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Enhancing Vigor of Cucumber (*Cucumis sativus* L.) Seeds Through Osmo-priming

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Abstract

Cucumber production relies heavily on high-quality seeds, yet prolonged storage often leads to a significant decline in seed vigor and viability, characterized by reduced germination and non-uniform growth. To address this challenge, invigoration techniques through seed priming offer a promising solution to revitalize aged seeds, which also aligns with global sustainability efforts in preserving genetic resources and ensuring food security. This study aimed to evaluate the effectiveness of various invigoration treatments in enhancing the viability and vigor of expired cucumber seeds from different storage periods. The research utilized a factorial split-plot design with two factors, consisting of invigoration treatments (Control, KH_2PO_4, PEG, and Distilled Water) and storage periods (9 years, 7 years, and 5 years). The variables observed included radicle emergence (RE) at 24 and 48 hours, germination capacity, percentage of abnormal seedlings, and vigor index. The results showed that all invigoration treatments significantly improved seed performance compared to the control. The KH_2PO_4 treatment was the most effective for rapid improvement within 24 hours, showing a strong correlation (R=0.99) with increased vigor and a reduction in abnormal seedlings (R=0.85). However, distilled water showed the best performance for 48-hour RE, particularly when applied to 5-year stored seeds, reaching an 88% germination rate and the highest vigor index of 80%. In conclusion, invigoration is a viable method to restore the quality of aged seeds, with KH_2PO_4 and distilled water being recommended for optimizing the performance of specific seed lots. Future studies could further explore the field establishment of these invigorated seeds under diverse environmental stress conditions.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an economically important vegetable widely cultivated in open fields and greenhouses. The cucumber variety Zatavy F1 is widely cultivated in Lampung, Indonesia, due to its exceptionally high market demand as a fresh vegetable (side dish/lalapan). In Lampung, cucumber production contributes to the regional vegetable output, which reached approximately 5.23 tons in 2023 (BPS Lampung, 2024). This variety is preferred by local farmers and consumers for several key reasons, including its high resistance to the Gemini virus and Downy Mildew, which are prevalent in Lampung's lowlands, its high yield potential of up to 70–80 tons per hectare, and its fruits characteristics of cylindrical shape with a deep green color that remains crisp, meeting the specific aesthetic and taste preferences of the local market. Successful cucumber production relies heavily on high-quality seeds that yield uniform, healthy seedlings. However, a major challenge is the decline in seed quality due to prolonged storage. Expired seeds generally have low vigor, characterized by reduced germination and non-uniform seedling growth. A loss of vigor and viability is characteristic of seeds stored past their expiration date, often hindering sprout

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development through difficulties in water absorption (Kamson et al., 2021). This degradation in seed quality is objectively measured by a significant reduction in germination rates or a complete failure to germinate (Amany et al., 2025).

The solution to enhancing the viability and vigor of aged seeds is invigoration. Invigoration techniques, which are pre-sowing seed treatments to enhance field performance (including germination, emergence, and early growth), generally encompass uncontrolled hydration (like pre-soaking), controlled hydration (known as seed priming), thermal treatments (such as chilling or drought), and coating (Farooq et al., 2009). Invigoration is a pre-sowing treatment that aims to improve seed quality by activating pre-germination metabolism (extending imbibition Phase II) (Demir et al., 2023). This process involves controlled hydration, where seeds are allowed to absorb water to a level sufficient to trigger initial metabolic processes like DNA repair, enzyme and hormone activation, but not enough to cause radicle emergence (Imbibition phase III). Invigoration can increase germination speed and uniformity, seedling vigor, and field performance, especially for low-vigor seeds or those stored under suboptimal conditions (Araújo et al., 2016). According to Ilyas (2012), seed priming is a commonly employed technique to improve seed germination potential. A notable method within this category is osmo-priming, a pre-sowing strategy that involves subjecting seeds to controlled partial hydration.

