



activities caused by respiration, which accompanies seed deterioration (Wang *et al.*, 2018). Temperature and relative humidity were described to be connected with oxygen in determining the seed longevity while in storage (Ventura *et al.*, 2008; Groot *et al.*, 2012; Xin *et al.*, 2014; Colville and Pritchard, 2019). It is known that seed longevity declines during storage, with the seed germination percentage in the end reducing or resulting in an increased rate of abnormal seedlings.

Some species seeds genetically store better than others. Pepper is considered to have seeds with short life span (Priestley, 1986). It is a warm climate summer crop and is produced intensively in sub-tropical regions where high relative humidity and temperature prevail, which are the basic causes of seed deterioration (Adebisi and Abdul-Rafiu, 2016) during storage. Loss of seed viability during storage is a primary cause of poor stand establishment and reflects the warm and humid ambient storage environment and limited availability of controlled environment storage facilities.

Long-term food production needs sustainable agricultural practices including organic production. The entire production process conforms to the rules of organic production. The main features of organic crop production are the removal of chemical crop protectants, the use of organic inputs instead of chemical fertilisers and the certification of the organic production system (Groot *et al.*, 2004). Hence the first step in organic farming is producing high quality organic seeds with the same quality standards as conventional farming (Groot and Raaijmakers, 2018). Quality organic seed production is crucial since preventive measures to keep plant healthy, i.e. using pesticides and herbicides, are not allowed and competition with weeds is fierce (Sripathy *et al.*, 2012).

There are numerous studies on longevity of conventionally-produced pepper seeds (Thanos *et al.*, 1989; Sundstrom, 1990; Sanchez *et al.*, 1993; Basay *et al.*, 2006; Demir and Ozcoban, 2007; Demir *et al.*, 2009; Caixeta *et al.*, 2014; Panayotov and Aladjadjian, 2014; Adam *et al.*, 2018; Verma *et al.*, 2018). Nevertheless, organic seed production systems are different than conventional ones and require specific measures for production (Anonymous, 2010). Thus, the longevity of organic and conventionally produced seeds may differ. The purpose of the study was to determine the longevity of organically produced pepper seeds and compare with conventionally produced ones.

## Materials and Methods

### *Seed production in organic and conventional systems*

Seed production was performed in 2015 in the organic and conventional parcels of Atatürk Horticultural Central Research Institute - Yalova / Turkey. 'Sürmeli', 'Kandil Dolma', 'Yagliik' and 'Corbaci' open-pollinated pepper cultivars were used as plant materials. In the Yalova region with temperate climatic conditions, the average air temperatures for the vegetation period in 2015 (March-October) ranged between 5.5 °C and 30.9 °C. During the vegetation period, different fertilizers were used depending on plant growth periods and the number of plant nutrients in the soil. Gentasol (30% total organic matter, 5% N, 3% K<sub>2</sub>O, 2% P<sub>2</sub>O<sub>5</sub>) and Ormin K (30% K<sub>2</sub>O, %5 organic matter) organic fertilisers were used for organic production and 20:20:20 (NPK) and potassium sulphate (50% K<sub>2</sub>O) compound fertilisers were used for conventional production.

According to the results of soil analysis, the soil structures of the production parcels have a clayey structure. The soil pH was determined to be between 7.73-7.79. Therefore, the structures of the parcels have slightly alkaline character. The amount of organic matter was 2.03% in the organic parcel and 3.51% in the conventional parcel.

Seeds of four different pepper cultivars were sown in the second week of March in 2015. The study was managed according to the randomised complete block design with 4 replicates and each replicate consisted of 150 plants. Seedlings were planted in organic and conventional parcels in the second week of May. Plant spacing was 0.8 m between rows and 0.4 × 0.5 m between plants with double rows. Weekly fertilisation was performed

depending on the plant growth and the number of plant nutrients in the soil. Gentasol 20 L per 1000 m<sup>2</sup> was applied to the organic parcel and 20:20:20 (N-P-K) 2 kg per 1000 m<sup>2</sup> of compound fertilizer was given by drip irrigation to the conventional parcel. Potassium fertilisation was done during the fruit maturation stage. Accordingly, Ormin K 2 kg per 1000 m<sup>2</sup> was used in the organic parcel and potassium sulfate 1.5 kg per 1000 m<sup>2</sup> was applied to the conventional parcel.

