

Germination of Expired Spinach (*Amaranthus hybridus* L.) Seeds with Various Coconut Water (*Cocos nucifera* L.) Concentrations

Nur Malinda¹, Elvi Rusmiyanto Pancaning Wardoy², Dwi Gusmalawati^{3✉}

^{1, 2, 3} Biology Study Program, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Pontianak, Indonesia

✉ **Corresponding Author** : Dwi Gusmalawati (e-mail: dwi.gusmalawati@fmipa.untan.ac.id)

Article Information	ABSTRACT
Article History Received : June 12, 2026 Revised : July 10, 2026 Published : July 20, 2026	Spinach (<i>Amaranthus hybridus</i> L.) productivity in Indonesia declined from 170,688 tons in 2023 to 168,400 tons in 2024, a decrease partly attributed to the use of expired seeds with reduced physiological viability. Coconut water (<i>Cocos nucifera</i> L.), which contains auxin and cytokinin, has potential as a natural priming agent to restore the germination capacity of expired seeds, yet its effect at different concentrations on expired spinach seeds using the top-of-paper method has not been established. This study aimed to determine the effect of various coconut water concentrations on the germination of expired spinach seeds. A Completely Randomized Design (CRD) with five treatments K0 (control/distilled water), K1 (25%), K2 (50%), K3 (75%), and K4 (100% coconut water) was applied, each replicated five times, and data were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT). Coconut water soaking significantly increased germination percentage and germination ability, and significantly reduced abnormal sprouts and dead seeds ($p < 0.05$). The highest germination percentage (96.8%) and germination ability (92%) were both recorded at 75% coconut water concentration (K3), statistically comparable to 50% (K2) and 100% (K4). The control treatment (K0) produced the highest abnormal sprouts (8.8%) and dead seeds (15.2%), while the 25% concentration (K1) gave the fastest average germination time (2.04 days). These findings indicate that soaking in 50–75% coconut water for 6 hours is an effective, low-cost priming method for restoring the viability of expired spinach seeds and offers a practical recommendation for small-scale seed quality improvement.
Keywords: <i>Coconut Water,</i> <i>Expired Seeds,</i> <i>Germination,</i> <i>Spinach,</i> <i>Top of Paper.</i>	

INTRODUCTION

Green spinach (*Amaranthus hybridus* L.) is a type of spinach widely cultivated and consumed by the public. Spinach is high in nutrients, including vitamin A and protein, and is a primary source of iron, which helps meet daily nutritional needs (Amir et al., 2012; Gusmalawati, 2023). It also contains other minerals, such as phosphorus and calcium, which are beneficial for maintaining health (Nurmas & Fitriah, 2011). As living standards and daily nutritional needs increase, market demand for spinach is increasing. According to data from the Central Statistics Agency (BPS), national spinach production will decline in 2024 compared to the previous year, from 170,688 tons to 168,400 tons. This decline in production can be caused by various factors, one of which is seed viability, namely expired seeds. Suboptimal crop production can directly impact seed quality, as unfavorable growing conditions often result in seeds that develop poorly,

have low germination rates, and suffer from decreased physiological and morphological quality (Lama & Kune, 2016).

Seed viability is the ability of seeds to remain viable and germinate, thus reflecting the potential for seed growth and survival (Henny et al., 2023). Low seed viability is physiologically characterized by increased abnormal sprouts, decreased germination rate, loss of germination potential, and seeds being more sensitive to suboptimal environments, which can reduce crop production (Widajati et al., 2013). Good seed quality can decline if stored in poor storage or if the seeds have passed their expiration date (Ernawati et al., 2017). This decline in seed quality can be improved in several ways, including storing them under optimal conditions, harvesting seeds at physiological maturity, and soaking the seeds in water (Utomo, 2006).