One common invigoration technique is osmo-priming. Osmo-priming as a type of invigorating technique involves soaking seeds in an osmotic solution, such as polyethylene glycol (PEG) or inorganic salt solutions like KNO_3 and KH_2PO_4 (chemo-priming), allowing the seeds to undergo metabolic "repair" before sowing (Anwar, 2020). Demir (2023) mentions that the effect of invigoration can be evaluated through the rapid Radicle Emergence (RE) test, which is a quick and simple vigor assay. While a full germination test takes a long time, the RE test measures the percentage of seeds whose radicle reaches a minimum length of 2 mm within a short period. Radicle emergence test has been found applicable to predict seedling emergence in various crops (Matthews et al., 2011). RE is validated as a vigor test to indicate field emergence in maize and oilseed rape (ISTA, 2024). Demir (2023) researches developed the best way RE and invigoration of cucumber seed. Mavi et al. (2016) concluded that the rate of RE would be worth investigating for the prediction of normal seedlings in commercially available lots. Studies on various crops, including rice and cucumber, show that the RE test has a high correlation with other seed quality parameters and field performance. Specifically, RE percentage is positively correlated with germination capacity and field emergence and strongly negatively correlated with mean germination time (MGT), meaning faster radicle emergence indicates higher seed vigor. This technique contributes to a substantial improvement in final emergence, a reduction in the mean emergence time, and enhanced germination performance in both tomato and kellus (Ali et al., 2020; Ahmadi et al., 2023).

Based on the problem of vigor decline in long-stored cucumber seeds and the potential of invigoration to improve it, this research was conducted. This study aimed to evaluate the effectiveness of invigoration treatments in enhancing the viability and vigor of expired cucumber seeds. This research aligns with the United Nations' Sustainable Development Goal 2, which is Zero Hunger, as improving seed quality from aged stocks is essential for maintaining agricultural productivity and preserving vital genetic resources. By revitalizing and replanting expired seeds, this study directly contributes to SDG 2 by ensuring that valuable plant genetic resources are not lost, thereby strengthening local food security. Furthermore, this approach aligns with SDG 12 (Responsible Consumption and Production) by promoting resource efficiency and reducing agricultural waste as it transforms degraded materials into productive assets. Cucumber seeds from three different lots, stored since 2016, 2018, and 2020, were used to represent varying degrees of deterioration. These seeds underwent invigoration treatments to assess their effects on germination and seed vigor parameters.

MATERIALS AND METHODS

Experimental Time and Place

This research was conducted from June to August 2025 at the Seed Science Laboratory, Seed Technology Study Program, Department of Food Crop Cultivation, Lampung State Polytechnic. Cucumber seeds (*Cucumis sativus*) variety Zatavy F1 were harvested in 2016, 2018, and 2020. The seeds were stored at -4^{o}C in a freezer for 9, 7, and 5 years, respectively.

Experimental Design

The treatments were arranged in a completely randomized factorial split-plot design. The first factor was invigorating treatment, which consisted of four levels: Control (I1), KH_2PO_4 (I2), PEG (I3), and Distilled Water (I4). The second factor was seed storage period, which consisted of three levels: 9 years (T1), 7 years (T2), and 5 years (T3). Invigoration treatments were assigned to the main plots, while storage period treatments were allocated to the sub-plots. The experiment was replicated six times, with 25 seeds per replicate, resulting in a total of 72 experimental units. The seeds were soaked in this solution for 24 hours.

Seed Preparation

Cucumber seeds were extracted from fruits and then soaked in 1% HCl for 1 hour, washed and dried until the moisture content reached 9% (Pradana et al., 2022). The germination test was carried out using the top paper test method (ISTA, 2024) using the top paper method with filter paper. Germination was performed in seed germination boxes (17 cm x 5 cm) containing 25 seeds per box with 6 replicates, utilizing one sheet of filter paper and one sheet of tissue towel. The seeds were germinated at room temperature of 25^{o}C and relative humidity 70 -80%.

Experimental Procedure

Observations were made on radicle emergence, germination capacity, abnormal seedlings percentage, and vigor index. Based on ISTA 2024, RE (radicle emergence) was calculated in frequent counts of radicle emergence after 24 hours and 48 hours. Observation was finished when the radicle approached 2 mm. Based on ISTA 2024, the first observation of the germination of cucumbers was on the 4th day, and the final count observation was on the 8th day. Abnormal seedling percentage was determined by counting the seeds that did not germinate normally on the 8th day. Vigor index was determined using the percentage of normal seedlings in the first count on the 4th day.