Fruits were harvested at maturity stage (60-65 days after anthesis) for each cultivar. The seeds were extracted and dried at 30 °C until the seed moisture content reduced to 7-8%. The moisture content of dried seeds was determined according to ISTA (2016). Seeds were kept in a fridge until use. The mean weight of 1000 seeds were determined after the drying process.

#### *Seed storage*

Organic and conventional seeds, dried to 7-8% moisture content, were vacuum sealed in laminated aluminium packets and stored for 48 months at 20±2 °C. The longevity of organic and conventional pepper seeds was evaluated by conducting germination percentage and mean germination time studies at the regular intervals of every 4 months during the period of 48 months.

The germination test was set with 4 replicates and each replicate had 50 seeds for each production system with four cultivars. Two pieces of Whatman No: 1 blotting paper were placed in 9 cm petri dishes. Then 4 ml of distilled water was added after the seeds were placed in the petri dishes (ISTA, 2016). The germination test was executed at 25 °C in the dark and the percentage germination was determined using daily counts for 14 days. The germination papers were moistened as needed. Normal seedlings were assessed at the end of the germination test.

Mean germination time (MGT, days) was calculated according to the formula below (Ellis and Roberts, 1980):

$$MGT = \frac{\sum n.t}{\sum n}$$

where n = number of newly germinated seeds (2 mm radicle emerged) at time t, t = days from planting, and  $\sum n$  = final germination.

#### *Seed longevity analysis*

Normal germination of organic and conventionally grown pepper seeds during storage (12 samples) were subjected to probit analysis as described by Ellis and Roberts (1981), with the convention that the probit of 50% normal seedling = 0. The seed viability equation was used to quantify seed deterioration rate as follows:

$$V = K_i - p/\sigma$$

Where v is the probit percentage of normal seedlings after a period of p (month) of storage at given seed moisture and temperature.  $K_i$  is the intercept on the probit viability axis before storage and is an index of the initial seed quality (combination effects of genetics and environmental factors on quality) in probit unit. The loss of viability is normally distributed in time; therefore, it can be quantified in terms of P50, half viability period or  $\sigma$  standard deviation of seed deaths in time (i.e. the time required to lose 1 probit of viability) at certain seed moisture and storage temperature. After converting normal percentages to probit in y axis, regression analysis was conducted and regression formulae ( $y=a-bx$ ) were developed for each cultivar and production system.

#### *Statistical analysis*

The laboratory tests in the study were established in accordance with the experimental randomised parcel design. The percentage values obtained from these tests were subjected to  $\sqrt{n}$  transformation. JMP 8.0 Statistical package program was used for analysis. Data were assessed with analysis of variance for the main effects, whereby the means of values were compared using the Duncan multiple range test and least significant difference test ( $p=0.05$ ).

## Results

Initial total seed germination of both organically and conventionally produced seeds were much higher than 91% for all cultivars. Especially 'Corbaci' and 'Yaglik' cultivars had values above 94% of total and 90% of normal. The difference between organic and conventional seeds was less than 3% in total and 4% in normal germination (Table 1). Thousand seed weights ranged between 6.41 and 6.90 g for the cultivars. Seed moisture contents of the cultivars varied between 7.6 and 8.0%.

**Table 1.** Changes in initial normal and total germination percentages, seed moisture content in storage and 1000 seed weight of organically and conventionally grown four pepper cultivars