Expired seeds are seeds that have passed their optimal shelf life, resulting in a decline in physiological quality, particularly viability. This seed aging process is caused by damage to cell membranes and the degradation of proteins and enzymes essential for germination. Seeds lose their metabolic capacity to maintain healthy cell structure and function, resulting in low germination rates, abnormal germination, and even embryonic death (Pirredda et al., 2023). Several methods can be used to increase seed viability, one of which is the hydropriming method (soaking in water). The hydropriming method can increase seed viability through a hydration-dehydration process, namely by soaking seeds in water to continue metabolic processes before seed germination (Najar and Bakhtiari, 2014). One ingredient that can be used for the hydropriming method is coconut water. Coconut water is an organic material containing minerals, cytokinins, auxins, phosphorus, and kinetin, which function to accelerate cell division and shoot and root growth (Fatimah, 2008).

Pagoda mustard (*Brassica narinosa*) seeds that had expired for 6 months were soaked for 6 hours in a solution of shallots, *Aloe vera*, and coconut water. Soaking in coconut water at a concentration of 50% had the best effect on increasing seed germination (Putu et al., 2020). Research by Sagita and Rahayu (2022) examined the germination of spinach (*Amaranthus* sp.) seeds after soaking in water hyacinth root extract for 2 hours, with seeds that had expired for 1 month, 3 months, and 6 months. The best concentrations for soaking spinach seeds were 75 g/L and 100 g/L, with seeds with an expiration date of 1 month yielding the best germination results.

Germination of spinach seeds soaked in coconut water using soil media affected plant height. Soaking in coconut water solution can increase germination in several types of expired seeds (Mangesa et al., 2021). Coconut water priming has increasingly been studied as a low-cost, natural alternative to synthetic plant growth regulators across various crops. Liu et al. (2022) reported that coconut water is rich in cytokinins, amino acids, and minerals that support cell division and early seedling development, reinforcing its potential as a priming agent. Nwonuala (2021) demonstrated that seed priming improved seedling emergence and early growth in bitter kola (*Garcinia kola*), indicating that priming benefits extend across diverse plant families. More recent studies have specifically confirmed coconut water's priming effect under stress conditions, such as in rice seeds subjected to chilling stress (Zhang et al., 2023), in artificially aged jujube seeds (Talpur et al., 2024), and most recently in improving germination percentage, uniformity, and mean germination time of rice seeds (Carganilla et al., 2025). However, these studies were largely conducted on non-leafy, non-naturally-expired, or artificially stressed seeds of crops such as rice, jujube, or bitter kola, and none have addressed spinach seeds that are naturally expired through normal shelf storage. In addition, prior studies on coconut water priming commonly used blotter or sand germination media, whereas the top-of-paper method using CD paper (opaque paper) which provides more uniform and controlled moisture conditions has not been applied to this particular crop-and-seed-condition combination.

There is no information available on soaking naturally expired spinach seeds in coconut water using CD paper (opaque paper), despite this method's advantage in providing controlled and reproducible moisture conditions for germination testing. This research is therefore necessary to determine the germination response of expired spinach seeds to various coconut water concentrations, which is expected to restore seed viability and fill this specific methodological and

crop-based gap in the literature. Based on this, the aim of this study was to determine the effect of soaking in various concentrations of coconut water on the germination of expired spinach seeds.

RESEARCH METHODS

Experimental Design

This study used a Completely Randomized Design (CRD) consisting of priming materials (coconut water and distilled water) with one factor, namely the concentration of young coconut water soaking. The treatment consisted of five treatments:

K0 = Control (distilled water)

K1 = 25% coconut water

K2 = 50% coconut water

K3 = 75% coconut water

K4 = 100% coconut water

Each treatment was replicated five times, resulting in 25 experimental units, each containing 25 seeds (Elfani & Jakoni, 2015).

Seed Material

The seeds used in this study were expired spinach (*Amaranthus hybridus* L.) seeds of the Maestro variety, commercially packaged under the East West Seed brand (Cap Panah Merah), obtained in an already-expired condition of approximately one year past the printed expiration date. Prior to treatment, the seeds were kept sealed in their original commercial packaging and stored at ambient room temperature to minimize further deterioration from humidity or pest exposure.

