment was the least effective in preserving viability as measured by the TZ test, but short-term storage for fewer than 8 months at 10°C would be sufficient if this was the only storage environment available to the seed grower.

Table 2. Analysis of Variance (ANOVA) for the effects of varieties, storage conditions and storage period on seed quality of five hemp varieties as measured by accelerated aging test (AAT), cold test (CT), seed moisture content (SMC), tetrazolium test (TZ), field emergence (FE), standard germination test (SGT), speed of germination index (SGI), and electric conductivity (EC) over an 18-month period.

Source of Variation	df	TZ	SGT	AAT	SGI	CT	FE	EC	Moisture
				Mean Square (MS)					
Variety (V)	4	301.3 ***	155.1 ***	31643.4 ***	476.3 ***	168.3 ***	567.9 ***	31936.9 ***	10.4 ***
Storage Condition (C) ‡	2	8.9 **	64.1 ***	118.5 ***	47.4 ***	10.6 ***	270.9 ns	59.5 ns	75.2 ***
Storage Period (P) §	5	57.3 ***	54.5 ***	1929.4 ***	371.8 ***	213.3 ***	16472.2 ***	3128 ***	53.5 ***
Interactions									
V x C		6.7 ***	26.2 ***	28.57 *	6.2 **	13.1 ***	96.5 ns	54.3 ns	0.5 ***
V x P		9.0 ***	25.1 ***	303.6 ***	70.7 ***	22.4 ***	292.8 ***	76.8 *	0.3 ***
C x P		4.7 **	17.4 ***	122. ***	13.9 ***	8.3 ***	146.8 ***	170.7 ***	4.2 ***
V x C x P		5.2 ***	10 ***	35.4 ***	9.4 ***	4 ***	93.9 ns	72.4 *	0.1 ns
Error		1.6	3.9	12	2.1	1.67	107	42.16	0.1

*, **, *** significant at 0.05, 0.01, and 0.001 probability levels, respectively.
† NS, not-significant.
‡ 5°C, 90% RH; 10°C, 75% RH; and 20°C, 30% RH.
§ 6 periods, 0, 4, 8, 12, 16, and 18 months.

Four of the five varieties (V1, V2, V3, and V5) did not show significant reductions in viability by TZ after 18 months. Across all temperatures, they retained viability of 98%, 96%, 97% and 94%, respectively. Only V4 dropped below 90%, finishing with 86% viability. Although V4 showed a significant difference in viability by TZ between 0 and 18 months, the biological significance of this is minimal, since a seed lot with 86% viability is still considered to be marketable.

Standard Germination

The ANOVA showed that germination rates differed significantly ($p \leq 0.001$) among varieties, storage conditions, duration of storage, as well as the interactions among these factors

(Table 2), indicating that the five varieties responded differently to the three storage conditions and the six storage periods. Germination was not consistent among varieties, under different temperatures, over the 18-month storage period.

Only at 10°C did germination show a significant decrease, from 97% to 91%. However, even viability of 91% is still acceptable in the marketplace. In some cases, the statistical difference may be significant, but it is high enough to meet the minimum quality standards of certification programs (e.g., 85%) and seed buyers. Across varieties, the average germination rates after 18 months at 5°C, 10°C, and 20°C were 97%, 91%, and 94%, respectively. This indicates that varieties stored at 10°C conditions resulted in the greatest deterioration and 5°C conditions were the most effective at maintaining seed quality. However, the magnitude of seed viability deterioration was not severe for the three storage conditions, as seed viability was above 90%, even after 18 months.

Accelerated Aging Test

The ANOVA results showed that variety, storage conditions, storage periods, and the interactions among them (V x C), (V x P), and (V x C x P) had significant effect on the germination by the AAT at $p \leq 0.01$ and 0.001 (Table 2). This indicates that varieties responded differently to storage conditions and storage periods. The five varieties produced different percentages of normal seedlings after stress exposure depending on the storage temperature and storage period. All five varieties showed significantly reduced AAT germination (vigor) after 4 months in the three storage conditions, despite maintaining high viability from the SGT and TZ tests.

At 5°C, varieties maintained the highest quality after 18 months, and showed the greatest deterioration at 10°C. Similarly, the CT, TZ, SGT, SGI, and EC tests also measured the greatest deterioration at 10°C. These results suggest that the lower RH of 75%, compared to the 5°C/90% RH did not compensate for the higher temperature (10°C), and that this combination of temperature and RH (10°C /75%RH) level is not optimal for long term hemp seed storage.

V4 and V5 showed the lowest initial germination by AAT among the five varieties at 55% and 65%, respectively. After 18 months, the percentage of normal seedlings of the seeds stored at 10°C was reduced to 7% (V4) and 9% (V5), whereas the seeds stored at 5°C were reduced to 27% and 28%, respectively.

Varieties V1, V2, and V3 had initial high vigor, with AAT results of 90%, 94%, and 93%, respectively. After 18 months of storage, they showed minimal deterioration with germination rates of 81%, 81%, and 85%, respectively. These results confirm that seeds with low initial quality deteriorate at a greater rate than seeds with high initial quality (Figure 1). Furthermore, the study showed that the AAT is an effective test for distinguishing between samples with different seed vigor (qualities). Additionally, these results highlight the effectiveness of the 5°C storage conditions in preserving initial seed vigor, and emphasize that the 10°C conditions were not optimal for storing low quality seeds.

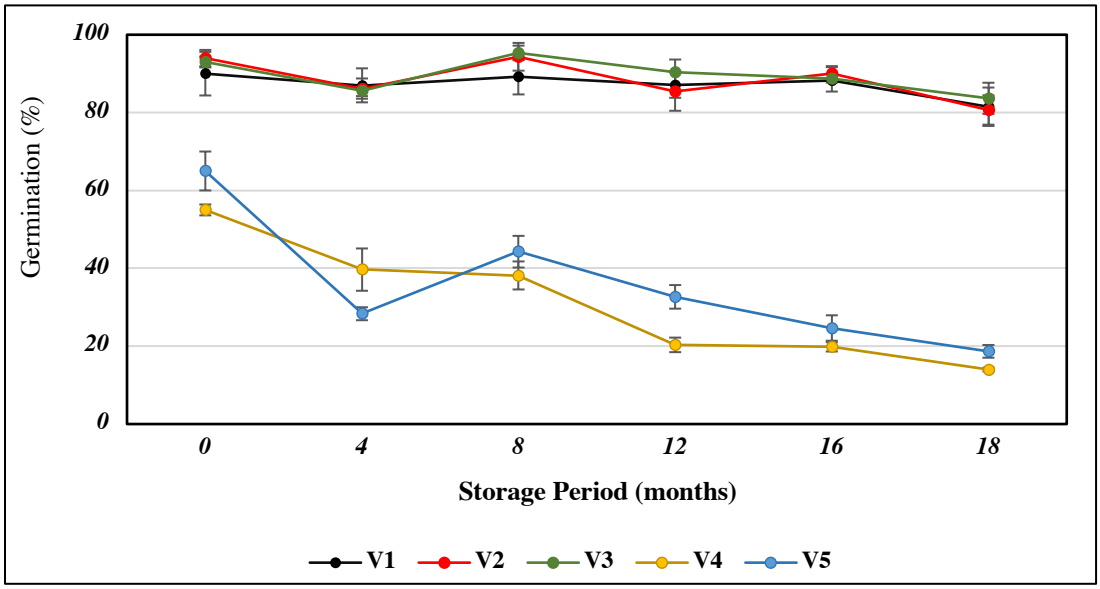

Figure 1. Accelerated Aging test results of five hemp varieties over 18 month-storage period. V4 and V5 began with the lowest initial vigor and showed greater deterioration than V1, V2, and V3, which began with higher initial vigor. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

Speed of Germination Index

The ANOVA showed that varieties, storage environment, and storage periods had significant effect on the speed of germination index (SGI) at $p \leq 0.001$ as measured by the SGI test. All interactions (V x C), (V x P), and (V x C x P) were also highly significant at $p \leq 0.001$ and $p \leq 0.00$, indicating that varieties responded differently to the storage conditions used in the study throughout the 18-month storage period. For example, after 18 months, V1, V2, and V3 had the

lowest germination speeds at 20°C, whereas V4 and V5 had the lowest germination speeds at 10°C.

The initial SGI of the five varieties was similar. However, the SGI of V4 and V5 gradually decreased throughout the 18-month storage period compared to the other three varieties, which maintained relatively high germination speeds (Figure 2). This indicates that the SGI test sufficiently differentiated between varieties with different qualities. However, it did not detect differences in germination speed among varieties with high quality, i.e., V1 - V3.

All varieties across all temperatures had reduced germination speed after 12 months of storage. Germination speed was maintained the best at 5°C, which didn't show a significant decrease until 16 months. This indicates that the storage conditions at 5°C/90% RH preserved germination speed longer than 10°C and 20°C. The 5°C environment had the highest humidity of the three storage conditions, but these results indicate that the low temperature compensated for the higher humidity.

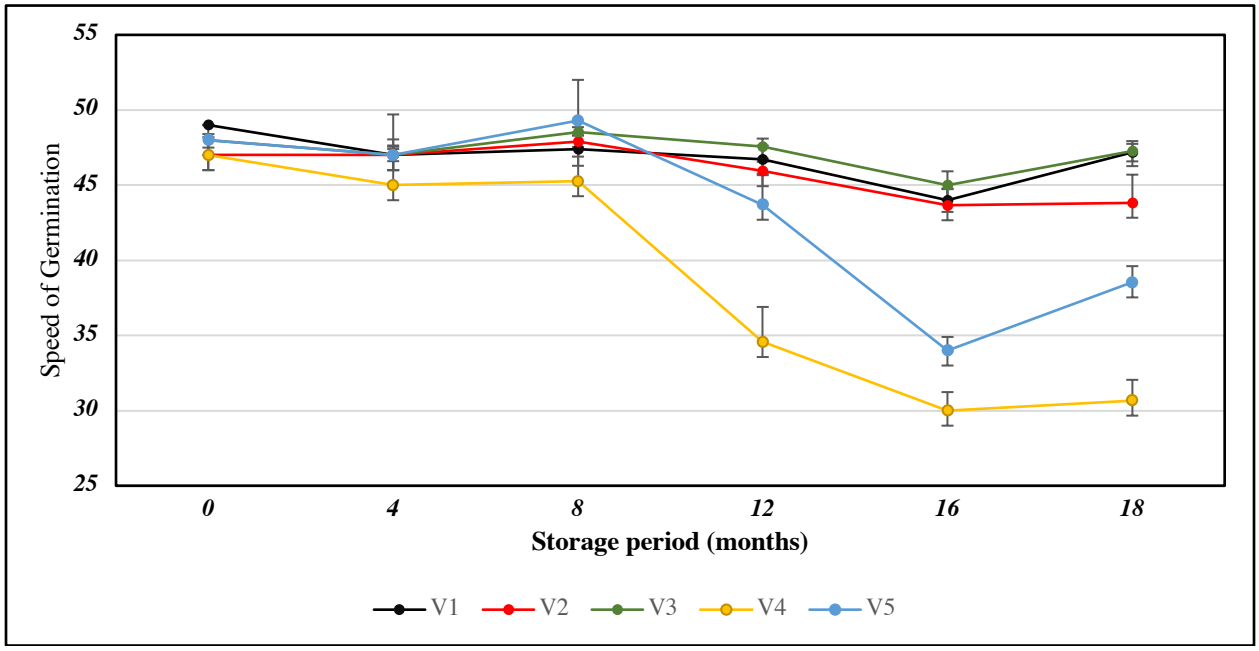

Figure 2. Speed of germination index (SGI) of five hemp varieties across three storage conditions after 18 months in storage. Varieties V4 and V5 had slower germination rate than V1, V2, and V3. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

Cold Test (CT)

The ANOVA showed that vigor as measured by the CT differed significantly ($p \leq 0.0001$) between varieties, storage conditions, and storage periods. Additionally, all interactions among factors were significant at $p < 0.001$, indicating that varieties had responded differently to the storage conditions and periods (Table 2).