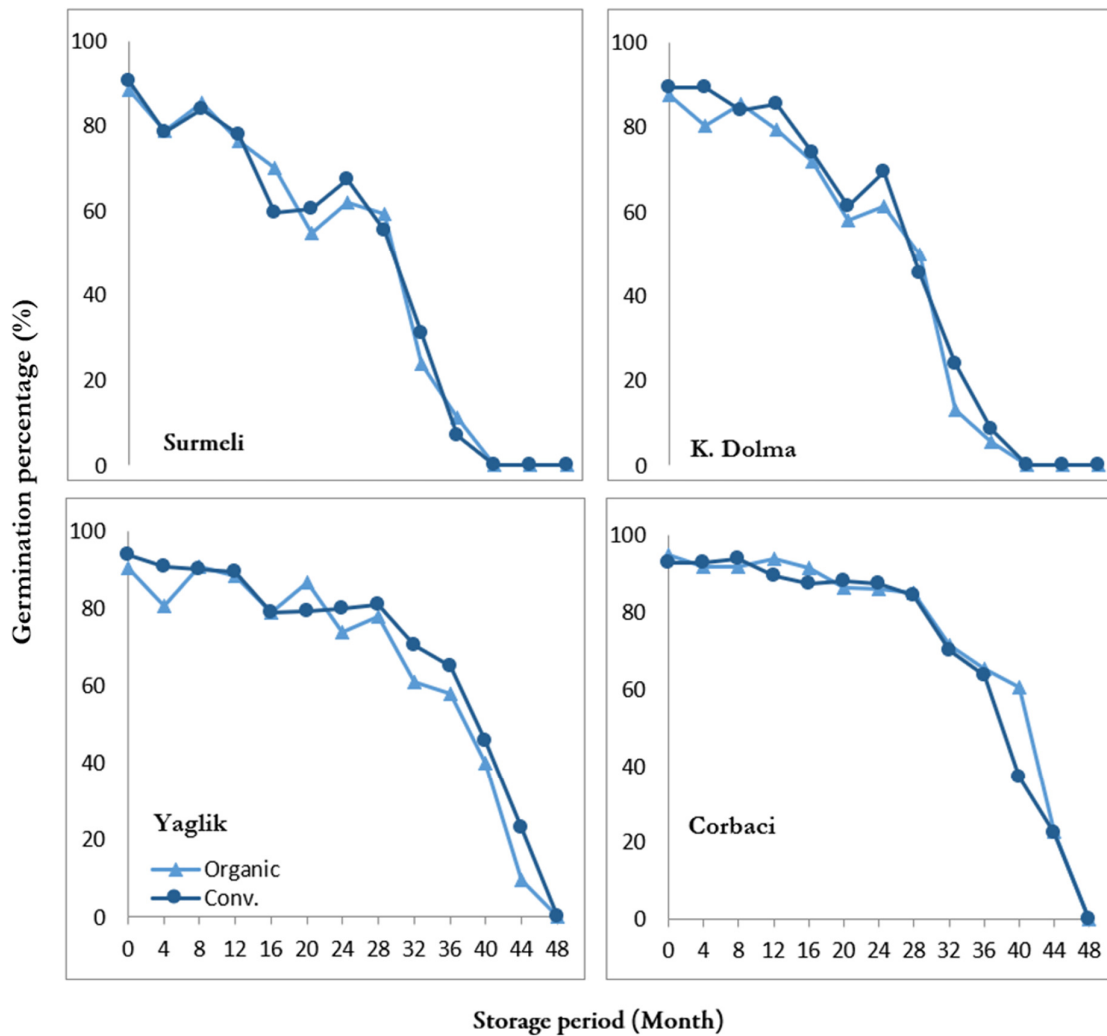
Cultivar	Seed types	Normal Germ. (%)	Total Germ. (%)	Seed mc. (%)	1000 Seed weight (g)
'Surmeli'	Organic	88a	92a	7,9	6,66
	Conv.	90a	92a	8,0	6,41
'K. Dolma'	Organic	87a	92a	7,6	6,79
	Conv.	89a	91a	7,7	6,56
'Yaglik'	Organic	90a	94a	7,7	6,65
	Conv.	94a	95a	7,7	6,73
'Corbaci'	Organic	95a	98a	7,8	6,90
	Conv.	93a	95a	7,7	6,78

The seed survival curves of organic and conventionally grown pepper seeds for the four cultivars are presented in Figure 1. The survival curves (normal germination plotted against storage period over 48 months) showed the typical sigmoidal pattern. Seeds of 'Surmeli' and 'K. Dolma' lost germinability completely after 40 months, while death of 'Yaglik' and 'Corbaci' seeds occurred by 48 months. Seed germination losses in 'Surmeli' and 'K. Dolma' were much faster in earlier sampling times of the storage than those of the other two species. In all cultivars, organic and conventional seed germination loss showed a very similar trend. The difference in germination was significant in just a few samplings, but not in the majority. Probit analyses of the survival curves are presented in Figure 2. Following probit analyses, survival curves converted to straight lines.