Soaking Seeds in Coconut Water

The soaking solution is made by mixing young coconut water and distilled water in the specified concentration ratios. Spinach seeds are soaked in distilled water and coconut water for 6 hours (Putu et al., 2020).

Seed Nursery

Germination testing used the top-of-paper method (ISTA, 2010). Twenty-five spinach seeds of each treatment were sown in petri dishes lined with CD paper and moistened with distilled water (until completely absorbed). The sown seeds were stored in the laboratory at 20°C to 30°C and observed on the 14th day.

Observation Parameters

The parameters observed in this study were germination percentage (GP), germination ability (GA), percentage of abnormal seedlings (PAs), dead seed percentage (DSP), average germination time (GT), hypocotyl length, and radicle length.

Germination Percentage (GP)

Germination percentage is the total number of seeds that germinated normally and abnormally divided by the total number of seeds sown. Germination percentage can be calculated using the ISTA (2019) formula as follows:

$$GP (\%) = \frac{\sum \text{Germinating seeds}}{\sum \text{The seeds that are sown}} \times 100\%$$

Description: GP. Germination Percentage

Germination Ability (GA)

Germination ability is calculated by observing seeds that germinate normally on the 14th day, calculated using the ISTA (2010) formula as follows:

$$GA (\%) = \frac{\sum \text{Normal sprouts}}{\sum \text{The seeds that are sown}} \times 100\%$$

Description: GA. Germination ability

Percentage of Abnormal Sprouts (PAs)

Abnormal sprouts were calculated by observing sprouts with deficiencies in the length of the primary root and hypocotyl that did not grow normally on the 14th day. Abnormal sprouts were calculated using the ISTA (2006) formula as follows:

$$PAs (\%) = \frac{\sum \text{Abnormal Sprouts}}{\sum \text{The seeds that are sown}} \times 100\%$$

Description. PAs: Abnormal Sprouts

Dead Seed Percentage (DSP)

Dead seeds are calculated by observing seeds that are rotten, not firm, and not fresh. The percentage of dead seeds is calculated using the ISTA (2010) formula as follows:

$$DSP (\%) = \frac{\sum \text{Dead Seeds}}{\sum \text{Seeds sown}} \times 100\%$$

Description: DSP. Dead Seed Percentage

Average Germination Time (AGT)

The average germination time is the time at which the radicle and cotyledons emerge, measured by counting the number of germinated seeds on each day of the observation period, from day 1 to day 14. The average germination time can be calculated using Sutopo (2002) formula, as follows:

$$AGT = \frac{N_1T_1 + N_2T_2 + \dots + N_{14}T_{14}}{\sum \text{Germinating seeds}}$$

Description: AGT. Average Germination Time; N. Number of seeds that germinated in a given unit; T. Number of seed testing times

Hypocotyl Length (cm)

Hypocotyl length was measured from the base of the hypocotyl to the cotyledons using a ruler/tape measure. This measurement was taken on the 14th day (ISTA, 2019).

Radicle Length (cm)

Radicle length was measured from the base of the radicle to the tip of the radicle using a ruler/tape measure. This measurement was taken on the 14th day (ISTA, 2019).

Data Analysis

The data obtained were analyzed using Analysis of Variance (ANOVA). If the results were significantly different, the Duncan Multiple Range Test (DMRT) was used. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Germination Percentage