The LSD mean separation test showed significant differences among germination means beginning at the 12-month storage period across the three storage conditions. However, this statistical significance does not necessarily imply biological significance because the drop in germination was not sharp. Like the TZ test and SGT, which showed significant differences among factors and interactions, the average viability and germination remained high by the industry and certification program standards, which typically set minimum germination standards at 85%. After 18 months, the CT results showed that the germination of all varieties across all storage conditions remained high, averaging 91%. Varieties V1, V2, V3, V4, and V5 had average germination rates of 95%, 91%, 96%, 86%, and 89%, respectively.

The lowest germination after the cold stress exposure occurred with variety V4 at 10°C, and V5 at 20°C, which dropped to 83% and 88%, respectively. The initial quality of these two varieties were the lowest among the five varieties used in the study, as measured by the AAT (55% and 65% germination, respectively). Although the CT was able to detect some reductions in vigor among varieties stored under different temperatures and periods of time, the differences were not as clear as the AAT in showing the deterioration in vigor across the five varieties. Therefore, the cold test is not recommended for measuring differences in quality of different hemp seed lots using the test parameters listed in this study.

The CT likely did not create enough stress on the seeds to differentiate between the different qualities. The conditions used to stress the seeds (5°C for 5d) were similar to those typically recommended for cold stratification, also known as prechilling, (5°C-10°C), which is commonly used to break seed dormancy in many species.

Future research could modify the parameters of this test by chilling the seeds at a lower temperature, such as 3°C. However, this may reduce the practicality of this test, as this temperature is less common in most seed testing laboratories, especially smaller labs. Another possible modification for this test could be to increase the time that the seeds are exposed to the 5°C cold temperature. This could add enough stress to differentiate between the vigor of seed lots. However, this test already takes twelve days (5 days for chilling and 7 days for

germinating), so an extension could result in backlog and delay in submitting test results. This could also be problematic for seed labs that have limited space in coolers and germination chambers. In addition, customers would have to wait longer to receive the results.

An additional barrier to the efficacy of this test was starting with high-viability seeds (initial TZ and germination across varieties averaged 98% and 97%, respectively). Medium or low-viability seeds could be better candidates for this test because they could be affected faster, or to a greater extent, than the high-quality seeds within the same parameters. Therefore, future research could include varieties or seed lots with a wider range of initial qualities.

Field Emergence (FE)

The ANOVA showed that FE was significantly affected by differences among varieties and storage period, but not by storage condition (Table 2). The two-way interactions between variety and storage period as well as variety and temperature were significant at $p \leq 0.001$, indicating that field emergence of the five varieties did not follow a similar pattern in response to the different temperatures or the storage periods used in the study.

Field emergence followed the cyclical climate patterns, with colder months producing lower germination rates than warmer months. The lowest germination occurred during the two coldest months, Feb 2021 and Feb 2022, which had average monthly temperatures of 5.5°C and 5°C (42°F and 41°F), respectively, with field emergence of 50% and 54% (Figure 3). Field emergence rates were the highest in Oct 2020 and June 2022, with average monthly temperatures of 12.2°C and 19.4°C (54°F and 67°F), respectively, and average emergence rates of 81% and 82% (Figure 3). The data from October 2021 is unavailable. These results confirm previous findings that planting hemp in temperatures below the optimal range (13.5°C - 18.5°C) will result in poor emergence (Cosentino et al. 2012; Roseburg et al. 2019).

Cosentino et al (2012) observed that the fastest field emergence for two industrial hemp varieties ‘Fibranova’ and ‘Tiborszallasi’ occurred with outside air temperatures between 13.5°C and 18.5°C (56.3°F and 65.3°F). These varieties showed reduced field emergence speed with temperatures under 10°C (50°F) and above 24°C (75.2°F). During the FE test, the only periods where the temperature was within this range were Oct. 2020 and June 2021, which were also the periods with the highest germination rates.

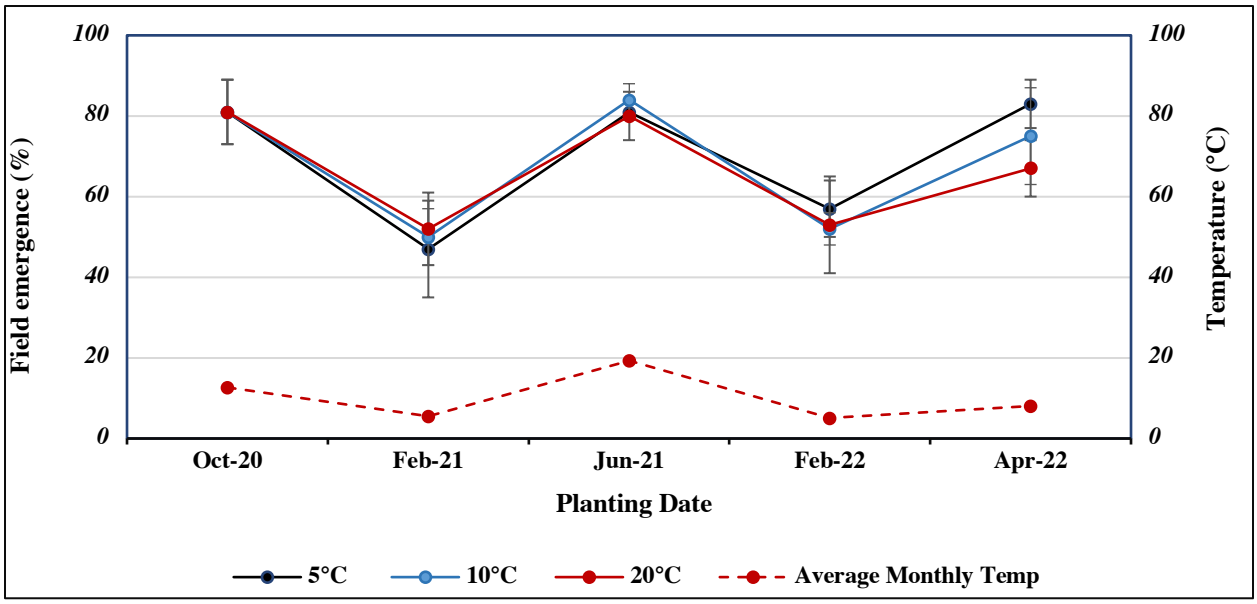

Figure 3. Field emergence (FE) percentage across five hemp varieties at 5°C/90% RH, 10°C/75% RH, and 20°C/30% RH; and monthly temperature (°C) in Corvallis, Oregon during field emergence period. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

Electric Conductivity (EC)

Variety (V) and storage period (P) significantly affected seed vigor at $p \leq 0.001$, as measured by the EC test. The interaction between the two factors was also significant ($p \leq 0.05$) indicating that varieties did not follow a similar pattern in response to the storage period. Storage condition (C) did not affect EC results significantly. The interaction between variety and storage condition (V x C) was not significant (Table 2), indicating that seeds stored at 5°C, 10°C, and 20°C had similar conductivity readings across all varieties.

The EC results did not show logical differences among varieties as affected by storage condition and storage period. For example, lower quality seeds were expected to have higher conductivity readings due to a higher concentration of solute leakage, such as sugars and amino acids, into the water. However, we found the opposite: lower quality seed lots had lower conductivity readings, which indicates lower leakage rates. Additionally, the decreasing quality shown by the AAT was not mirrored in the EC test with increasing leakage and higher conductivity. Rather, EC stayed relatively stable over 18 months and showed no significant difference between means across varieties and temperatures.

The unexpected results of the EC test possibly occurred because the seed coats of the newly harvested seeds remained intact, preventing solute leakage. As the seed ages, phospholipids cause a true breakdown (autoxidation) of lipids in the seed coat, allowing solutes to pass through the membrane into the water. If the seed membranes of the varieties used in this study prevented solute leakage, then this test would not be able to detect deterioration within these seed lots. Therefore, we do not recommend this test to differentiate between hemp seed lots with different qualities.

Seed Moisture Content

The ANOVA showed that varieties, storage conditions, and storage period, as well as the two-way interactions among them had significant effects on the seed moisture content (SMC) at $p \leq 0.001$ (Table 2). This indicates that the SMC of each variety responded differently to storage periods and conditions. Seeds stored at 5°C and 10°C showed similar patterns in moisture throughout the 18-month storage period, but all varieties ended with the highest moisture content at 10°C/75% RH and the lowest at 20°C/30% RH (Figure 4).

The results showed that seeds stored in all conditions had significant reduction in moisture content at the 8-month storage period. At 22°C, a significant decrease in moisture content occurred after 4 months of storage compared to 5°C and 10°C (Figure 4). This shows that a low relative humidity environment reduces seed moisture content rapidly.

The final SMC of all varieties after 18 months at 5°C, 10°C, and 20°C were 5.6, 6.3, and 3.2, respectively (Figure 4.) This indicates that the containers that were used to store the seeds (polypropylene 'Ziploc' bags kept inside of a lidded, plastic 'Tupperware' box) permitted an interaction between the seeds and the humidity of the surrounding environment. Due to their hygroscopic nature, seeds will continuously gain and lose moisture until they reach an equilibrium with the environment (Copeland and McDonald, 2001). This was reflected by the fluctuation and eventual stability of the moisture content across varieties (Figure 4). The environment with the lowest relative humidity, 20°C, produced seeds with the lowest moisture content. If the containers were moisture-proof and provided a true barrier between the seeds and their surrounding environment, the SMC would have remained stable throughout the storage

period. These results reveal the importance of choosing moisture-proof containers for seed storage.

The 10°C conditions resulted in seeds with higher moisture content than the 5°C conditions, despite having 15% lower RH. Additionally, seeds stored at 10°C had lower vigor and viability than seeds stored at 5°C or 20°C, as indicated by the AAT, CT, SGT, TZ, SGI, and EC tests. This could signify that the 15% decrease in RH did not compensate for the 5°C increase in temperature. The lower seed quality and higher moisture content could be correlated, although it is not certain. It is possible that the slightly higher moisture levels resulted in accelerated deterioration of the seed membrane and a reduction in quality (viability and vigor). Additionally, higher moisture levels increase the activity of storage microorganisms such as *Aspergillus* and *Penicillium*. These storage fungi are saprophytes that feed on dead tissues. They produce toxic metabolites and eventually invade the seed embryo, resulting in deterioration (Copeland and McDonald, 2001). This study confirms previous findings that higher MC produces lower quality seeds.