**Table 2.** Probit model parameters of four pepper cultivars produced in organic and conventional systems obtained from Figure 2

Cultivar	Seed types	Model parameters				Regression coefficient (b)
		Ki	P <sub>50</sub>	R <sup>2</sup>	$\sigma$	
'Surmeli'	Organic	1,36	23,3	0,84	17,1	0.058
	Conv.	1,39	23,1	0,81	16,6	0.060
'K. Dolma'	Organic	1,49	21,4	0,84	12,4	0.069
	Conv.	1,61	23,7	0,88	16,6	0.067
'Yaglik'	Organic	1,60	34,9	0,74	21,6	0.045
	Conv.	1,66	39,7	0,85	23,9	0.041
'Corbaci'	Organic	1,88	43,4	0,81	23,0	0.043
	Conv.	1,87	40,2	0,81	21,3	0.046

**Ki:** The intercept on the probit viability axis of the regression line, or an index of the initial seed quality **P<sub>50</sub>:** Half viability period;  **$\sigma$ :** The days required to lose 1 probit of seed viability, **R<sup>2</sup>:** The coefficient of determination of the probit regression line

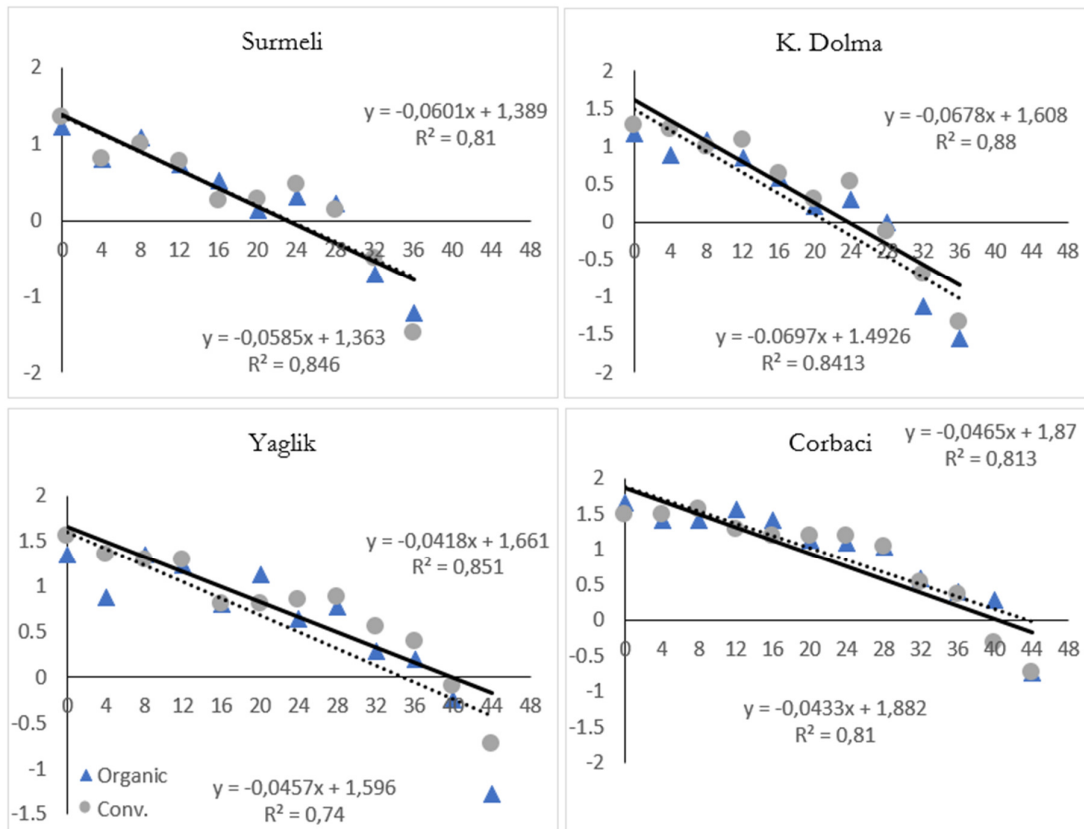


**Figure 1.** Survival curves of seeds of organically ( $\Delta$ ) and conventionally ( $\bullet$ ) grown pepper cultivars, 'Surmeli', 'K. Dolma', 'Yaglik' and 'Corbaci' stored at  $20\pm 2$  °C with precise storage moisture contents

