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Invigoration of Expired Soybean Seeds Using Plant Extract: Effects on Viability and Growth

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ABSTRACT

Expired seeds exhibit reduced viability and are difficult to germinate; however, appropriate invigoration can partially restore performance. We evaluated the effects of plant extracts on the viability and growth of expired soybean seeds using a factorial randomized complete block design with two factors and three replicates. Factor 1 comprised extract type—distilled water (control), young coconut water (25%), bamboo shoot extract (25%), and mung bean sprout extract (25%). Factor 2 was immersion duration: 6, 12, and 18 h. Across outcomes, 25% bamboo shoot extract produced significantly higher vigor index, maximum growth potential, and pollen fertility than the control, 25% coconut water, or 25% sprout extract. A 6-h immersion maximized vigor index, maximum growth potential, plant height at 5–6 weeks after planting, and pollen fertility compared to 12 or 18 h. The combination of 25% bamboo shoot extract and 6-h immersion accelerated the onset of flowering.

Key words: expired seed, invigoration, viability, plant extract, soybean

INTRODUCTION

Soybean is an economically important legume that provides protein and functional food components, including isoflavones, which have been associated with a reduced risk of osteoporosis, cardiovascular diseases, atherosclerosis, type 2 diabetes mellitus, colorectal and prostate cancer, as well as alleviation of menopausal symptoms (Gómez-Zorita et al., 2020; Hasanah & Sembiring, 2018; Hasanah et al., 2020a; Hasanah et al., 2020b; Kim, 2021; Hu et al, 2020).

Demand continues to outpace domestic production in many regions (Baroh et al., 2022; Hasanah et al., 2019). In Indonesia, domestic soybean production reached 555,000 tons as of December 2023, while national demand was approximately 2.7 million tons (Badan Pusat Statistik, 2023). Soybean seeds deteriorate rapidly during storage. Storage temperature is a critical determinant of seed longevity: higher temperatures accelerate deterioration and reduce safe storage time, whereas cooler temperatures extend viability. Specifically, seeds stored at 25–30 °C and 80% relative humidity spoil quickly; conversely, storage for up to 10 years is possible at temperatures below 5 °C and 50% relative humidity (Gebeyaw, 2020; Martín et al., 2022; Pamungkas et al., 2025).

Expired seeds, defined as lots with reduced or degraded quality, show limited growth and yield potential when used for cultivation. Loss of viability during storage is influenced by seed moisture content, ambient temperature and humidity, microorganisms, and air composition (Han, 2024).

Invigoration treatments are used to mitigate this decline. Invigoration involves partial hydration combined with the use of plant growth regulators to restore vigor after physiological maturity (Bhattacharya et al., 2015; Bhattacharya et al., 2019). Common natural sources include young coconut water, bamboo shoot extract, and mung bean sprout extract. Coconut water is rich in natural phytohormones and stimulates growth (Tammu et al., 2022; Vijayalakshmi et al., 2022; Windarto et al., 2024).

Auxins and cytokinins, present in coconut water, bamboo shoots, and bean sprouts, regulate cell division and organ formation. Auxins further promote cell elongation, xylem and phloem differentiation, and root formation while suppressing leaf abscission, whereas cytokinins delay chlorophyll degradation (leaf senescence) and stimulate shoot initiation and expansion (Hai et al., 2020; He et al., 2021; Hussain et al., 2021; Setiawati et al., 2018).

Gibberellins, abundant in these extracts, promote stem elongation, leaf expansion, photosynthesis, and root growth (Castro-Camba et al., 2022; Shah et al., 2023). Coconut water at 25% can enhance soybean growth parameters (Putra et al., 2022), and priming for 4 to 6 h optimizes germination and yield

(Tandoh et al., 2025).

This study aimed to determine whether expired soybean seeds could be invigorated using natural extracts at 25% concentration and immersion durations of 6–18 h.

MATERIALS AND METHODS

Experimental Phases and Locations

The germination phase was conducted in the Medan Johor District, Medan, Sumatra Utara, Indonesia, while the growth and yield phases took place in a screen house at the Faculty of Agriculture, Universitas Sumatera Utara, Medan.

Plant Material and Experimental Design

Expired soybean seeds (Anjasmoro variety) labeled to expire on March 25, 2020 (1 year and 10 months past their expiration date at the time of planting) were stored at 2 °C prior to the experiments. A factorial randomized block design with two factors and three replicates was used. Factor 1 comprised the following extracts: distilled water (control), 25% young coconut water, 25% bamboo shoot extract, and 25% mung bean sprout extract. Factor 2 was immersion duration: 6, 12, 18 h. Thirteen plants per treatment were sampled.