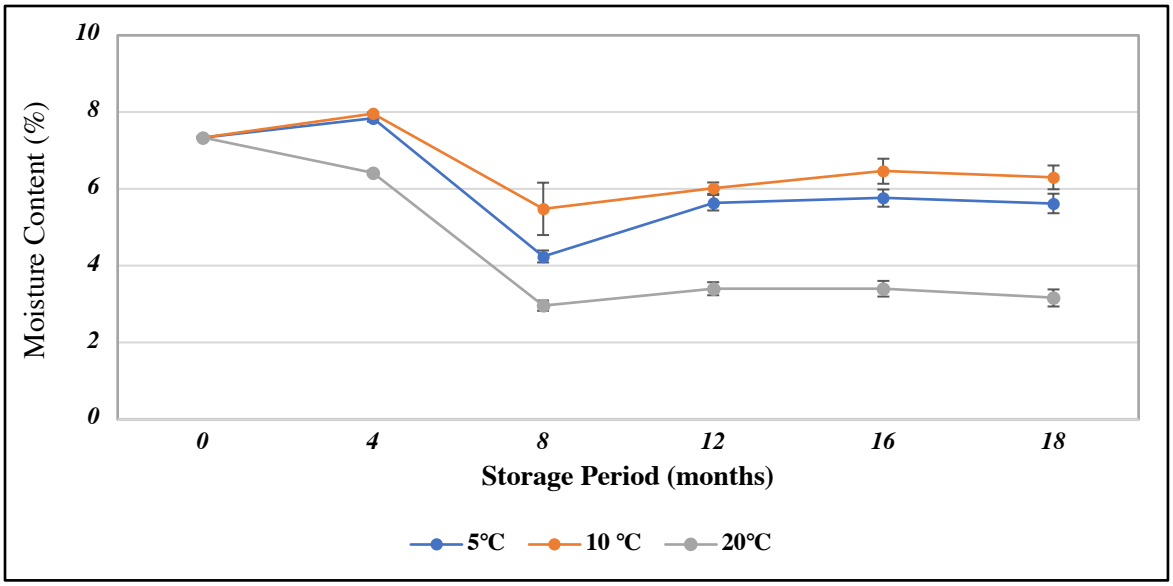

Figure 4. Average seed moisture content of five hemp seed varieties stored for 18 months in three different storage conditions: 5°C/90% RH, 10°C/75% RH, and 22°C/30% RH. rue 3

Viability and Vigor

The results of this study indicate that high viability does not necessarily guarantee high vigor (Figure 5). At initial testing, before storing the seeds, V4 and V5 had viabilities by TZ of 96% and 98%, respectively. However, their initial percentage of normal seedlings was determined by the AAT test to be 55% and 65%, respectively. The lowest vigor results after 18 months occurred with V4 at 10°C, with only 7% germination after the stress exposure of the AAT. However, the same variety at 10°C had a standard germination rate of 90% at the same period.

After 18 months in storage, viability as measured by TZ and SGT averaged 93% and 94%, respectively, across varieties and storage conditions. One would be inclined to conclude, based solely on these viability results, that these hemp seeds retained high quality over time. However, the viability results do not represent the full picture of these seeds' quality. The AAT test showed significantly reduced vigor across all varieties and temperatures, beginning at 4 months. After 18 months, vigor averaged 56% over all varieties. These results highlight the importance of differentiating between viability and vigor when discussing seed quality. It is probable that field emergence will correlate more with vigor than with viability since the standard germination tests are conducted under optimum conditions, which are unlikely to occur under field conditions.

Commercially available seeds only require labelling of germination percentage (viability). However, germination percentages do not account for seed vigor and therefore are not a comprehensive indicator of seed quality. If seed growers establish management practices based upon germination percentage alone, they risk encountering indicators of low vigor: slow germinating seeds, a high percentage of abnormal seedlings, and poor tolerance to environmental stress.

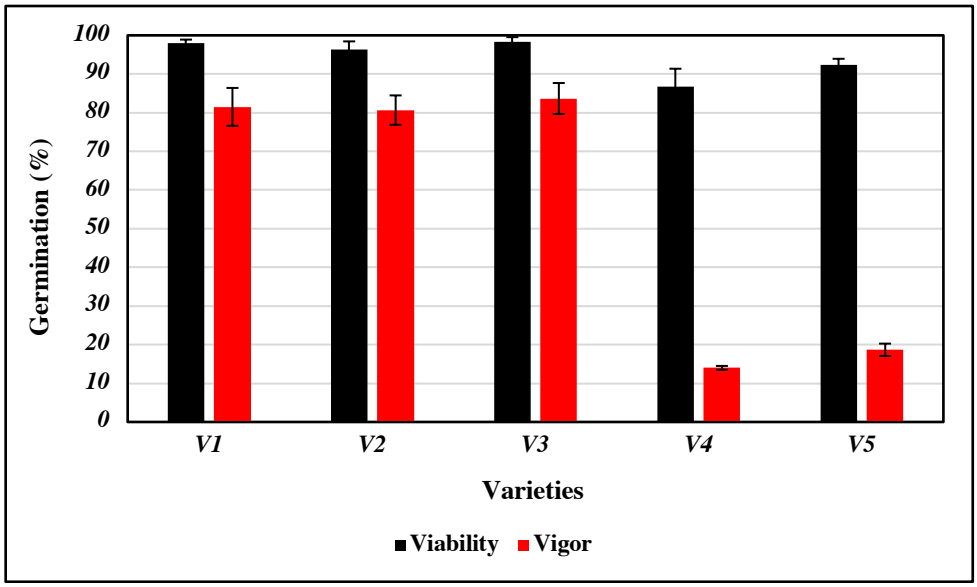

Figure 5. Average viability (standard germination test), and vigor test (Accelerated aging test) of five hemp varieties stored for 18 months at three different conditions.

Conclusions

Storage temperature and relative humidity are crucial factors that affect seed longevity in storage. This study found that seeds stored at 5℃ and 90% RH had the highest quality after 18 months, followed by seeds stored at 20°C and 30% RH, and then seeds stored at 10°C and 75% RH. This indicates that the low temperature of the 5°C cooler compensated for the high RH, and that the 10°C conditions with higher RH (75%) were not optimal for long term storage. Seeds stored at 20°C/30% RH had higher quality than the 10°C/75% RH, indicating that the low RH (30%) storage environment compensated for the higher temperature (20°C). A storage temperature of 20°C could reduce electricity costs if the RH is low and stable. In general, seeds with high viability and vigor maintain their quality in both short and long-term storage better than seeds with low initial quality.

None of the storage environments in this study prevented vigor deterioration of seeds with low initial quality. If storage of low-quality seeds is required for longer than 4 months, low temperature and RH are necessary. Additional precautions, such as using moisture-safe containers and a dehumidifier, would prolong seed quality. Short-term storage of less than 4 months would not require as rigorous of precautions if the storage environment is optimal.

The AAT test differentiated between hemp seed varieties with different qualities more effectively than any other vigor test used in the study. Therefore, we recommend the AAT when needing to rank seed lots with different qualities. The AAT test detected low initial vigor of V4 and V5. These varieties showed rapid and significant vigor deterioration over 18 months (14% and 18%), while the viability by SGT and TZ remained high (93% and 96%) (Figure 5). Vigor offers a more complete picture of seed quality than viability, upon which farmers can make more accurate, well-informed management decisions.

The Electric Conductivity and Cold Test did not effectively differentiate between hemp varieties with different seed qualities. Based on the results of this study, these tests cannot be recommended for measuring vigor of hemp seeds.

Future studies should consider the following:

1) Altered parameters for the Cold Test, such as using a wider range of varieties with initial seed qualities, lower temperature for the stress period (> 5°C), or a duration of stress exposure longer than 5 days.
2) A deterioration comparison between moisture-proof containers vs. those that are not moisture proof.
3) Longer duration of storage for seed lots with different qualities to determine if, or when, seeds would completely lose viability and vigor.

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APPENDIX

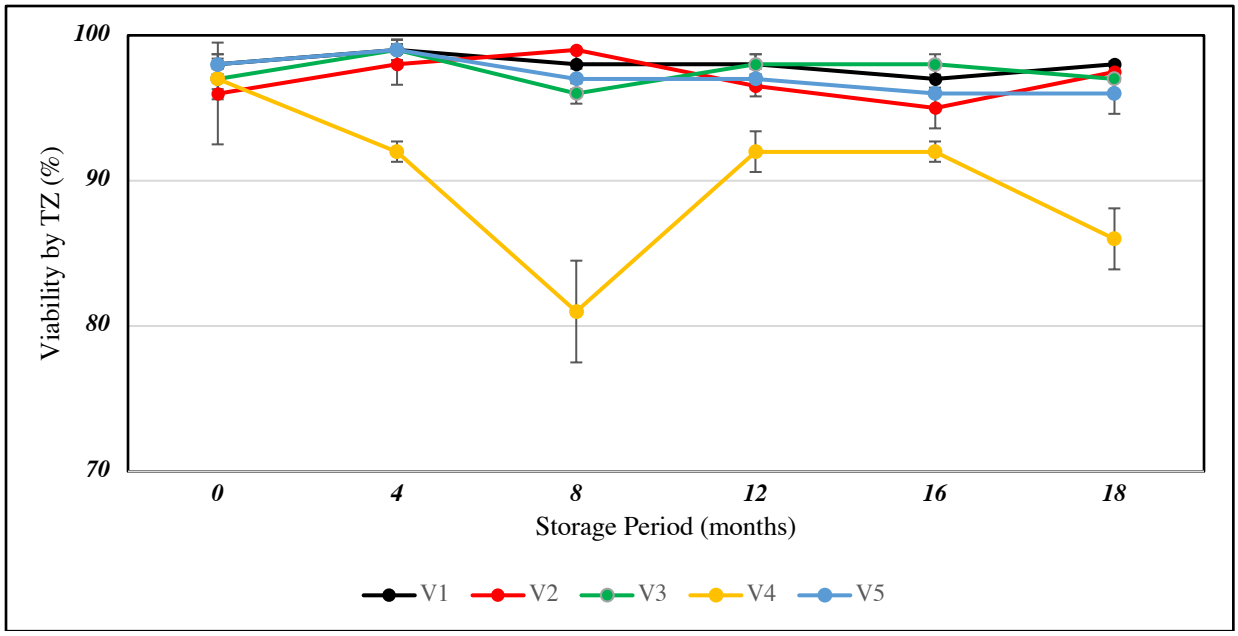

Figure 6. TZ test results for five varieties of hemp seeds stored at 5°C /90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

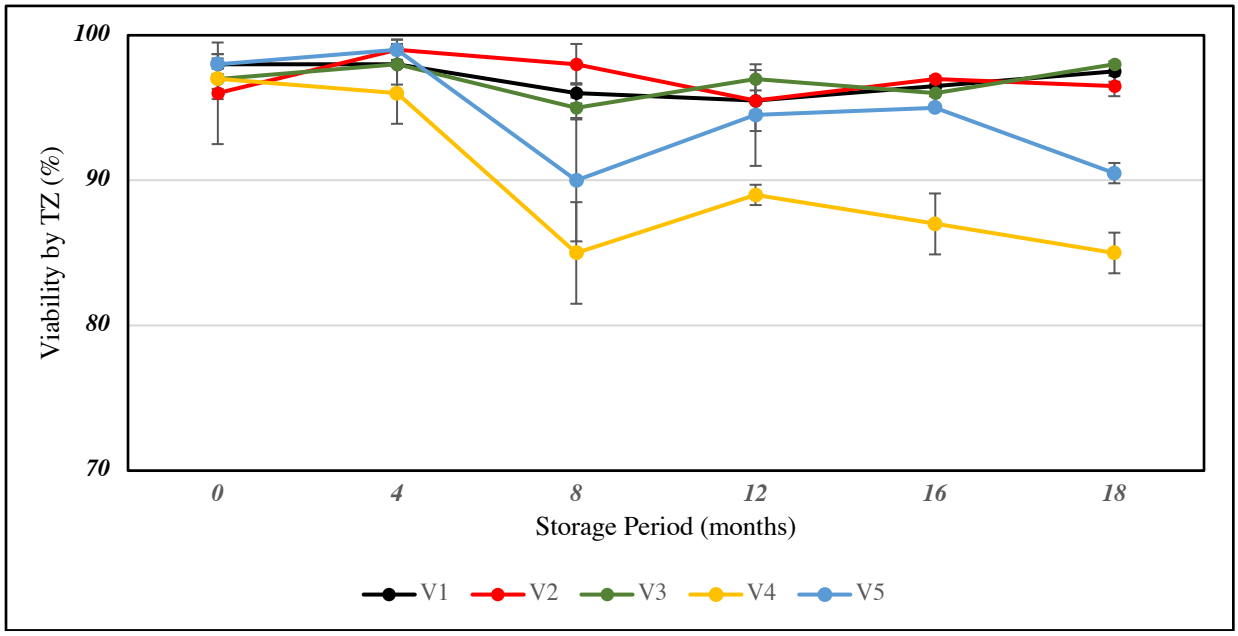

Figure 7. TZ test results for five varieties of hemp seeds stored at 10°C /75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