The greatest value of  $K_i$  was obtained from Corbaci as 1.87-1.88 in conventional and organic seeds, respectively (Table 2). The other cultivars were ranked as 'Yaglik', 'K. Dolma' and 'Surmeli' regarding  $K_i$  values. The minimum  $K_i$  value was observed in the Surmeli cultivar. Lower  $K_i$  values were related to shorter longevity as indicated by P50 and  $\sigma$  (Figure 4). There were differences among the cultivars but statistically there were no differences between organic and conventional seeds. 'Corbaci' also had the longest longevity as seen by P50. It was followed by 'Yaglik' and 'Surmeli' which had the lowest values of 23.3 and 23.1 months in organic and conventional seeds. The  $\sigma$  values were larger (viability loss was slower) for 'Corbaci' and 'Yaglik' cultivars than for 'Surmeli' and 'K. Dolma'. There was no difference between  $\sigma$  values in relation to organic and conventional seeds (Table 2). Coefficient of regression (b) values also showed the rate of ageing was lowest in 'Corbaci' and 'Yaglik', but higher in 'Surmeli' and 'K. Dolma'.

Regarding seed vigour, mean germination time increased (Figures 3) as storage time extended. As the seeds were aged during storage, mean germination time increased from about 4-6 days to about 9-10 days. The difference between organic and conventional seeds was not significant in the majority of the samples. The greatest differences were seen in the 'Yaglik' cultivar in which organic seeds showed a more vigorous behaviour

during storage. Organic and conventional seed vigour was very similar in most of the samples, but the highest differences were seen in the ‘Yaglik’ cultivar.