Preparation of Extracts and Crop Management

Young coconut water (250 mL) was diluted to 25% with 750 mL of distilled water. Bamboo shoot extract was prepared by blending 250 g of shoots with 750 mL of distilled water (25%). Mung bean sprout extract was prepared in a similar manner. Seeds were pre-soaked in water for approximately 15 min and then immersed in treatment solutions for 6, 12, or 18 h.

Two seeds were sown per 10-kg polybag, and seedlings were thinned to one per polybag at 1 week after planting (WAP). Fertilizers were applied at rates of 50 kg urea ha^{-1}, 100 kg triple superphosphate (TSP) ha^{-1}, and 75 kg KCl ha^{-1}. Urea was applied in two splits: 25 kg ha^{-1} at planting and 25 kg ha^{-1} 4 WAP. TSP and KCl were applied at planting.

Plants were watered daily and weeds removed weekly. Mealybugs (*Bemisia tabaci*), armyworms (*Spodoptera litura*), and grasshoppers were controlled with deltamethrin (2 mL L^{-1}). Harvesting was carried out by cutting the main stem with scissors at a height of approximately 5 cm above soil surface. Harvest occurred 82–92 days after planting.

Study Variables and Statistical Analysis

The variables assessed in this study were vigor index (VI), maximum growth potential (MGP), plant height at 2–6 WAP, pollen fertility, and time to first flowering. VI was calculated on day 7 of germination using the following formula (Lestari et al., 2020):

$$\text{VI} = \frac{\text{Seeds that produced normal seedlings at the initial assessment}}{\sum \text{Seeds planted}} \times 100\%$$

MGP was calculated on day 14 after germination using the following formula (Lestari et al., 2020):

$$\text{MGP} = \frac{\sum \text{Seeds grown}}{\sum \text{Seeds planted}} \times 100\%$$

Plant height was recorded weekly from 2 to 6 WAP using a ruler by measuring the distance from the soil surface at the stem base to the apical growing point. Pollen fertility (PF) was assessed at first flowering by sampling fresh flower buds before anthesis. Antlers were gently excised, and pollen was dusted onto a clear microscope slide. One drop of 1% Lugol's iodine solution was added, and the preparation was covered with a cover slip. Slides were examined under a digital light microscope at 40× magnification. Pollen grains that were well-stained and round were classified as fertile, whereas grains that were unstained or withered were classified as sterile (Setiawan, 2019). PF was calculated as follows:

$$\text{Pollen Fertility (\%)} = \frac{\text{Stained/round pollen}}{\text{Total pollen}} \times 100\%$$

Data were analyzed using analysis of variance. When the F-test was significant, means were separated using Duncan's multiple range test ($\alpha \leq 0.05$). For immersion duration, orthogonal polynomial regression was fitted to model trends.

RESULTS

Vigor Index

All extracts increased VI relative to the control. The 6-h immersion yielded the highest VI, significantly higher than 18-h immersion. No significant extract × duration interaction was detected (Table 1). Increasing immersion duration reduced VI (Figure 1).

Table 1. Vigor Index (%) of Expired Soybean Seeds by Extract Type and Immersion Duration.

Extract	Duration of immersion (hours)			Mean
	6	12	18	
Distilled water (control)	33.33	26.67	16.67	25.56[b]
Coconut water (25%)	73.33	60.00	30.00	54.44[a]
Bamboo shoot extract (25%)	90.00	63.33	53.33	68.89[a]
Bean sprout extract (25%)	63.33	63.33	53.33	60.00[a]
Mean	65.00[a]	53.33[ab]	38.33[b]	

Note: Means followed by the same letter in the same row and column are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

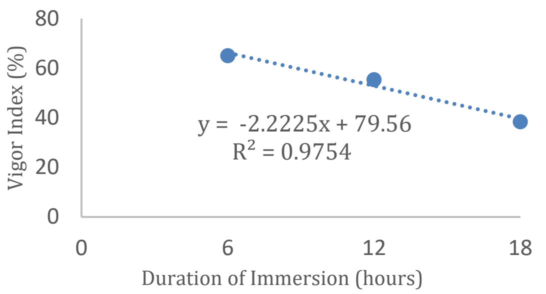

Fig .1. Linear correlation between duration of immersion and vigor index

MGP

Compared with the control, all extracts significantly increased MGP, with bamboo shoot extract showing the highest value. Immersion for 6 or 12 h outperformed 18 h. No significant interaction between extract type and immersion duration was detected (Table 2). Longer immersion was associated with reduced MGP (Figure 2).

Table 2. Maximum Growth Potential (%) by Extract Type and Immersion Duration.