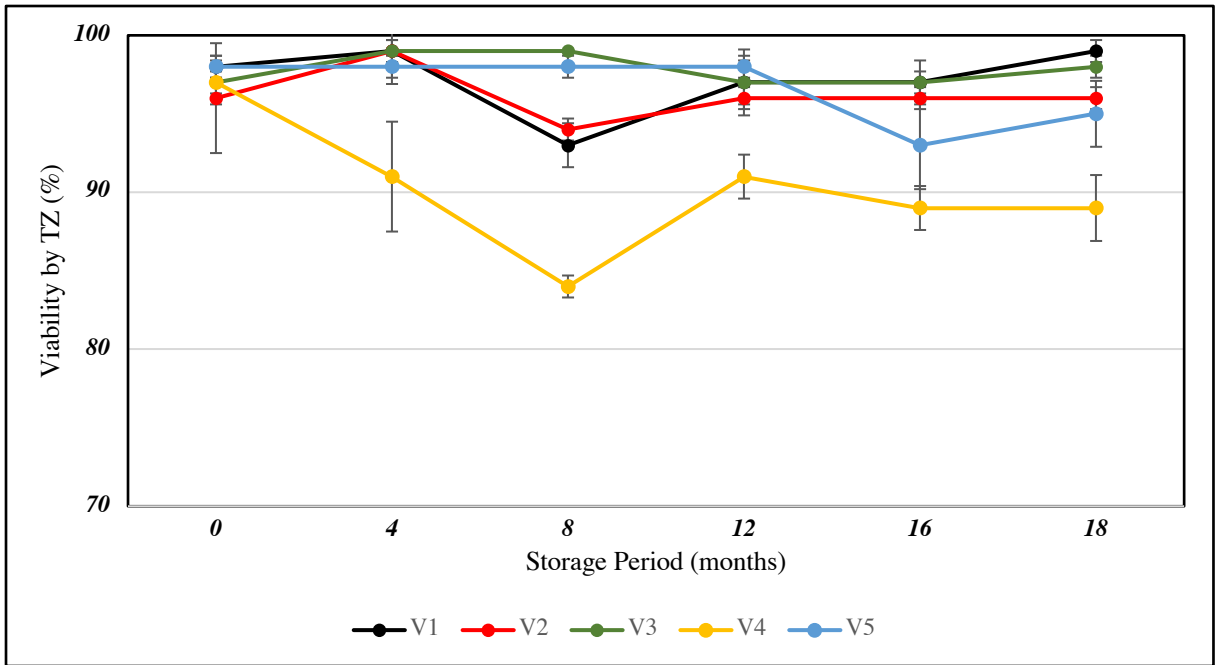

Figure 8. TZ test results for five varieties of hemp seeds stored at 20°C /30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

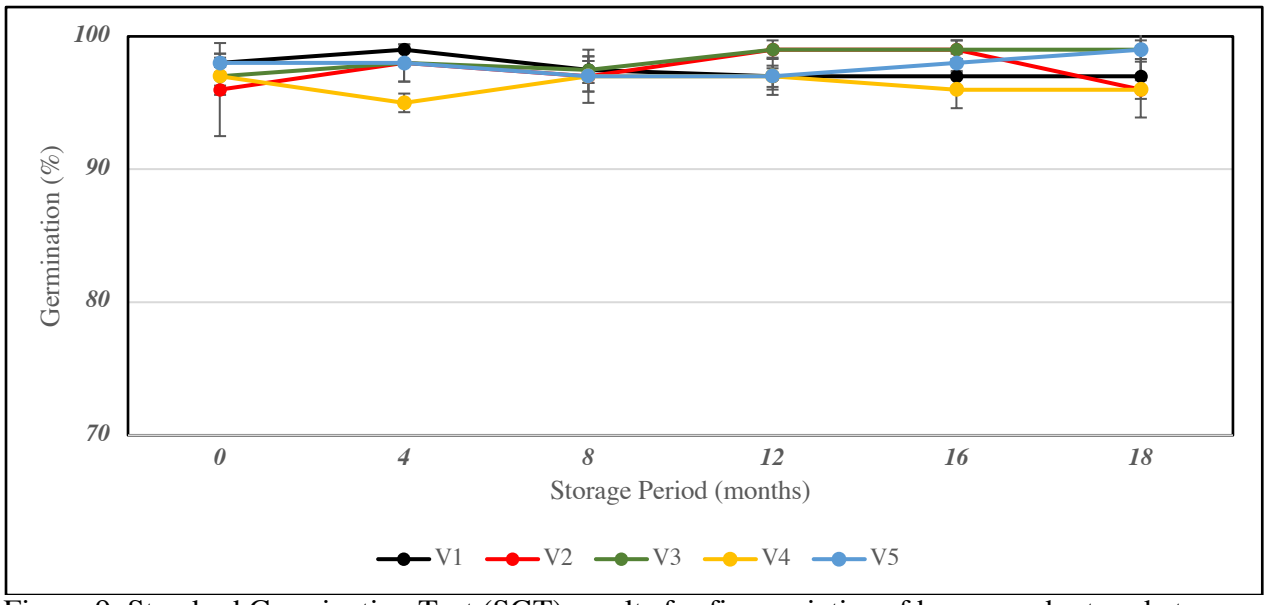

Figure 9. Standard Germination Test (SGT) results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

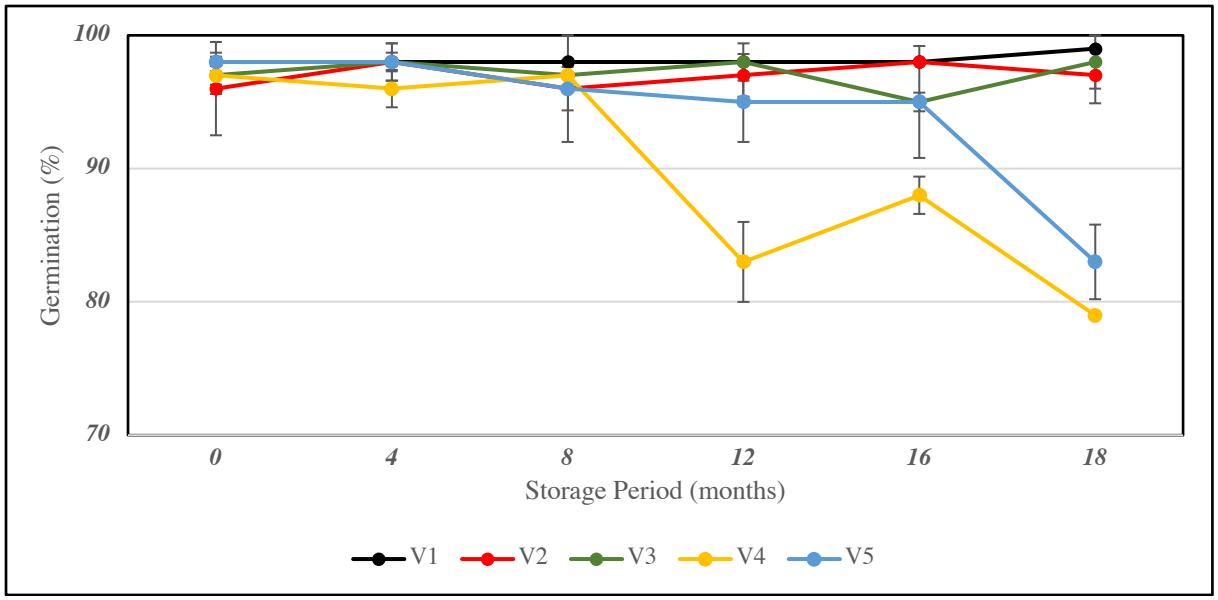

Figure 10. Standard Germination Test (SGT) results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

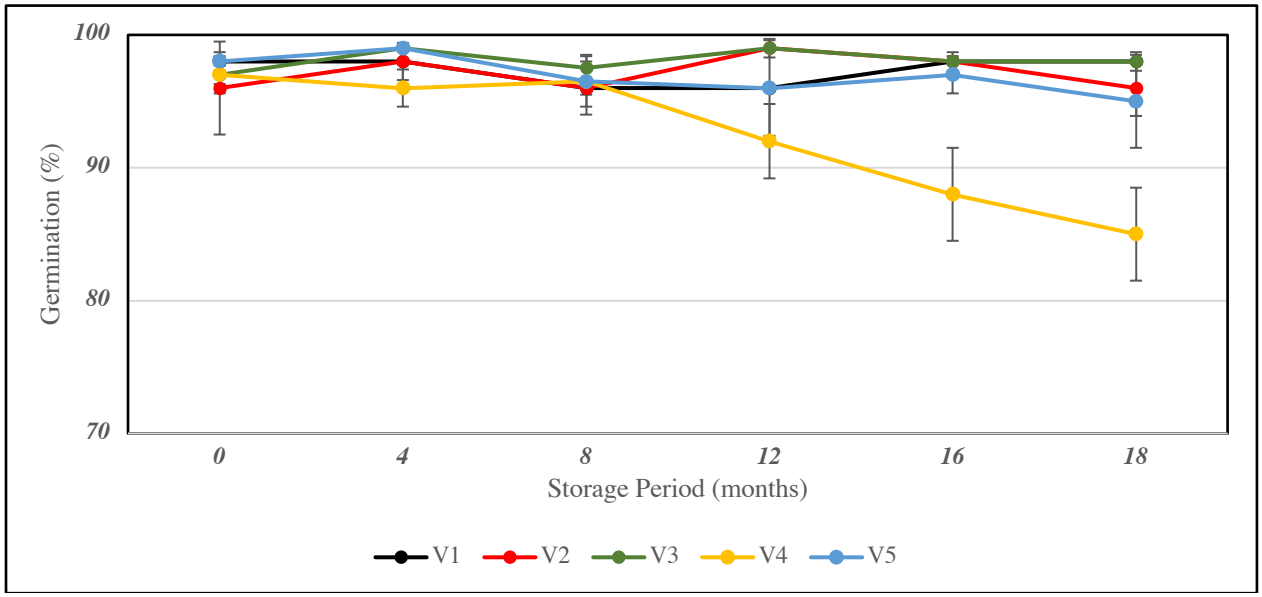

Figure 11. Standard Germination Test (SGT) results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