The results showed that a 75% coconut water concentration (K3) gave the best germination percentage of 96.8%, statistically comparable to 50% (K2) and 100% (K4). This pattern reflects the dual hormonal contribution of coconut water: auxin, which accelerates cell elongation and shoot initiation, and cytokinin primarily in the form of kinetin and zeatin which stimulates cell division and reactivates the cell cycle in the embryonic axis (Rosniawaty et al., 2018). According to Fatimah (2008), coconut water can increase germination percentage when the concentration matches the physiological requirement of expired seeds, allowing the germination process to proceed smoothly. Mechanistically, this occurs because cytokinin promotes the synthesis of enzymes such as α -amylase, which hydrolyzes stored starch in the endosperm into simple sugars that fuel the resumption of respiration and ATP production in seeds whose metabolic machinery had deteriorated during expiration (Deliana et al., 2024). The germination percentage in the control treatment had the lowest value (84.8%) because the seeds used had expired, and, lacking any exogenous hormonal input, could not fully reactivate this metabolic pathway. Juanda et al. (2017) stated that the germination ability of expired seeds decreased termed deterioration causing a decline in seed quality and viability. This is consistent with the broader pattern reported by Nwonuala (2021), who found that priming treatments generally restore vigor loss associated with seed aging across diverse plant families, not only in spinach (Figure 1).

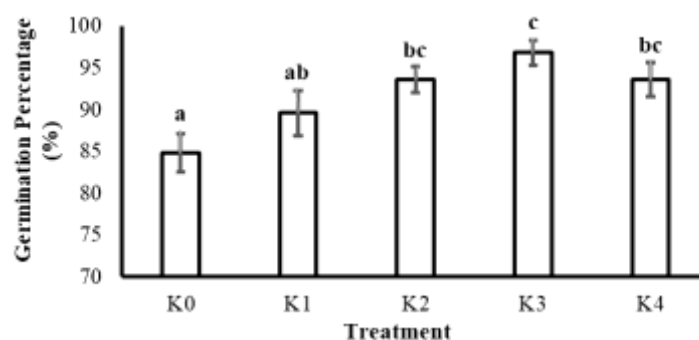


Figure 1. Germination Percentage of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

The results of the study showed that a 50% concentration of coconut water gave the best germination percentage of 93.6% (Figure 1). According to (Fatimah, 2008), coconut water can have an effect in increasing the germination percentage if the concentration used is according to the needs of expired spinach seeds so that the germination process can take place smoothly, this is because coconut water contains auxin and cytokinin hormones which function to increase cell division and accelerate the growth process of shoots and roots. The germination percentage in the control treatment had the lowest value of 84.8%, this was because the seeds used had expired. Juanda et al. (2017), stated that the germination ability of expired seeds decreased or was called deterioration, causing a decrease in seed quality and viability.

Germination Ability

ANOVA analysis results showed that coconut water soaking significantly affected germination ability ($F = 5.341$, $p = 0.004$, ANOVA). Duncan's further test showed that treatment K2 (91.2%) was significantly different from treatment K0 (76%), but not significantly different from treatments K1 (83.2%), K3 (92%), and K4 (87.2%). The germination ability analysis results showed that the highest value was found in treatment K3 (92%), and the lowest value was found in treatment K0 (76%) (Figure 2).

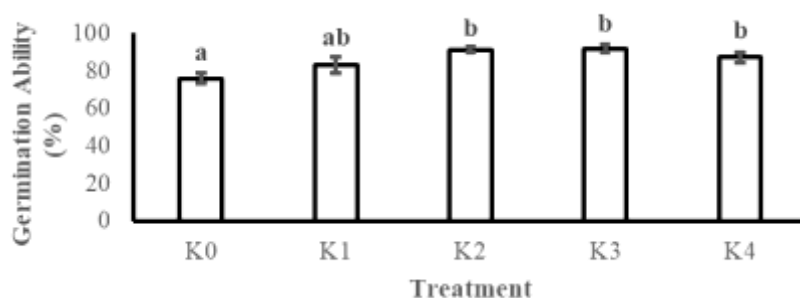


Figure 2. Germination Ability of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