Extract	Duration of Immersion (hours)			Mean
	6	12	18	
Distilled water (control)	36.67	30.00	16.67	27.78[b]
Coconut Water (25%)	76.67	73.33	40.00	63.33[a]
Bamboo shoot extract (25%)	90.00	80.00	60.00	76.67[a]
Bean sprout extract (25%)	76.67	66.67	63.33	68.89[a]
Mean	70.00[a]	62.50[ab]	45.00[b]	

Note: Means followed by the same letter in the same row or column are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

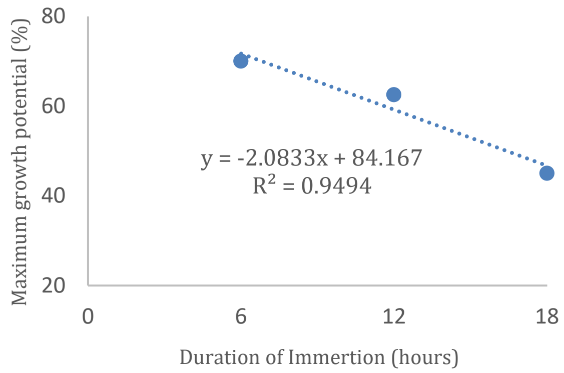

Fig. 2. Linear correlation between duration of immersion and maximum growth potential.

Plant Height

Extract type did not significantly affect plant height; however, all extracts modestly increased height relative to the control. Immersion for 6 or 12 h produced taller plants compared to 18 h, with a linear decline in height at longer immersion times (Table 3). Based on Figure 3, it can be seen that increasing the duration of immersion reduces the plant height 6 WAP.

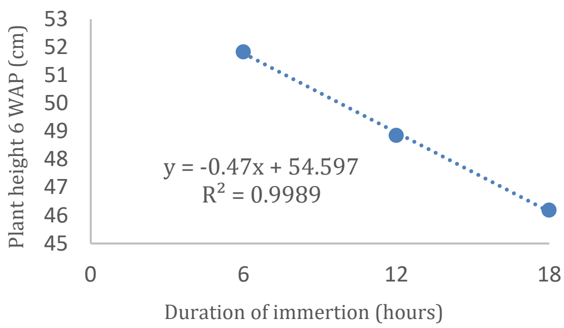

Fig. 3. Linear correlation between duration of immersion and plant height 6 WAP.

Table 3. Plant Height (cm) at 2 to 6 Weeks after Planting by Extract Type and Immersion Duration.

Week after planting	Extract	Duration of immersion (hours)			Mean
		6	12	18	
3	Distilled water (control)	21.00	19.17	19.94	20.04
	Coconut water (25%)	21.72	21.06	21.00	21.26
	Bamboo shoot extract (25%)	21.56	21.89	19.94	21.13
	Bean sprout extract (25%)	20.78	21.17	20.22	20.72
	Mean	21.26	20.82	20.28	
4	Distilled water (control)	25.78	23.11	23.33	24.07

	Coconut water (25%)	24.67	26.06	24.17	24.96
	Bamboo shoot extract (25%)	26.39	26.22	25.28	25.96
	Bean sprout extract (25%)	26.78	26.78	23.56	25.70
	Mean	25.90	25.54	24.08	
5	Distilled water (control)	38.50	30.11	31.56	33.39
	Coconut water (25%)	33.39	34.83	32.17	33.46
	Bamboo shoot extract (25%)	37.61	34.72	32.78	35.04
	Bean sprout extract (25%)	38.11	35.39	30.61	34.70
	Mean	36.90^{a}	33.76^{b}	31.78^{b}	
6	Distilled water (control)	53.28	42.56	45.83	47.22
	Coconut water (25%)	48.56	50.28	46.61	48.48
	Bamboo shoot extract (25%)	54.28	51.17	47.33	50.93
	Bean sprout extract (25%)	51.22	51.39	45.00	49.20
	Mean	51.83^{a}	48.85^{ab}	46.19^{b}	

Note: Means followed by the same letter in the same row are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

Pollen Fertility

All extracts increased PF relative to the control, with the 25% bamboo shoot extract achieving the highest mean PF. A 6-h or 12-h immersion increased PF compared to 18 h (Table 4). The interaction between extract type and immersion duration was not significant (Figure 4).

Table 4. Pollen Fertility (%) by Extract Type and Immersion Duration.

Extract	Duration of immersion (hours)			Mean
	6	12	18	
Distilled water (control)	43.50	26.73	20.29	30.17^{b}
Coconut water (25%)	46.51	47.98	24.30	39.60^{ab}
Bamboo shoot extract (25%)	70.51	40.71	46.88	52.70^{a}
Bean sprout extract (25%)	39.99	41.41	23.63	35.01^{b}
Mean	50.13^{a}	39.21^{ab}	28.78^{b}	

Note: Means followed by the same letter in the same row and column are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

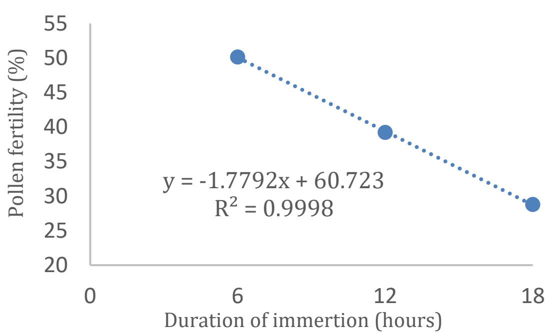

Fig. 4. Linear correlation between duration of immersion and pollen fertility (%).