Statistical Analysis

Data analysis was performed using MINITAB 22 Statistical Software with ANOVA test at α=5% for testing the comparison of treatment means. If the results showed a significant effect, further testing was conducted using Tukey Test/Honestly Significant Differences (HSD) at α=5% level to determine the difference in means between treatments. A linear regression analysis using Google Spreadsheet was used to determine relationship between radicle emergence test and normal seedling germination and vigor index.

RESULTS AND DISCUSSION

Radicle Emergence

Analysis of variance showed highly significant effects on the radicle emergence in 24-hour and 48-hour treatment of the invigoration and storage period. Interaction between invigoration and storage period showed a highly significant effect below 0.01 (Table 1). The invigoration treatment and storage period showed a highly significant effect on radicle emergence at both 24 and 48 hours, germination capacity, abnormal seedling percentage, and the vigor index. This suggests that both factors, when acting independently, have a strong influence on these seed quality characteristics. The Interaction effect (I*T) was not significant on either germination capacity or the abnormal seedling percentage. This implies that the effect of the invigoration treatment on these two specific parameters is consistent across different storage periods. Demir (2023) mention that

Table 1. Effects of invigoration treatment based on ANOVA

Source	Radicle Emergence (%)		Germination Capacity (%)	Abnormal seedling (%)	Vigor Index (%)
	24 Hours	48 Hours			
I	**	**	**	**	**
T	**	**	**	**	**
I*T	**	**	ns	ns	**
CV	1.47	0.68	6.41	21.14	9.91

Remarks: I = Invigoration, T = Storage Period, I*T = Interaction between invigoration and Storage Period. ** = Very Significant, ns = No Significant, CV = Coefficient of Variation.

radicle emergence is centered on the concept of seed vigor, which invigoration aims to enhance and which radicle emergence helps to measure. Essentially, invigoration treatments improve seed vigor, and a primary indicator of high vigor is rapid and uniform radicle emergence. Ozden et al. (2018) noted that this may also apply to other crops where the number of early-emerging normal seedlings is considered a marker of high quality, as, based on our observations and those of many seed analysts, seeds that produce normal seedlings early are among the earliest to produce radicles.

Invigoration Treatment

The I2 (KH_2PO_4), I3 (PEG), and I4 (distilled water) treatments consistently performed better than the control (I1) across all parameters. Specifically, I2 (KH_2PO_4) treatment showed the highest radicle emergence at 48 hours (98.88%) and germination capacity (82.44%). It also had the lowest percentage of abnormal seedlings (17.55%) and vigor index (62.88). Meanwhile, I4 also showed the highest vigor index than others treatment. Overall improvements over the control, KH_2PO_4, PEG and distilled water showed the higher result of radicle emergence, germination and vigor index (Table 2). The seeds stored from 206 shoed the lowest value of radicle emergence at 24 hours. Invigoration positively affected the germination process of aged seeds, in contrast to non-aged seeds where invigoration methods caused germination reduction compared to control, by 4.12% (water) and 9.3% (KH_2PO_4) (Jovičić, 2022). Kamanga (2024) states that seed invigoration results in the germination both in the laboratory and the field, reducing the number of days to a single count of RE and increasing the number of normal germinated seedlings, which is associated with seed strengthening which helps in improving seed quality. According to Pavan (2021), chemicals such as PEG-6000ppm and potassium dihydrate orthophosphate KH_2PO_4 are used as osmotic solutions in this priming phase, allowing the seeds to easily absorb water and initiate germination while preventing radicle protrusion through the seed coat.

Interaction Between Invigoration and Storage Period

The data showed that the seeds stored from 2016 had the lowest viability gemination and vigor index. Invigoration applied in all storage periods showed a general improvement performance over the time. The best treatment, which is distilled water, showed germination capacity from 88%. The lowest value was observed in the seeds stored from 206 treated with PEG. The most effective combinations for germination capacity were I2T2 (84.66%) and I4T2 (86%), showing that these treatments were highly effective in the second year of storage. Regarding Abnormal Seedling Percentage, the I2T2 (15.33%), I4T2 (14%), and I4T3 (12%) treatment combinations showed higher percentage of normal seedlings and lower percentage of abnormal ones. The I4T3 combination (distilled water and 2020 storage period) resulted in the highest vigor index of 80%, which was statistically significantly better than all other combinations (Table 3). Ozden (2020) mention that a longer ageing period increases the level of aging and decreases the potential for normal germination in all seed lots. However, the differences in how each seed lot responds to aging show the