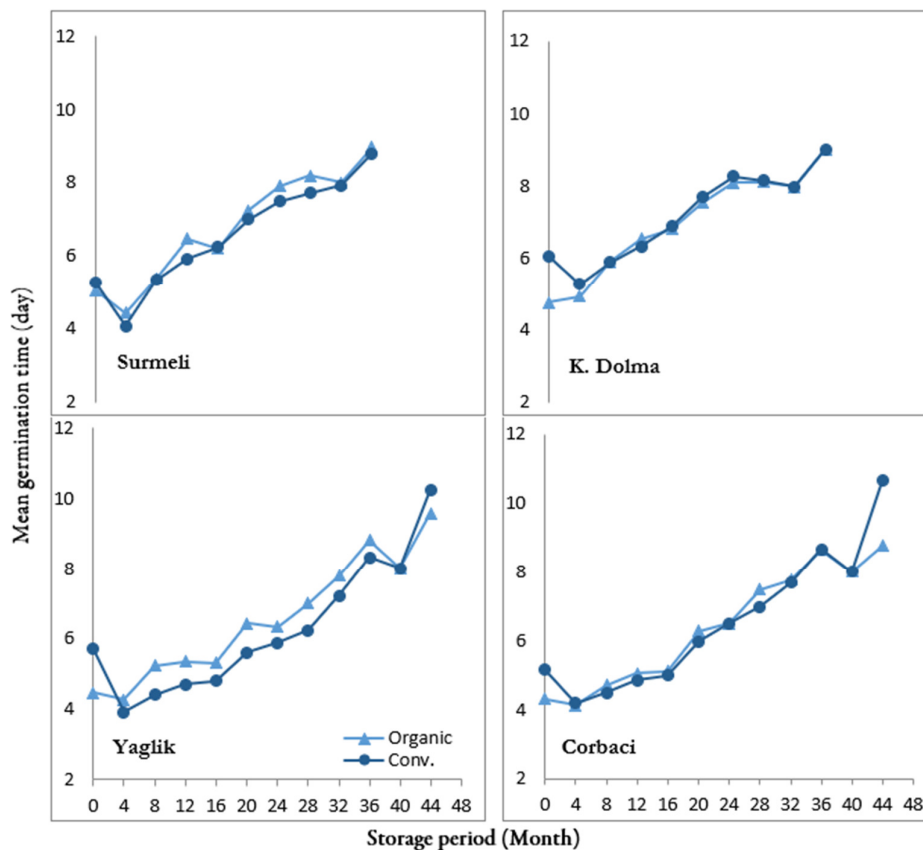


**Figure 2.** Probit values of germination percentages of survival curves in Figure 1. The estimates of  $K_i$ ,  $P_{50}$ ,  $\sigma$  and regression values were shown in Table 2

### Discussion

The results of the study showed that the seed longevity of organic and conventional seeds was similar in four pepper cultivars. Differences were seen between the cultivars in terms of longevity. ‘Surmeli’ and ‘K. Dolma’ cultivars had faster seed deterioration compared to those of ‘Yaglik’ and ‘Corbaci’. The longevity of any seed lot depends on various factors. One of which is the genetic structure (Wiebach *et al.*, 2019; Zhou *et al.*, 2019). Some species deteriorate faster than others. Pepper is also considered to be a faster deteriorating species (Passam and Lambropoulos, 1997). This is in overall agreement with comparisons of species concerning seed longevity by Priestley (1986). Ellis (1991) stated that some oily seeds such as lettuce and onion have shorter life-span than starchy ones such as peas, cereals and beans. But the conclusions of some reports contradict this finding. In our earlier work, watermelon seeds with higher oil content than pepper survived longer (Ozcoban and Demir, 2002). It can be stated that as the duration of seed storage rises, high amounts of peroxidation and oxidation of the lipids in the seed tends to reduce the volume of unsaturated fatty acids and soluble sugars (Li *et al.*, 2005). The rapid decline in seed viability in some cultivars or species compared to others requires special attention for some cultivar seeds. Incidence ageing is common in hot and humid environments particularly for pepper seeds that are grown in such regions.

Organic farming is gaining broad recognition as a system and the demand for organic products is constantly increasing due to perception by consumers as healthier and safer for the environment (Knight and Newman, 2013). Farmers are challenged by obtaining organic seeds with high quality. Consecutively, there has been significant progress in increasing the availability of organic seeds. Investments in organic seed research are rising (Anonymous, 2016). Therefore, the storage potential of organic seeds needs attention. Vegetables including peppers are grown by transplants and high-quality transplant production depends on the use of high-quality seeds (Groot *et al.*, 2005) because fast and uniform emergence is provided by use of highly vigorous seeds. The necessity to store seeds for use in the next production year may cause deterioration during the storage period, which is the main reason for low emergence and transplant size (Sano *et al.*, 2016). In our study, the gradual increase in MGT and reduction indicates that seeds lose emergence and transplant production potential (vigour) as storage extends. This can be an important aspect for production of organic transplant production (Rosso, 2005). High quality seeds are vital for successful transplant production in modules and crop establishment and weed competitiveness during the early stages of crop growth. Improved seed longevity in organic seeds is even more crucial (Groot *et al.*, 2004).

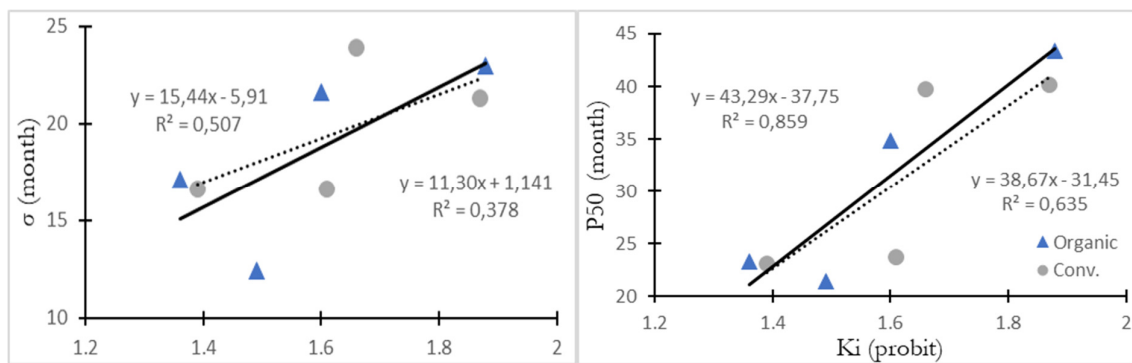


**Figure 3.** Changes in mean germination times of four pepper cultivars grown organically(Δ) and conventionally(●) systems during storage. Calculation was not included when seed germination was null