Time to First Flower

Extract treatments—especially 25% bamboo shoot extract—shortened the time to first flower compared with the control. Longer immersion tended to delay flowering, although the effect was not significant (Table 5).

Table 5. Time to first flower (days after planting) by extract type and immersion duration in expired soybean seeds.

Extract	Duration of immersion (hours)			Mean
	6	12	18	
Distilled water (control)	92.67^{de}	85.33^{bcd}	95.00^{e}	91.00
Coconut water (25%)	84.33^{bc}	96.00^{e}	83.00^{ab}	87.78
Bamboo shoot extract (25%)	78.67^{a}	86.33^{bcd}	94.33^{e}	86.44
Bean sprout extract (25%)	86.00^{bcd}	84.00^{bc}	90.00^{cde}	86.67
Mean	85.42	87.92	90.58	

Note: Values followed are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

DISCUSSION

Treatment with natural hormones—particularly 25% bamboo shoot extract—increased VI, MGP, PF, and plant height at 2 and 4–6 WAP, while shortening the onset of flowering relative to the control. These responses are consistent with the relatively high concentrations of auxin, gibberellins, and cytokinins in bamboo shoot extract (Hasibuan et al., 2020) and with established physiological mechanisms whereby gibberellins upregulate hydrolytic enzymes (e.g., amylase) to mobilize reserves, support membrane stabilization and repair after storage, and promote *de novo* protein synthesis required for germination and early growth. In line with these mechanisms, Avezum et al. (2023) have shown that seed growth potential depends on enzymes such as amylase, whose activity is upregulated by gibberellin to supply energy for radicle emergence and weaken physical barriers that limit germination. Functionally, auxin supports root initiation and elongation, while gibberellin activates amylase and drives reserve mobilization, influencing the development of roots, stems, and flowers. Cytokinins promote cell division and can enhance germination.

The earliest flowering was observed with the 25% bamboo shoot extract treatment. However, flowering in expired seed lots was delayed, likely reflecting structural and physiological deterioration associated with storage aging (Adetunji et al., 2021; Ranganathan and Groot, 2023), including abnormalities in the nucleus, protoplasm, mitochondria, lysosomes, and ribosomal plastids. Suboptimal environmental conditions may have compounded these effects, as daytime temperatures approached 38.5 °C in the screen house and 34.5 °C in the field. A single, early application of gibberellin may have been insufficient to restore flowering time in expired seeds.

A 6-h immersion increased VI, MGP, plant height at 2–6 WAP, PF, and the onset of flowering. Consistent with these findings, it has been shown that optimal soaking durations maximize germination, while excessive soaking can damage the embryo or induce abnormal germination (Bhatla et al., 2023; Mokrani et al., 2022). Soaking facilitates the entry of water and oxygen, promoting metabolic activation and uniform growth (Oben et al., 2014).

A 25% bamboo shoot extract combined with 6-h soaking enabled optimal uptake of plant growth regulators, providing sufficient signaling without inducing osmotic or hormonal stress. Bamboo shoots are rich in gibberellins, which promote flowering by activating the florigen pathway. This includes FLOWERING LOCUS T, a mobile signal that moves from leaves to the shoot apical meristem to initiate inflorescence development (Liu et al., 2021). Cytokinins support meristem activity and interact with gibberellins to coordinate floral meristem development, while auxin, at appropriate levels, helps maintain apical meristem function and proper floral primordium development. Consistent with these mechanisms, seed-soaking/biopriming studies have shown that solutions enriched in auxins, cytokinins, gibberellins, and antioxidants can activate apical meristems, enhance mitotic activity, facilitate auxin-mediated cell expansion and gibberellin-mediated cell elongation, and strengthen root systems and resource uptake—collectively improving shoot growth and biomass (da Silva et al., 2024; Dugesar et al., 2025; Ellouzi et al., 2024).

CONCLUSIONS

The 25% bamboo shoot extract was the most effective treatment, producing higher VI, MGP, and PF compared to 25% young coconut water, 25% bean sprout extract, and the control. A 6-h soaking was superior to 12 and 18 h for VI, MGP, plant height at 5–6 WAP, and PF. The combination of 25% bamboo shoot extract with 6-h soaking accelerated the onset of flowering.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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