Table 2. Effects of the invigoration and storage period on the seed quality

Treatment	Radicle Emergence (%)		Germination Capacity (%)	Abnormal Seedling (%)	Vigor Index (%)
	24 Hours	48 Hours			
I1	53.77 b	96.22 b	68.88 c	31.11 a	60.00 b
I2	98.00 a	98.88 a	82.44 a	17.55 c	62.88 b
I3	95.77 a	97.77 ab	73.55 b	26.44 b	53.77 c
I4	96.66 a	98.88 a	82.00 a	18.00 c	72.88 a
T1	75.66 c	98.00 ab	69.16 c	30.83 a	51.66 c
T2	82.83 b	96.16 b	78.66 b	21.30 b	64.66 b
T3	99.66 a	99.66 a	82.33 a	17.66 c	70.83 a

Remarks: I = Invigoration, T = Storage Period. I1= Control, I2=KH_2PO_4, I3=PEG, I4=distilled water, T1= 9 years, T2=7 years, T3=5 years. Means followed by the same letters in the same column are not significantly different according to HSD (α 5%).

Table 3. Interaction effects of the invigoration and storage period on the seed quality

Treatment	Radicle Emergence (%)		Germination Capacity (%)	Abnormal Seedling (%)	Vigor Index (%)
	24 Hours	48 Hours			
I1T1	14.00 c	97.33 a	64.00 f	36.00 a	56.00 cd
I1T2	48.00 b	92.00 b	68.00 ef	32.00 a	56.66 cd
I1T3	99.30 a	99.30 a	74.66 de	25.33 bc	67.33 bc
I2T1	96.00 a	99.33 a	76.66 bcde	23.33 bcde	49.33 de
I2T2	98.00 a	97.33 a	84.66 abc	15.33 def	62.66 c
I2T3	100.00 a	100.00 a	86.00 ab	14.00 ef	76.66 ab
I3T1	96.66 a	98.00 a	64.00 f	36.00 a	40.00 e
I3T2	98.00 a	96.00 ab	76.00 cde	24.00 bcd	62.00 c
I3T3	100.00 a	99.33 a	80.66 bcd	19.30 cdef	59.33 cd
I4T1	96.00 a	97.33 a	72.00 def	28.00 abc	61.33 cd
I4T2	94.00 a	99.33 a	86.00 ab	14.00 ef	77.33 ab
I4T3	100.00 a	100.00 a	88.00 a	12.00 f	80.00 a

Remarks: I1= Control, I2=KH_2PO_4, I3=PEG, I4=distilled water, T1= 9 years, T2=7 years, T3=5 years. Means in followed by the same letters in the same column are not significantly different according to HSD (α 5%)

different levels of vigor that the seeds had before the test. The longer the seeds are stored, the more their viability and vigor will decrease. According to Pramoro (2019), the duration of storage and room temperature are the main factors that cause seed deterioration and decrease seed vigor. Mavi et al. (2016) suggest that the radicle emergence test provides small laboratories with the opportunity to gain early information on the eventual count of normal seedlings. The results of this study is in line with the results obtained by Brar et al. (2019), in which invigoration using KH_2PO_4 at 0.5% significantly reduced cell membrane leakage and increased germination. According to Jovičić (2022), in seeds that have aged, a solution of monopotassium phosphate (KH_2PO_4) was found to significantly boost the length of both shoots and roots. Interestingly, when the seeds were treated with only water, it led to an increase in root length but not shoot length when compared to the control group. The study also indicated that the conditions under which the seeds deteriorated were the primary cause for the observed variations in the fresh weight of the shoots and roots of the resulting seedlings.