The results showed that a 50–75% coconut water concentration produced the best germination ability (91.2–92%). This is because the auxin and cytokinin hormones contained in coconut water are at an optimal balance for normal seedling development at this concentration range. A 100% coconut water concentration decreased germination ability to 87.2% (Figure 2). This decline is best explained through the concept of hormonal and osmotic imbalance: at excessively high concentrations, auxin uptake into the seed becomes disproportionate relative to cytokinin, promoting the conversion of auxin precursors into ethylene, a hormone that antagonizes auxin-driven elongation and can trigger premature senescence in germinating tissue (Campbell, 2002). In addition, undiluted coconut water has a higher solute concentration, which lowers its water potential and can impose mild osmotic stress on the imbibing seed, slowing water uptake and enzymatic activation rather than accelerating it. This osmotic mechanism is consistent with the findings of Carganilla et al. (2025), who reported that coconut water concentrations of 50% or higher did not improve and sometimes reduced germination performance in rice due to osmotic stress, mirroring the plateau and slight decline observed at K4 (100%) in the present study. These results collectively indicate that the beneficial effect of coconut water on germination ability is concentration-dependent, following a bell-shaped physiological response rather than a linear one.

Abnormal Sprouts

The ANOVA analysis showed that coconut water soaking significantly affected abnormal sprouts ($F = 3.783$, $p = 0.019$, ANOVA). Duncan's further test revealed that the K0 treatment (8.8%) was significantly different from the K2 (2.4%) and K3 (4.8%) treatments, but not significantly different from the K1 (6.4%) and K4 (6.4%) treatments. The analysis of abnormal sprouts showed that the highest value was found in the K0 treatment (8.8%) and the lowest value was found in the K2 treatment (2.4%) (Figure 3).

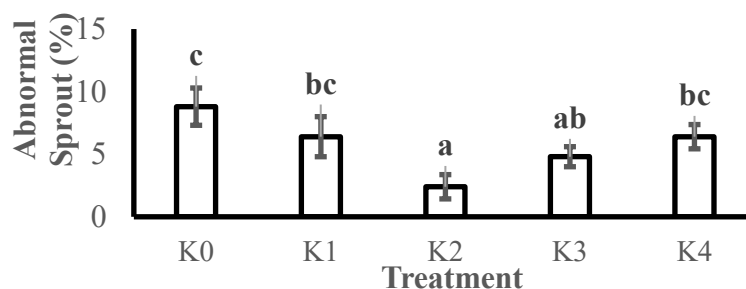


Figure 3. Percentage of Abnormal Germination of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

Observations on the control treatment showed the highest abnormal sprout rate, at 8.8% (Figure 3). This is thought to be caused by cell damage in expired seeds. Copeland (1976) stated that abnormal sprouts are caused by damage to germinating seed cells, thus inhibiting sprout growth. Another factor that causes stunted sprouts is fungal pathogen infection, which causes the

seeds to rot and die. Kamil (1982) stated that the influence of external factors such as pathogen infection (fungi or other microorganisms) during the germination test process will affect seed germination and viability. Based on this, it shows that expired spinach seeds with the control treatment have a higher abnormal sprout rate compared to the treatment given coconut water.

Dead Seedlings

The ANOVA analysis showed that soaking in coconut water significantly affected dead seedlings ($F = 4.897$, $p = 0.006$, ANOVA). Duncan's further test showed that the K0 treatment (15.2%) was significantly different from the K2 (6.4%), K3 (3.2%), and K4 (6.4%) treatments, but not significantly different from the K1 treatment (10.4%). The analysis of dead seedlings showed that the highest value was found in the K0 treatment at 15.2% and the lowest value in the K3 treatment at 3.2% (Figure 4).