Mean germination times were extended in both seed production types. The MGT test is a well-known vigour test that is related to longevity of pepper seeds (Demir *et al.*, 2008). Seed vigour loss precedes germination loss during storage (Powell *et al.*, 1991). Our work also confirms this finding. Continuous loss of germination time and an increase in MGT during storage was seen in Figure 3. At the initial stages of sampling (4-12 months storage), germination levels in Yaglik and Corbaci were very high. But the time to germination and MGT values are increased gradually (Figure 3). This obviously shows that seed vigour changes precede

germination loss during storage, as seen in many different crop seeds (Abba and Lovato, 1999; Garcia *et al.*, 2006; Demirkaya *et al.*, 2010; Goyoaga *et al.*, 2011; Groot *et al.*, 2015; Demir *et al.*, 2016; Yin *et al.*, 2017; Zhou *et al.*, 2019).

There is a considerable variation in seed longevity among orthodox species which makes it possible to determine trends in relative seed longevity associated with climate, production systems or country of origin, for instance (Walters *et al.*, 2005; Probert *et al.*, 2007). There is much less variation within a species or even a genus. Some studies assumed that the rate of loss, i.e. standard deviation of distribution of seed death over time, does not differ between seed lots of the same species stored at the same moisture content and temperature (Ellis and Roberts, 1980). Thus, it was suggested that only the initial quality of a seed lot of Ki determines the longevity of a seed lot of a particular species in storage. Our results showed that Ki describes the seed quality and longevity potential of any seed lot that is produced by organic or conventional methods (Figure 4). It is an indicator of the combination effect of genotype and the pre-storage environment on seed quality, and in turn longevity (Rajjou *et al.*, 2008; Kochanek *et al.*, 2010; Zhou *et al.*, 2019). Both production systems had different Ki values but similar  $\sigma$ , p50, and regression coefficient values in similar storage conditions. This indicates that utmost attention should be paid to pre-storage seed production practices during organic and conventional seed production to obtain the maximum quality and longevity.



**Figure 4.** The relationship between Ki and  $\sigma$  and P50 values derived from Table 2

The four pepper cultivars used in this work have different fruit shapes. The one with shortest longevity of ‘Surmeli’ is a long green type and ‘K. Dolma’ is a bell shape. Both lose germinability faster during storage. Different seed lots even within the same genotype can differ substantially in longevity during experimental storage under similar conditions (same temperature and seed moisture) as a result of differences in the seed production environment conditions of harvest and the post-harvest drying and storage conditions (Ellis and Roberts, 1981). The seed lots were produced under either organic or conventional conditions and dried under identical conditions. The other difference can originate from maturation stages. Even though we harvested fruits when they were in the bright red mature stage, some cultivars may need longer maturation time on the mother plant to obtain maximum longevity (Zanakis *et al.*, 1993).

## Conclusions

In conclusion, this study indicates that organic pepper seeds had the same seed longevity as conventionally grown ones. The basic differences in longevity originated from cultivar differences and genetic backgrounds. Accordingly, in spite of the fact that all the cultivation processes were the same within the production systems, the responses of the cultivars to longevity can still be different. Pre-storage factors and genetic structure should be taken into account when considering pepper seed longevity.



### Authors' Contributions

KCY and ID were planned the experiments, discussed and interpreted the obtained results, like wise, KCY was designed manuscript according to writing style for the journal, EO was statistically analyzed the data and created the figures, ZG was controlled the manuscript in terms of grammatically and modal/formal convenience, errors, etc. All authors read and approved the final manuscript.

### Acknowledgements

This study was supported by the Ministry of Agriculture and Forestry association with Degirmen Eco-Investment Company – Turkey.

### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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