Correlation Values between Radicle Emergence and Viability Parameters

Regression analysis examined the relationship between the percentage of radicle emergence 24 hours and the percentage of germination as affected by four different seed treatments (Control, KH_2PO_4, PEG, and distilled water). All regression showed

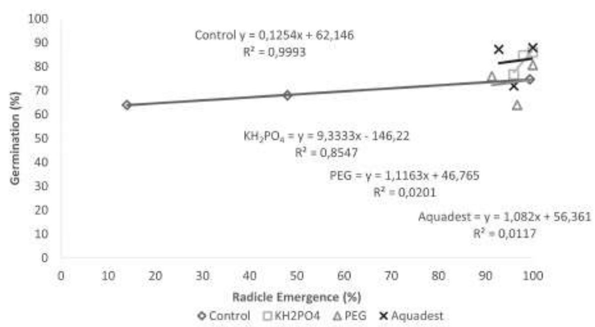

Figure 1. Linier regression between germination with 24 hours Radicle Emergence

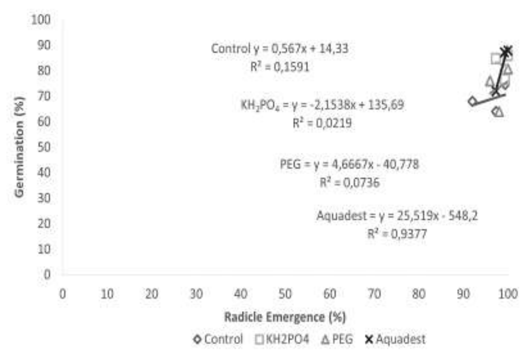

Figure 2. Linier regression between germination with 48 hours Radicle Emergence

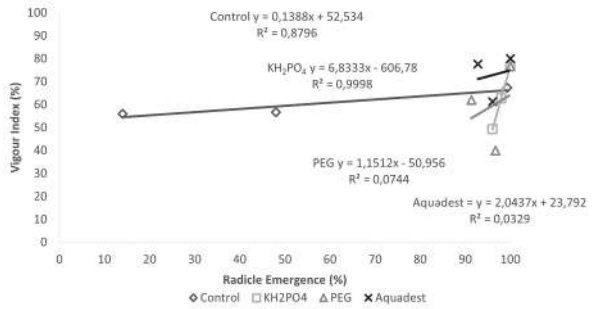

Figure 3. Linier regression between vigour index with 24 hours Radicle Emergence

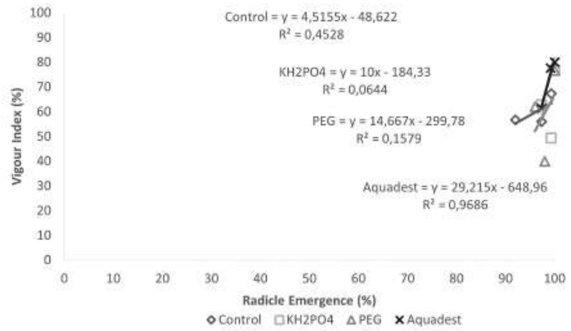

Figure 4. Linier regression between vigour index with 48 hours Radicle Emergence

positive correlation between germination and radicle emergence. The treatment using distilled water and PEG showed weak correlation with coefficients of correlation of R=0.01 and R=0.02, respectively. The best positive and strong correlation was demonstrated in control and KH_2PO_4, with a coefficient of correlation of R = 0.99 (Figure 1). Pandey (2017) mentions priming with chemicals appears beneficial as there was high percent vigor when compared to priming with water (hydropriming). Using chemicals, the germination increased by 95%, compared to only 65% before treatment. The regression analysis revealed a strong positive correlation between the percentage of radicle emergence (RE) after 48 hours and the final germination percentage. The high R-value of 0.93 indicates that 93% of the variability in germination can be explained by the variation in 48-hour RE (Figure 2) demonstrating a robust and reliable relationship. Ozden et al. (2019) mention that lower germination percentage and lower RE showed values were also indicated by seed ageing during storage. Notably, the aquadest (distilled water) treatment exhibited the most favorable results, suggesting that a non-stressful, optimal hydration environment during RE 48 hours is a critical predictor of successful germination. Seed priming induces an overlap of various mechanisms, including efficient water absorption to an extent that allows activation of pre-germinative metabolism, but insufficient water to complete radicle emergence (Jovičić, 2022).