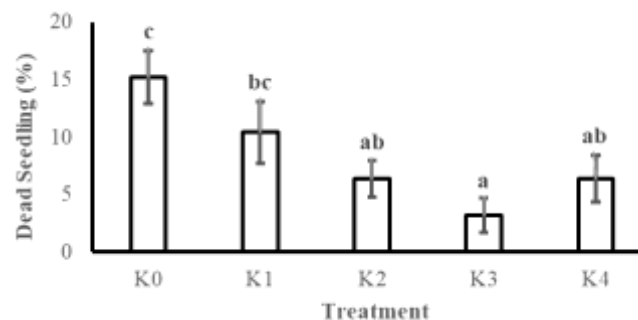


Figure 4. Percentage of Dead Seeds of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

Based on the control treatment, it was shown that 15.25% of the sprouts died in the control treatment (Figure 4.4). This was caused by expired seeds experiencing cell damage. According to Haq et al. (2024), expired seeds germinate very slowly because they are difficult to germinate. Kamil (1982) stated that the influence of external factors such as pathogen infection (fungi or other microorganisms) during the germination test process will affect seed germination and viability. Based on this, it shows that expired spinach seeds with the control treatment had a higher value of dead sprouts compared to the treatment given coconut water.

Average Germination Time

ANOVA analysis results showed that coconut water soaking significantly affected the average germination time ($F = 1.770$, $p = 0.175$, ANOVA). Duncan's further test revealed that treatment K2 (2.12 days) was significantly different from treatment K0 (2.06 days), but not significantly different from treatments K1 (2.04 days), K3 (2.10 days), and K4 (2.19 days). The analysis of average germination time showed that treatment K1 had the fastest germination time (2.04 days), and treatment K4 had the longest germination time (2.19 days) (Figure 5).

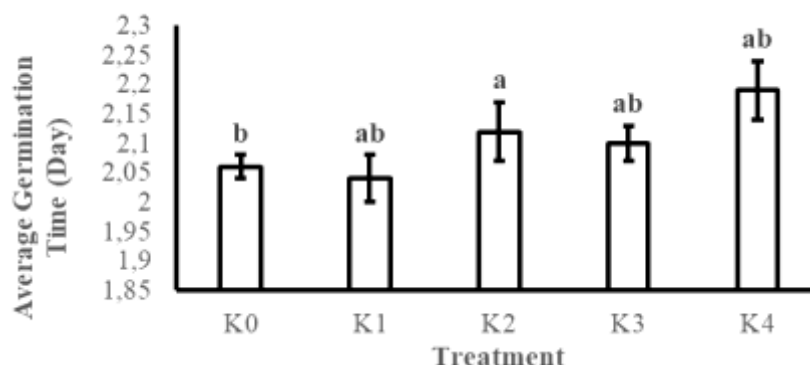


Figure 5. Average Germination Time of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

Observations at a 25% coconut water concentration showed the fastest average germination time (2.04 days), while 100% concentration showed the longest (2.19 days). This finding is best understood through the physiology of imbibition, which proceeds along a water potential gradient from the soaking medium into the seed. At lower concentrations (25%), the solution's water potential remains close to that of pure water, allowing rapid and unimpeded water uptake that quickly triggers the resumption of respiration and mobilizes stored reserves for radicle emergence. As concentration increases toward 100%, the higher solute load reduces the water potential gradient, slowing imbibition, and the correspondingly higher auxin load may induce localized oxygen depletion (anoxia) around the embryonic axis during the six-hour soaking period, further delaying the onset of active respiration (Utomo, 2006). This concentration-dependent trade-off between hormonal stimulation and osmotic/anoxic inhibition explains why the fastest and most uniform germination did not occur at the highest hormone concentration, but at a lower concentration where water availability remained the dominant driving factor. A pattern also reported by Carganilla et al. (2025) in their study of coconut water priming duration and concentration in rice.