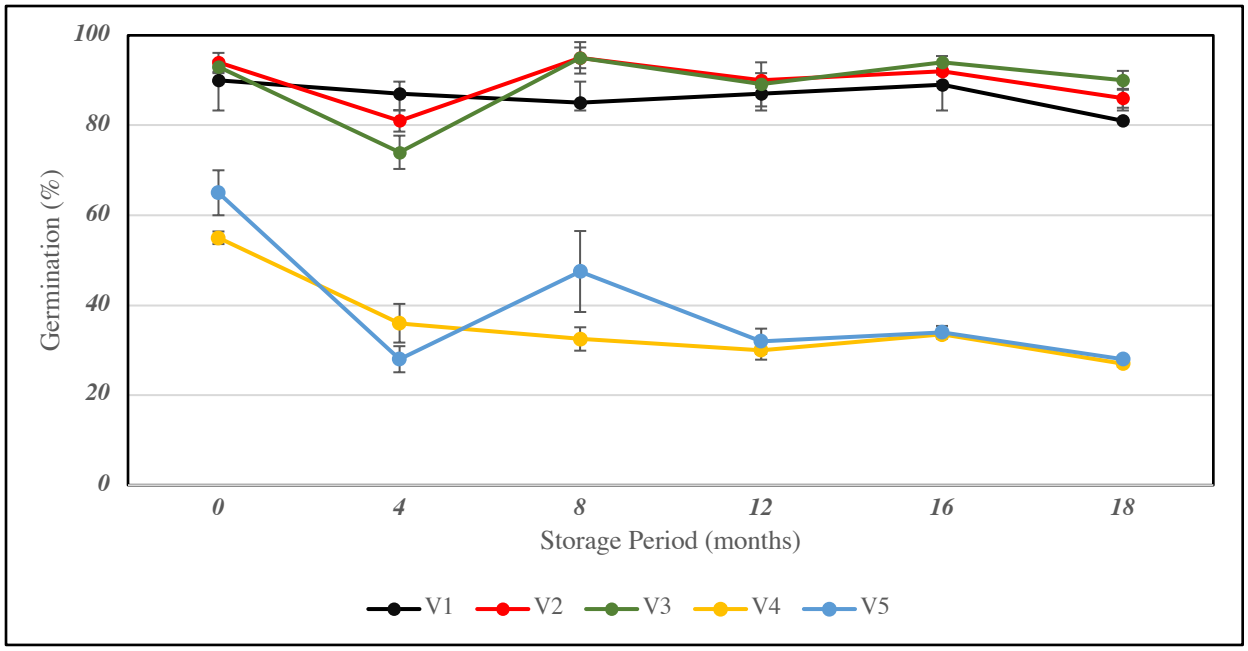

Figure 12. AAT results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

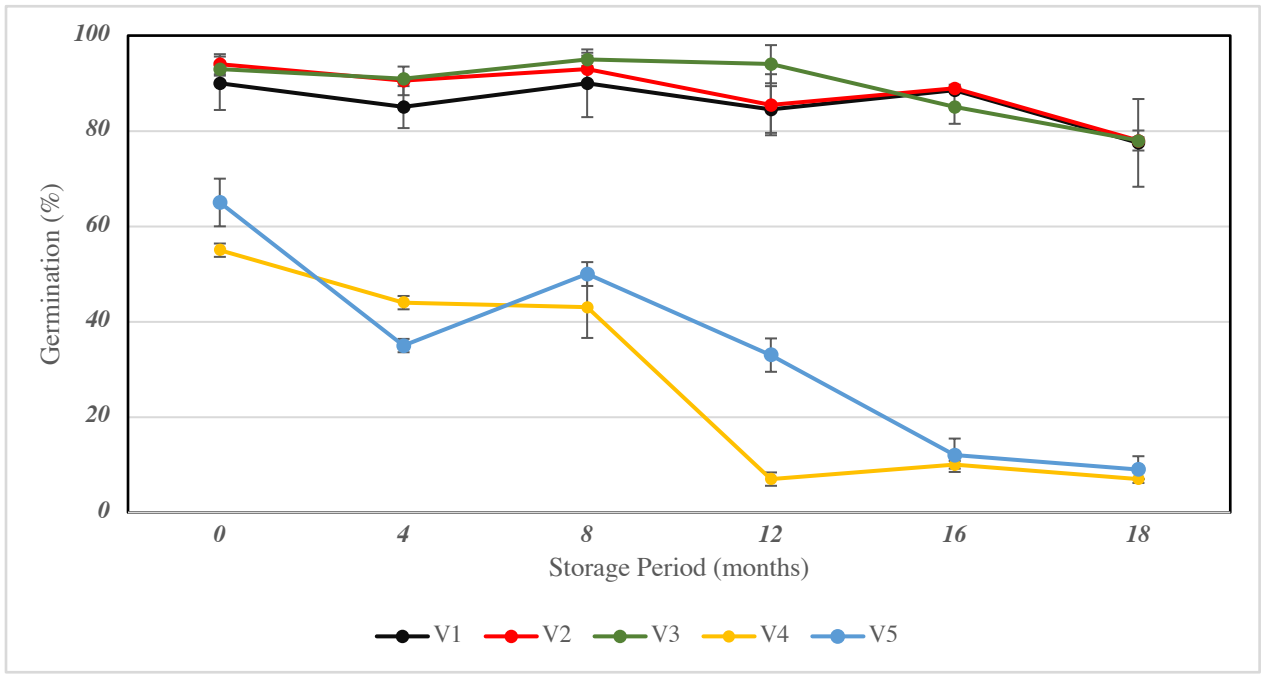

Figure 13. AAT results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

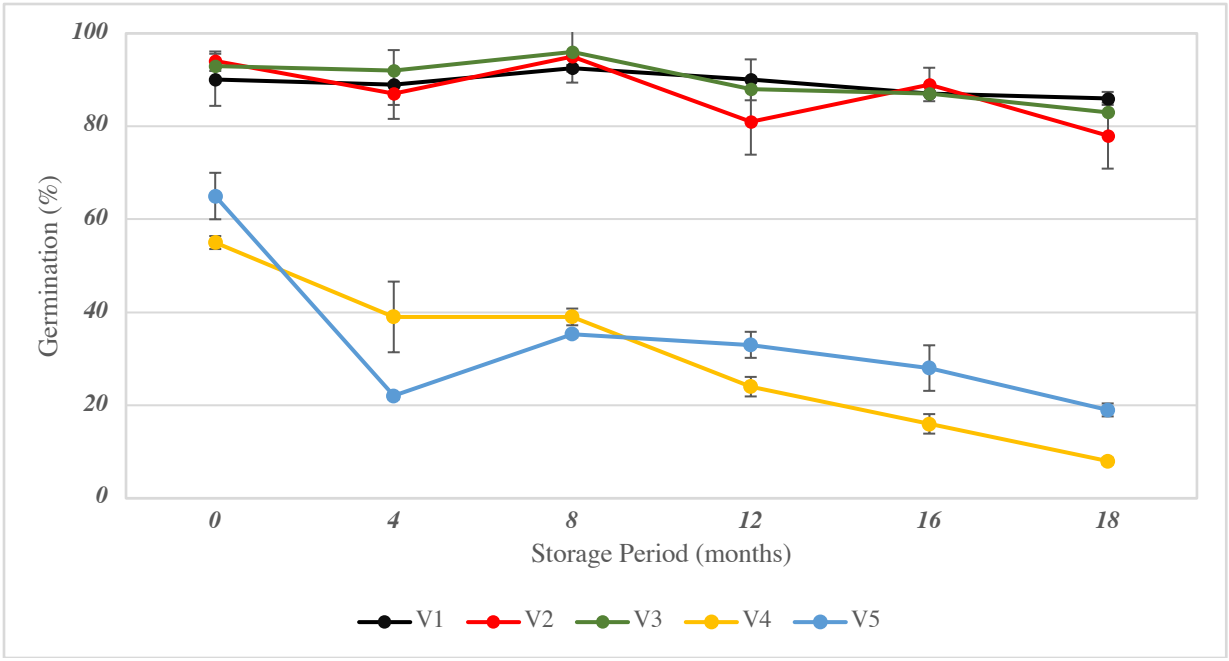

Figure 14. AAT results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

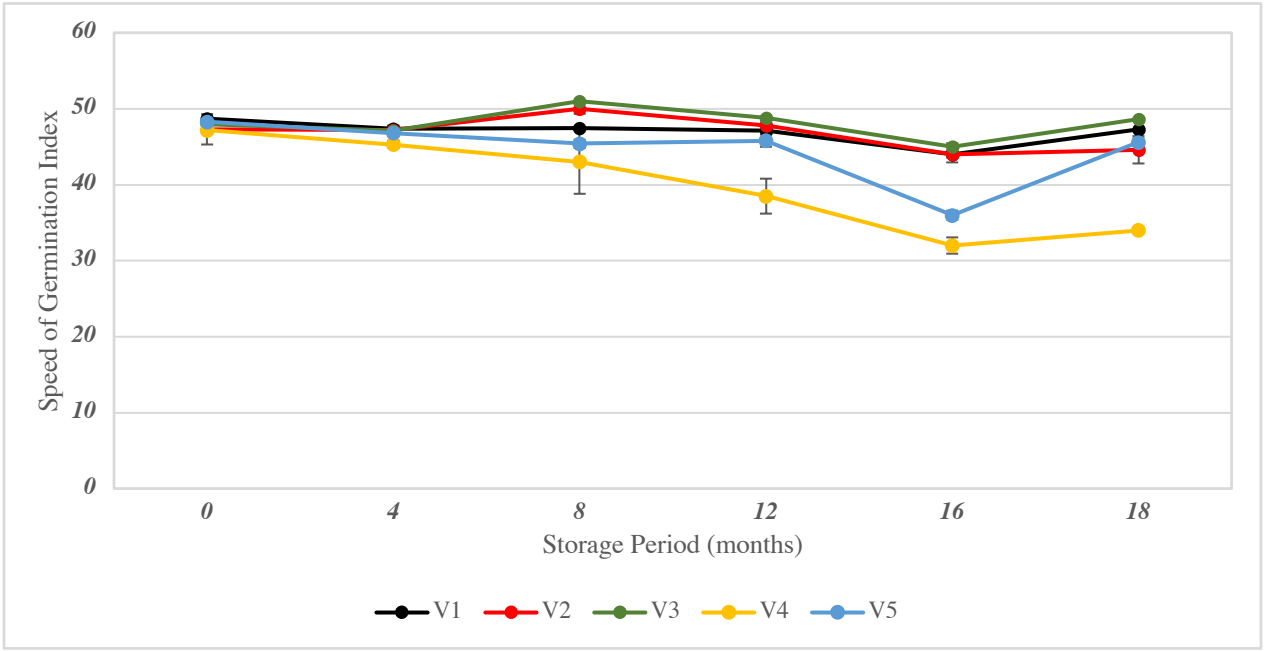

Figure 15. SGI results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

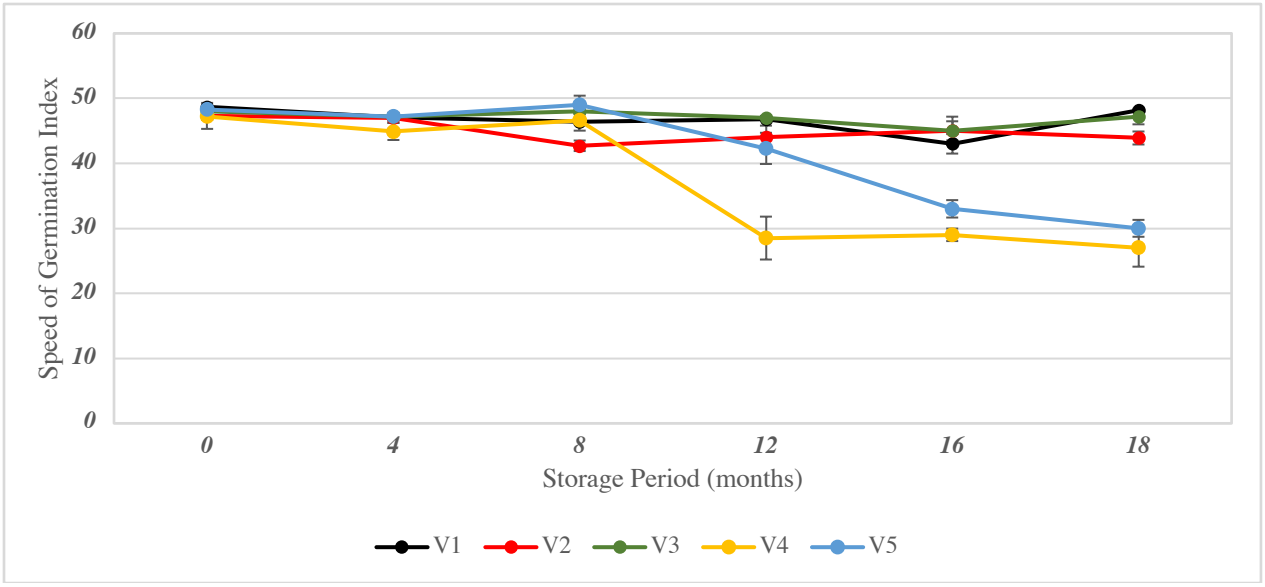

Figure 16. SGI results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