The analysis of the vigor index following a 24-hour RE treatment showed that the potassium dihydrogen phosphate (KH_2PO_4) treatment had a positive and strong correlation with the increased seed vigor, with a regression coefficient of R= 0.99 (Figure 3). A similar result was observed in the regression analysis for abnormal seedlings after 24 hours of RE, where the KH_2PO_4 treatment was found to decrease the number of abnormal seedlings, with an R = 0.85 (Figure 5). The application of osmo-priming using chemical agents was effective in improving seed vigor that had declined during the 24-hour RE

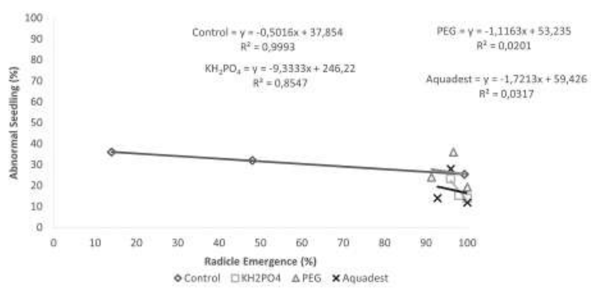

Figure 5. Linier regression between abnormal seedling with 24 hours Radicle Emergence

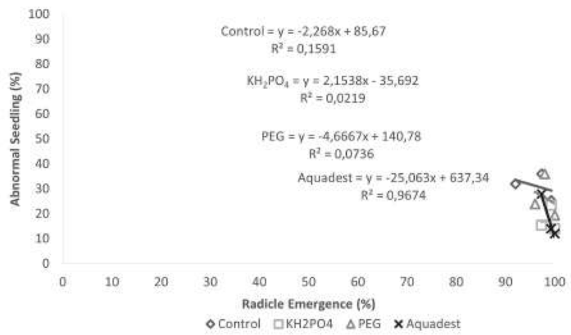

Figure 6. Linier regression between abnormal seedling with 48 hours Radicle Emergence

observation period. Ozden (2020) notes that the prediction of seedling emergence by a single count of RE after 24 h in germination tests at 25°C supports previous findings reporting that RE can distinguish differences in seedling emergence and growing potential. Nugraheni (2023) states that the beginning of germination indicates high seed vigor, while low radicle emergence indicates low seed vigor, and if the seeds have high vigor index, it aligns with germination capacity. The priming-induced enhancement in seeds is due to activation of DNA repair mechanisms, synchronization of the cell cycle in G2 and preparation to cell division (Bewley et al., 2013). Cell division starts just after radical emergence, thus, seed priming, which prolongs Phase II of seed germination and is finished just before Phase III (radicle emergence), thus does not affect cell division in itself but prepares the seed for cell division. After entering phase 3, radicle emergence becomes one of the vigor parameters.

During the 48-hour RE observation, the R values for each treatment showed a slight decrease. However, the 48-hour RE treatment with distilled water (aquadest) showed the highest result, with an R = 0.98 (Figure 4), indicating a strong positive relationship in influencing seed vigor. This trend was also observed for abnormal seedlings at 48 hours of RE, where the R = 0.96 (Figure 6). This indicates that the distilled water treatment at 48 hours of RE has a beneficial effect in suppressing the occurrence of abnormal seedlings. Since water is the main initiator of all activities in living cells, all metabolic processes occur much faster after the seed re-swelling during enhanced seed germination (Catiempo et al., 2021). Based on the data, an inverse correlation was observed between germination capacity and vigor index with the incidence of abnormal sprouts; higher values for the former correlated with a lower frequency of the latter.

CONCLUSIONS

The invigoration treatments using KH_2PO_4 increased seed vigor by increasing radicle emergence, germination capacity, and vigor index by 98.88%, 82.44%, and 62.88%, respectively. KH_2PO_4 invigoration treatments improved seed performance compared to the control, resulting in 45.13% higher RE, 16.44% higher germination capacity, and 4.58% higher vigor index. At the 24-hour observation, the highest correlation value between Radicle Emergence (24h) and Vigor Index was R = 0.99, which was due to KH_2PO_4 treatment. At 48-hour observation, the highest correlation value between Radicle Emergence (48h) and Vigor Index was R = 0.96, which was observed in distilled water treatment.

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