Hypocotyl Length

The results of the ANOVA analysis showed that coconut water soaking had no significant effect on hypocotyl length ($F = 1.426$, $p = 0.262$, ANOVA). Based on the results of quantitative descriptive analysis, the average longest hypocotyl of expired spinach seeds was found in the K3 treatment (2.15 cm), while the average shortest hypocotyl of sprouts was found in the K1 treatment (2.03) (Figure 6). These results suggest that K3 is able to increase the growth of the hypocotyl length of sprouts, because the K3 treatment is able to play a role in activating the enzyme's work power thereby stimulating hypocotyl growth. Putra et al. (2022), coconut water at a concentration of 75% increases the germination of soybean seeds. Coconut water, at a concentration of 75%, has been shown to affect germination, even though the seeds used are seeds with low viability. The high germination value at a concentration of 75% is because the hormones auxin, gibberellin and cytokinin contained in coconut water are at the most acceptable concentration for germination at that concentration (Wati, 2013). According to Krishnamoorthy (1981), plant hormones play a role in cell division, controlling endosperm reserves during the early embryonic growth phase, plant growth and elongation, and the development of generative organs. According to Srilaba et al. (2018), enzyme activity can form new materials, such as increasing the size of the hypocotyl.

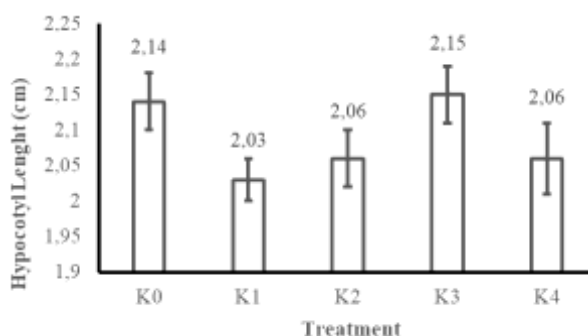


Figure 6. Hypocotyl Length of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

Radicle Length

The results of the ANOVA analysis showed that coconut water immersion had no significant effect on radicle length ($F = 0.356$, $p = 0.837$, ANOVA). Based on the results of quantitative descriptive analysis, the average length of the longest radicle of expired spinach seeds was in the K3 treatment (2.16 cm), while the K1 treatment had the lowest average radicle length (1.29 cm) (Figure 7). This indicates that different concentrations have not been able to stimulate the growth of the radicle length of expired spinach seeds. The observed radicles of the sprouts had short primary roots and no secondary roots. This also indicates that the resulting sprouts were

abnormal sprouts. Prabhandaru and Saputro (2017) stated that abnormal sprouts are characterized by short roots, no primary or secondary root growth, no cotyledons, and damaged sprouts.

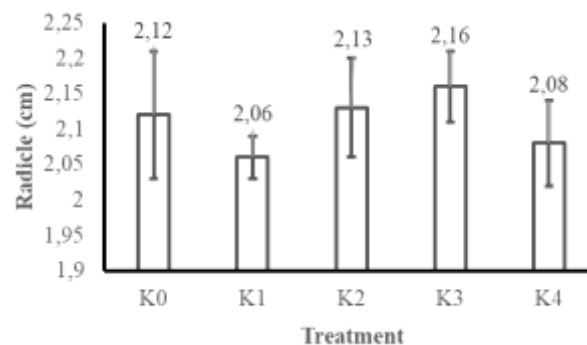


Figure 7. Radicle Length of Expired Spinach Seeds with Various Coconut Water Concentrations; K0 = Control, K1 = 25%, K2 = 50%, K3 = 75%, and K4 = 100%, Bar = Standard Error

CONCLUSION

This study demonstrates that soaking expired spinach (*Amaranthus hybridus* L.) seeds in coconut water at concentrations of 50–75% effectively restores germination percentage and germination ability to above 90%, while substantially reducing abnormal sprouts and dead seeds compared to the untreated control, following a concentration-dependent response consistent with the combined hormonal and antimicrobial action of coconut water discussed above. Beyond confirming the priming potential of coconut water, these findings contribute new evidence for a naturally expired, leafy vegetable seed evaluated using the top-of-paper method with CD papera crop-and-method combination not previously reported and offer a low-cost, accessible priming recommendation (50–75% coconut water for six hours) particularly relevant for smallholder farmers and community seed banks facing aging seed stock. Further research is recommended to validate these results under field and soil-based conditions, to narrow the optimal concentration range between 50% and 75%, and to directly measure endogenous hormone levels in soaked seeds to empirically confirm the physiological mechanisms proposed in this study.

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