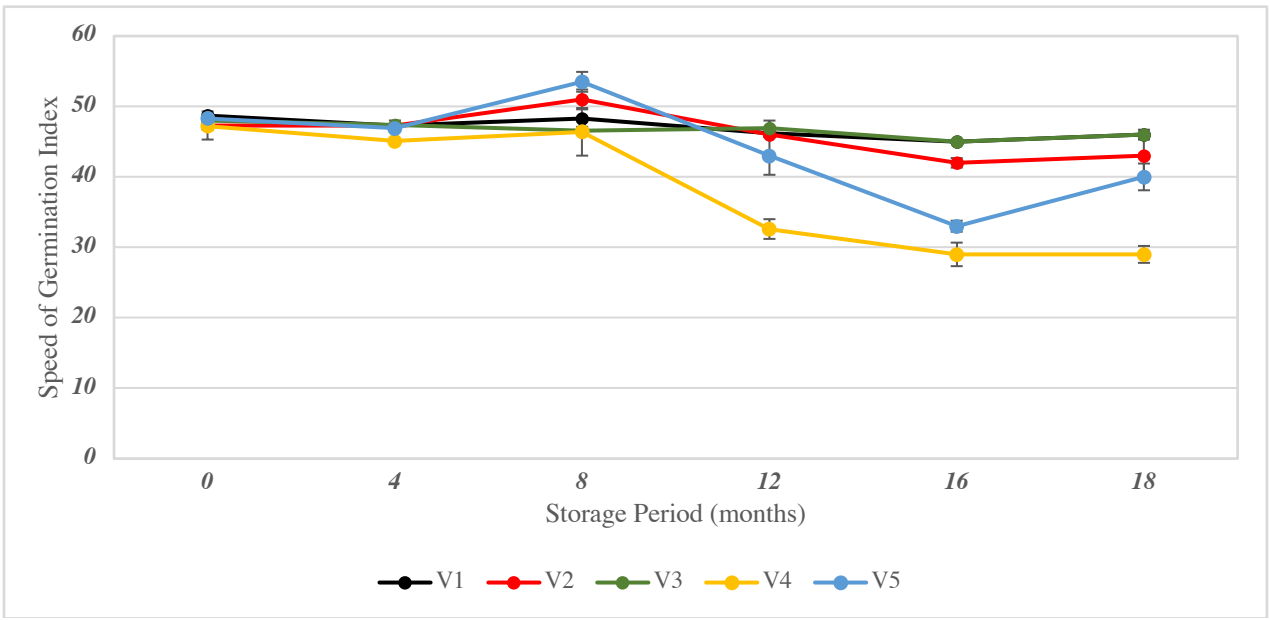

Figure 17. SGI results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

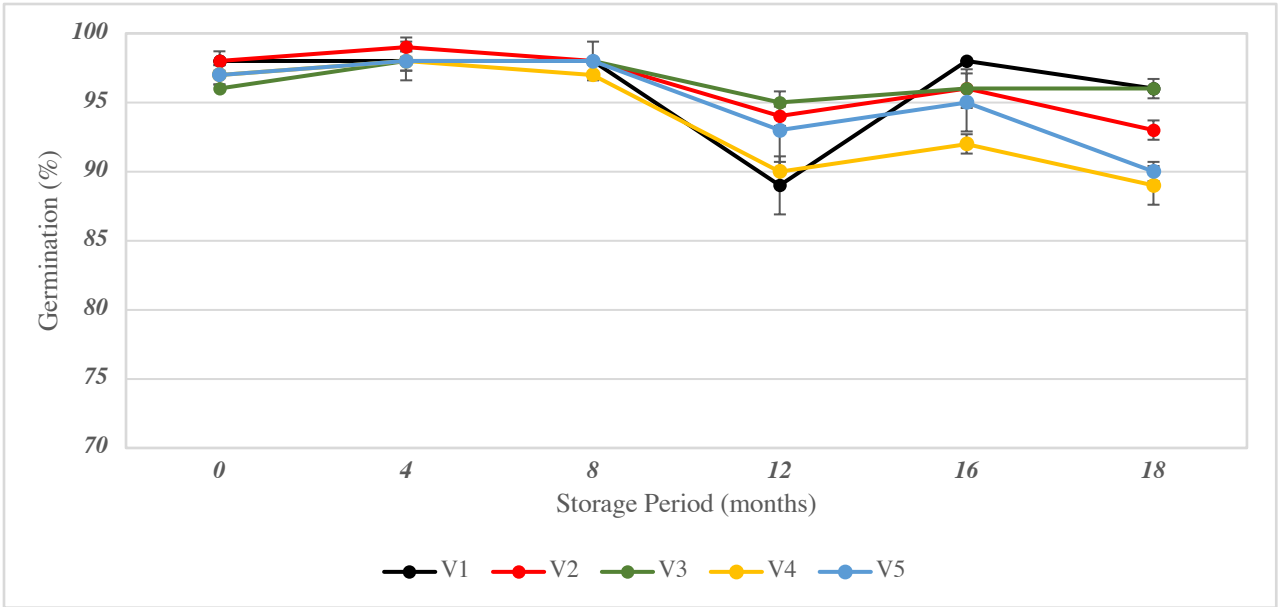

Figure 18. CT results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

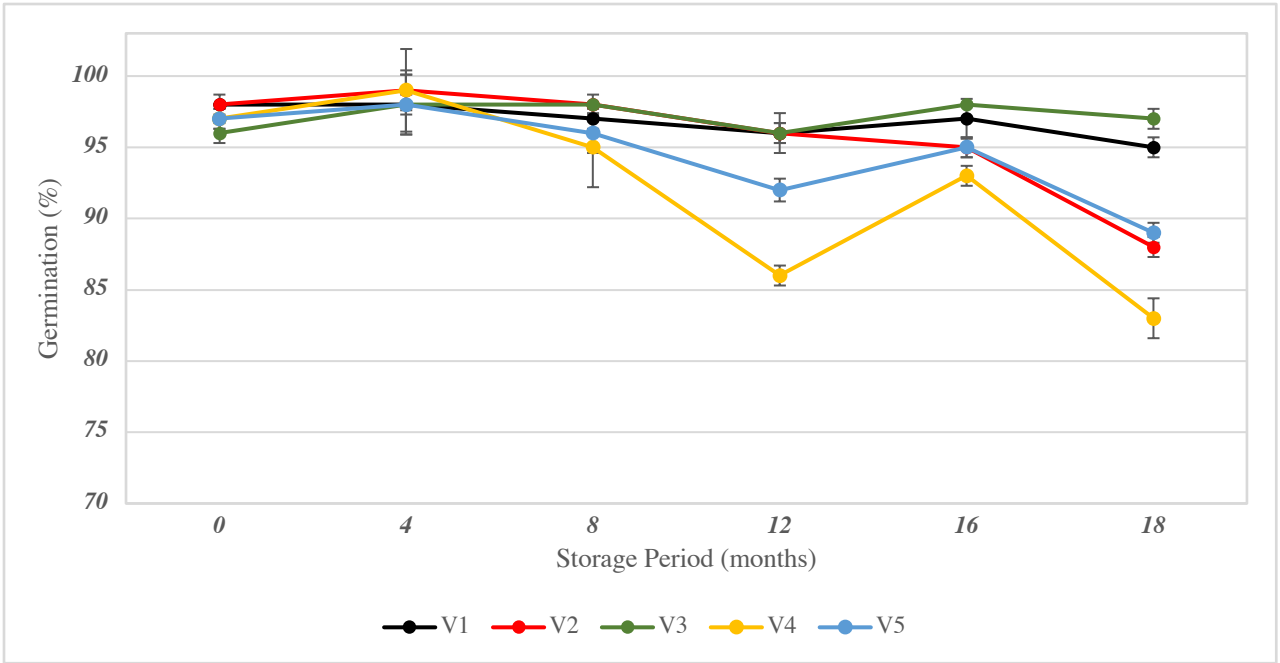

Figure 19. CT results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

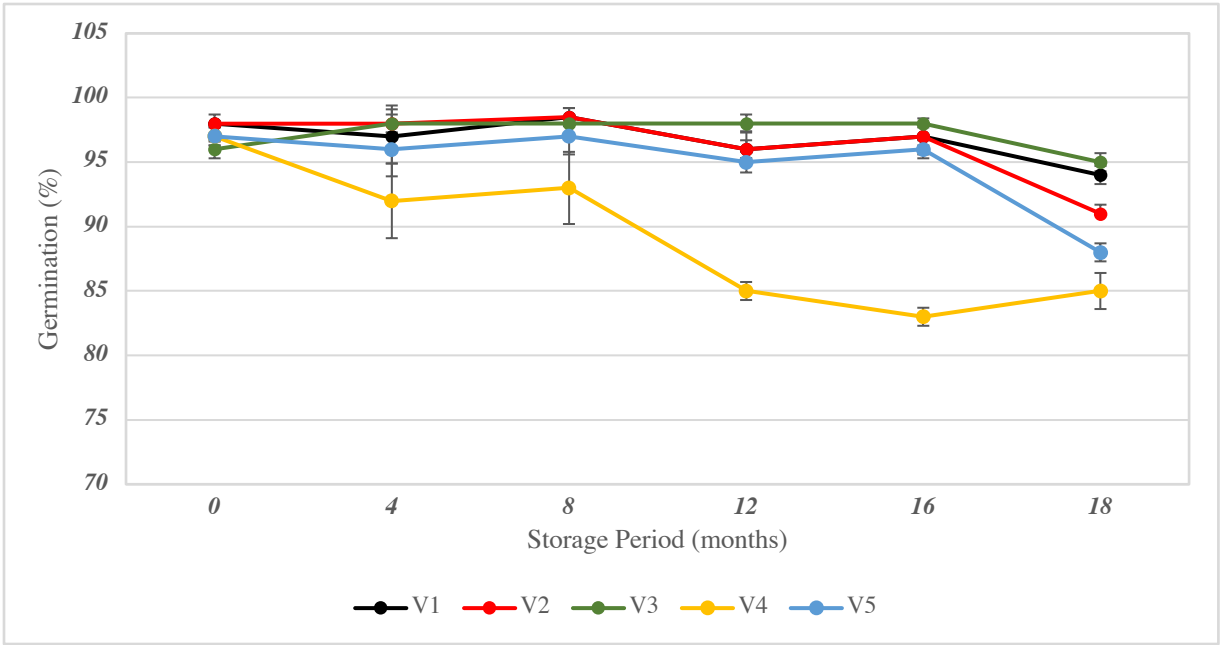

Figure 20. CT results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

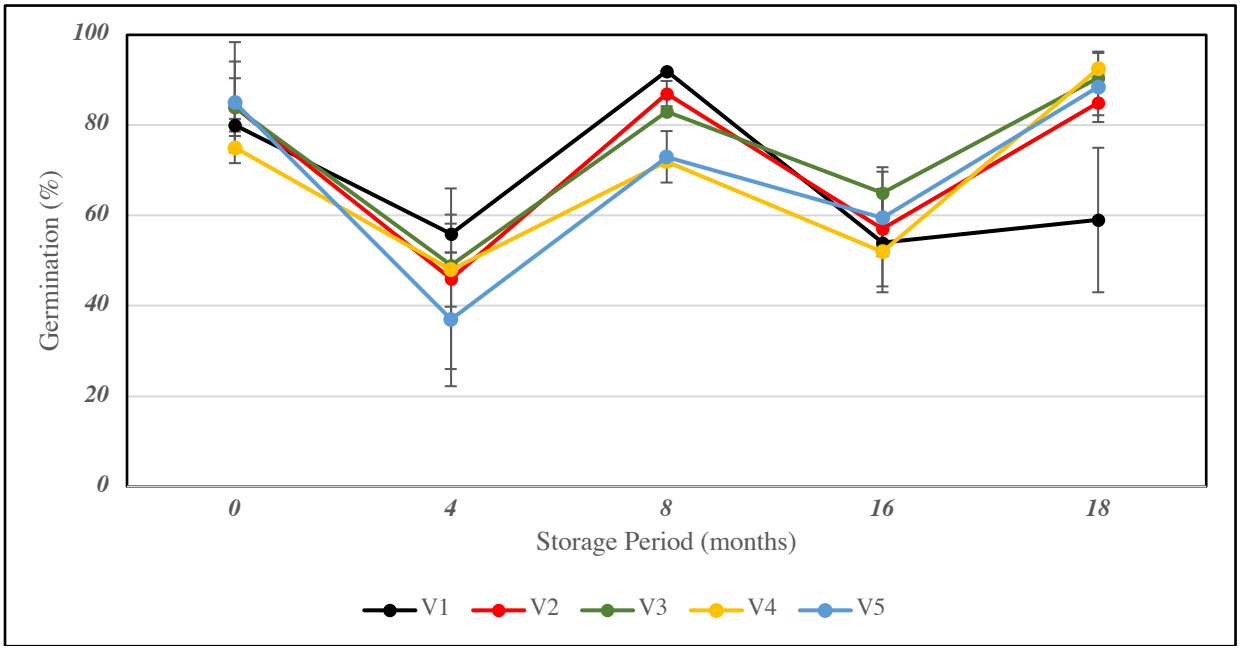

Figure 21. FE results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

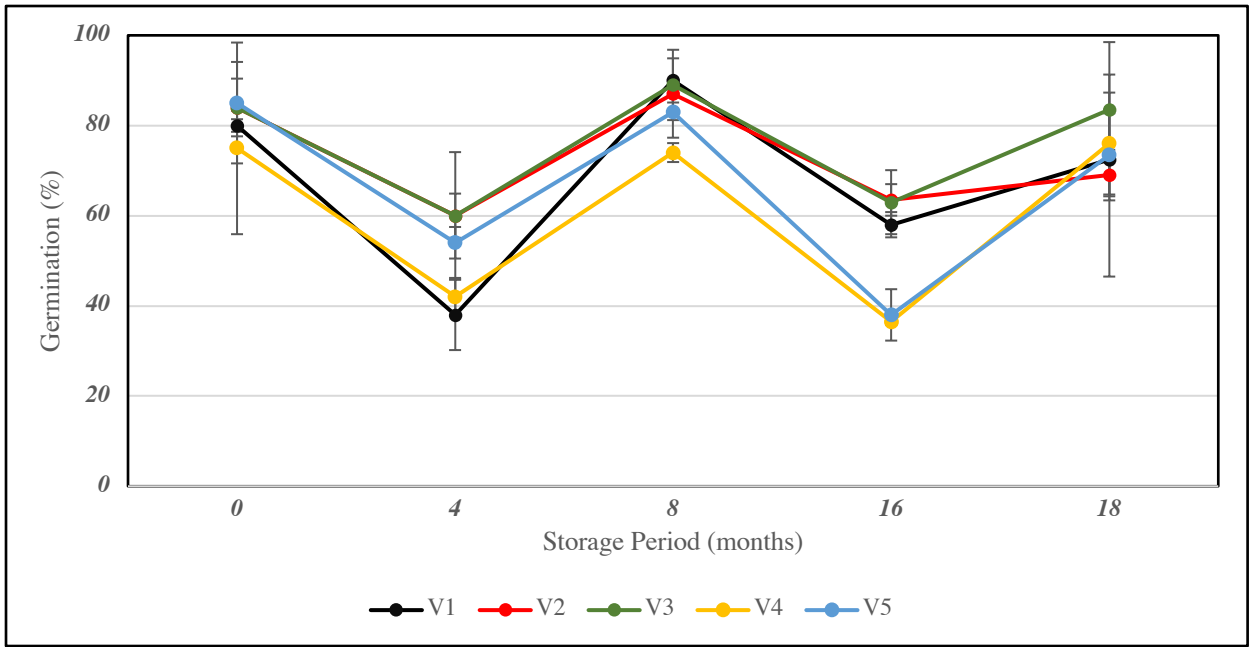

Figure 22. FE results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

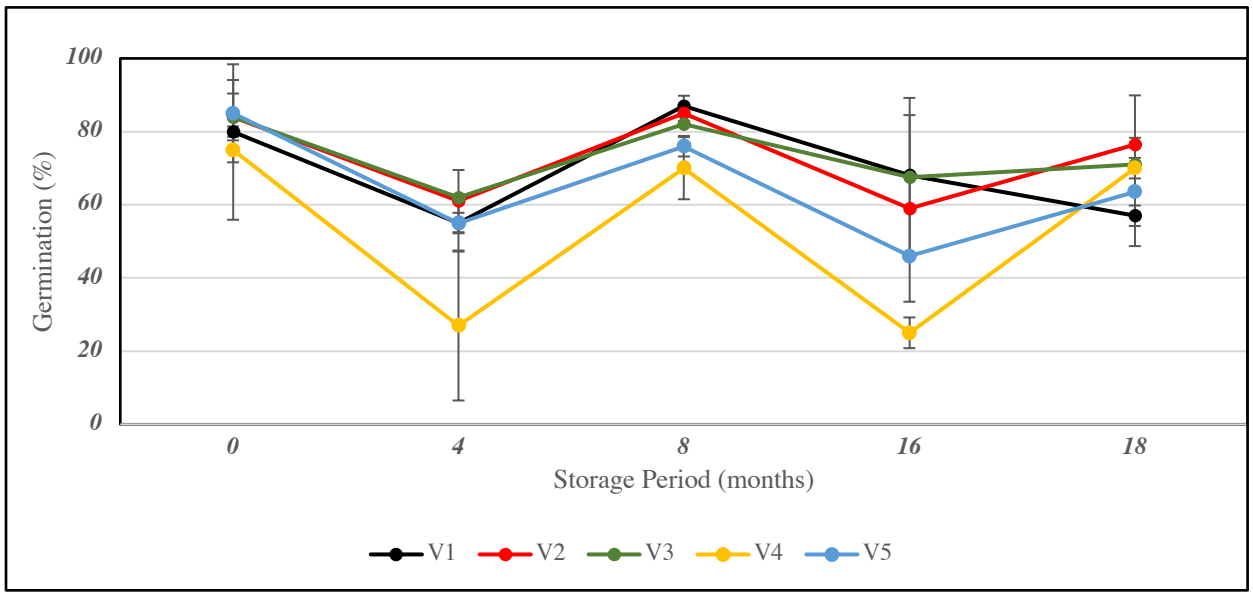

Figure 23. FE results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

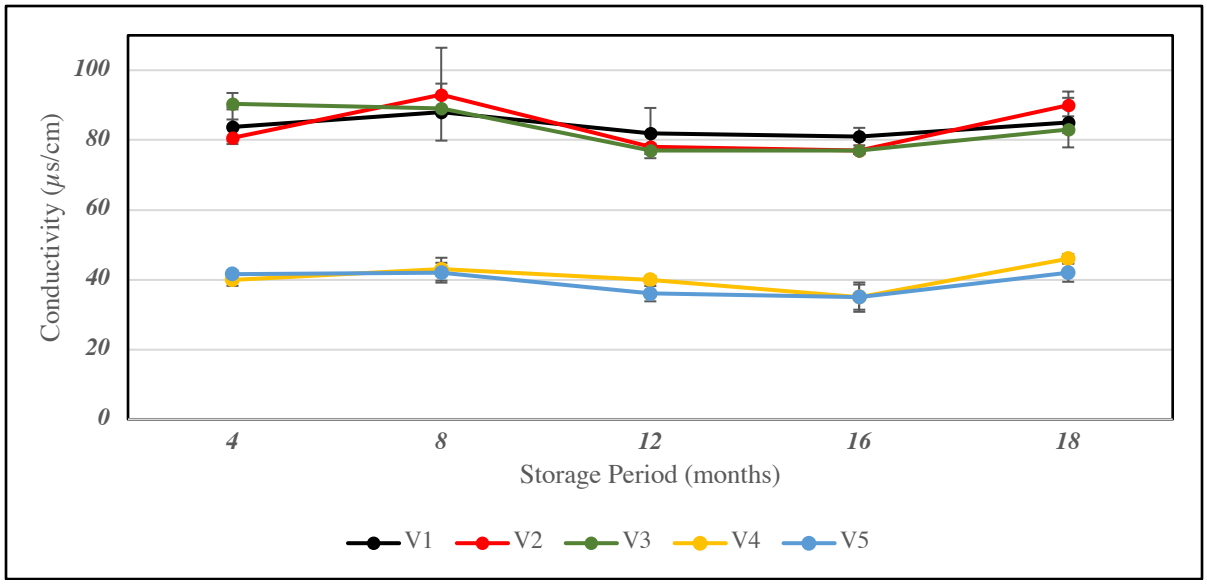

Figure 24. EC results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

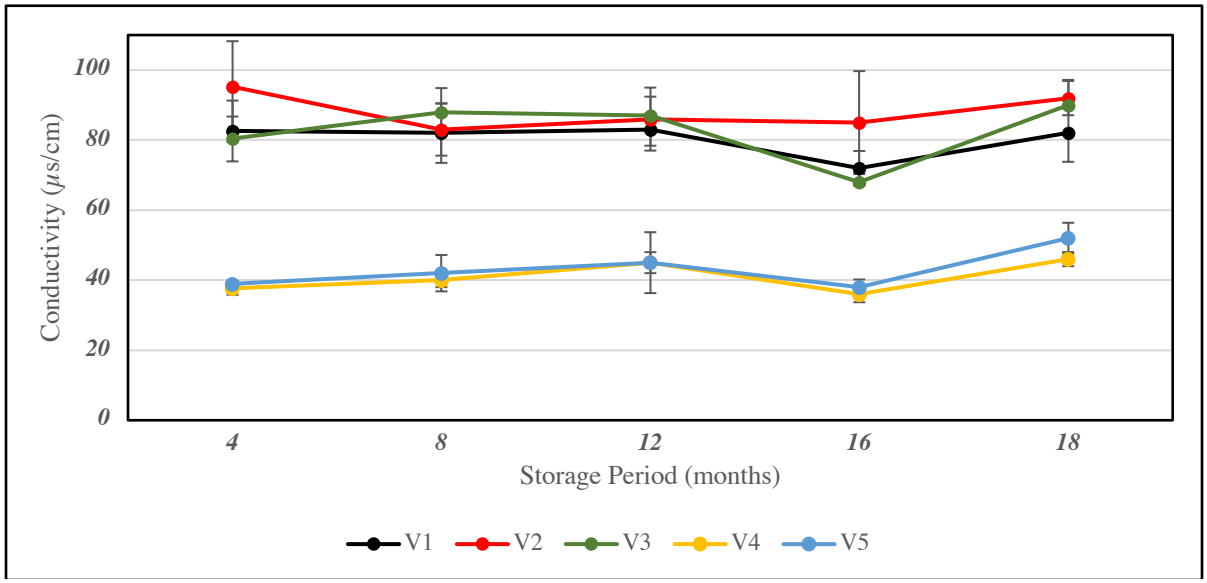

Figure 25. EC results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

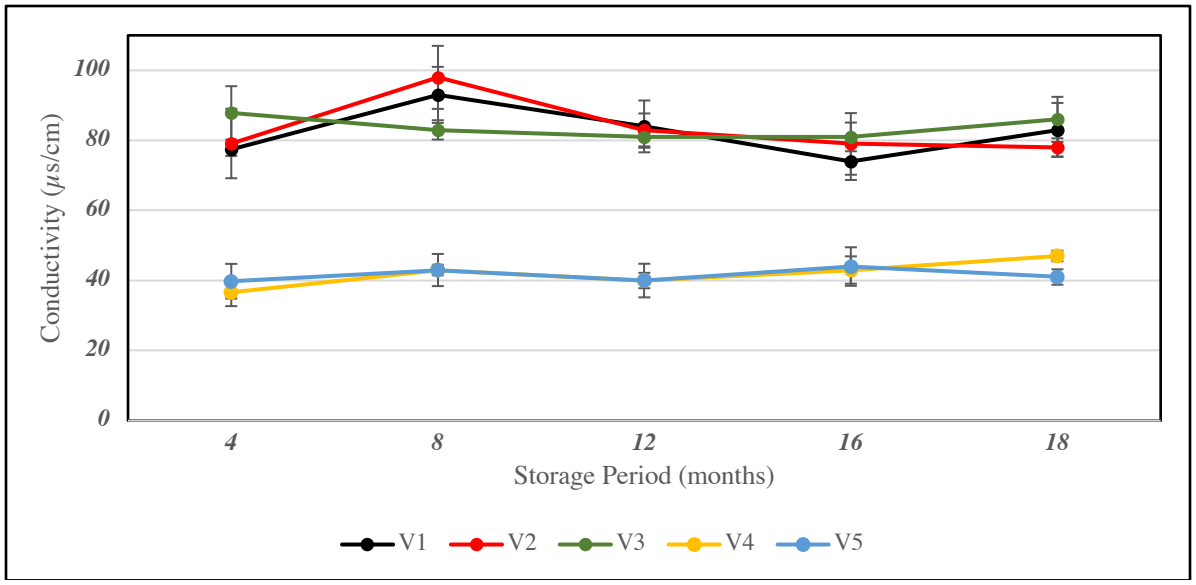

Figure 26. EC results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

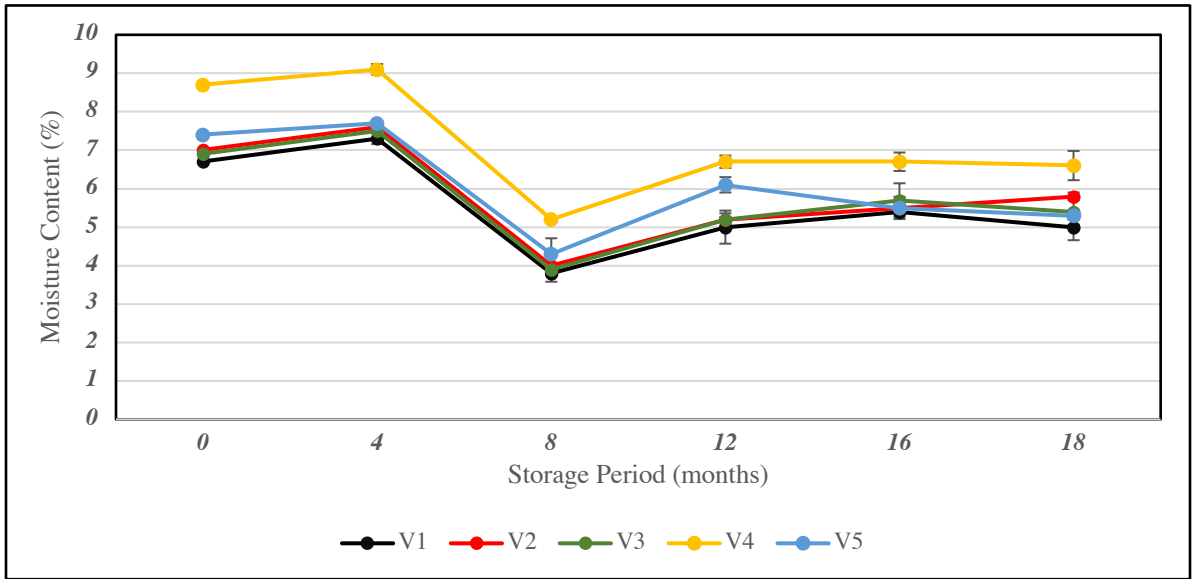

Figure 27. SMC results for five varieties of hemp seeds stored at 5°C/90% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

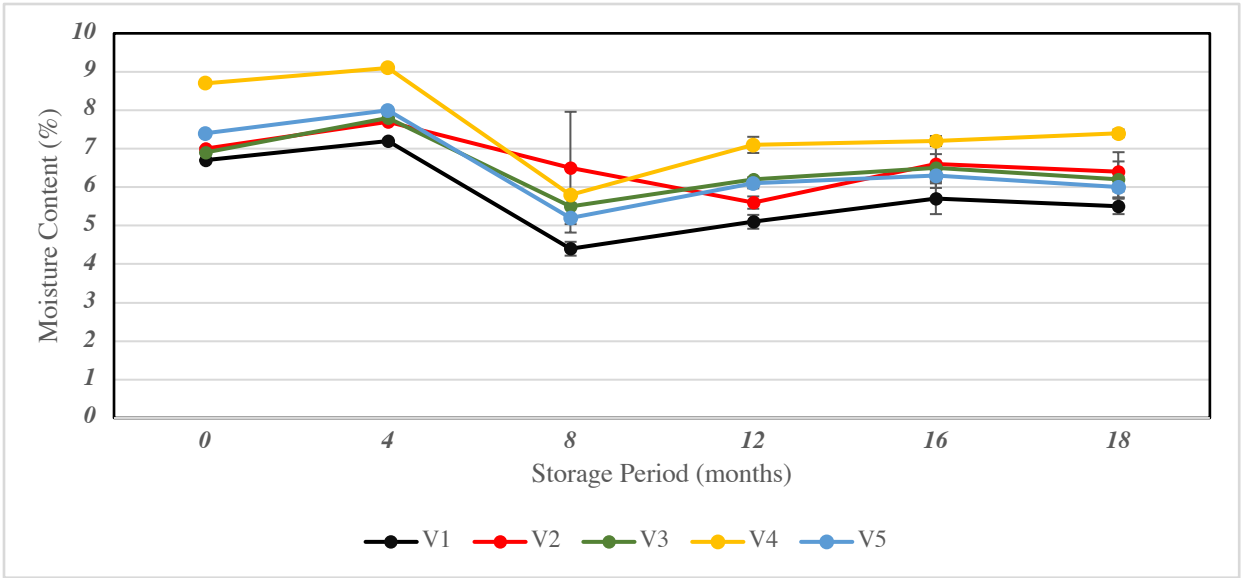

Figure 28. SMC results for five varieties of hemp seeds stored at 10°C/75% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.

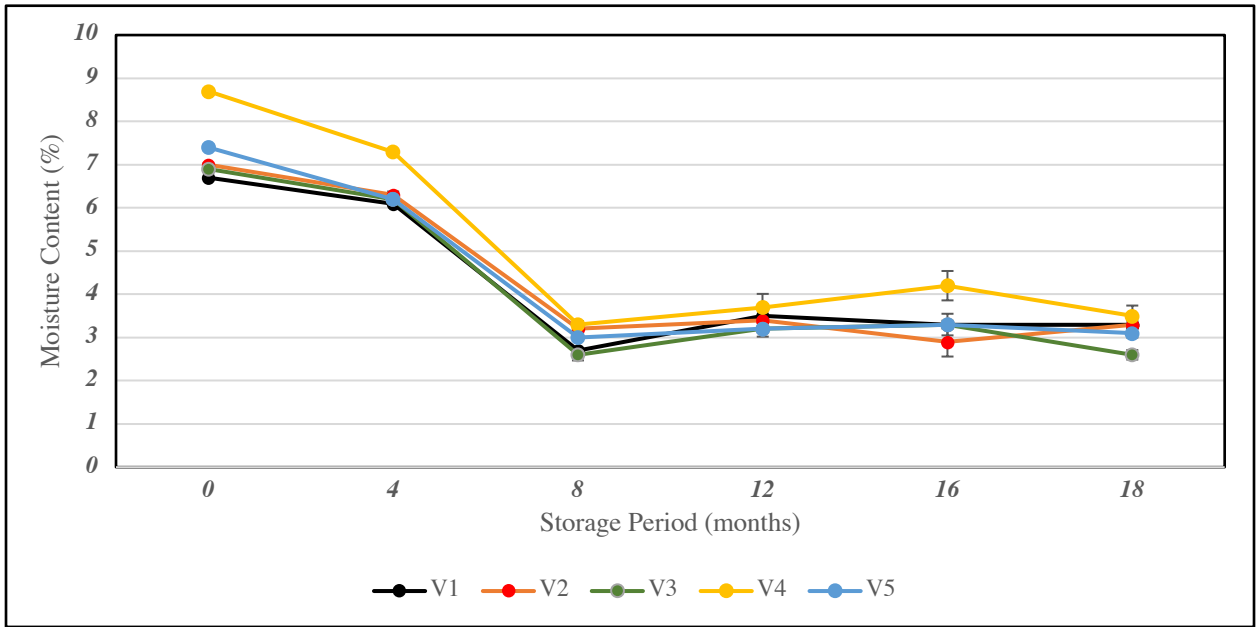

Figure 29. SMC results for five varieties of hemp seeds stored at 20°C/30% RH for 18 months. Means with overlapped error bars are not significantly different from each other at $P \leq 0.05$.