

PRIMING OF CHILI SEEDS (*Capsicum annum* L.) WITH FERN EXTRACT (*Davallia denticulata*) EXTRACTED WITH SEVERAL TYPES OF SOLVENTS

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ABSTRACT

Seed priming is a pre-sowing technique involving controlled hydration to accelerate and enhance germination. *Davallia denticulata* extract is a potential natural biostimulant for seed priming. This study evaluated the effects of solvent type and extract concentration on the germination physiology of kopay chili (*Capsicum annum* L.) seeds. A factorial Completely Randomized Design was employed, with two factors: solvent type (distilled water, methanol, ethanol, butanol) and extract concentration (0, 25, 50, and 100 mL/L). Solvent type, extract concentration, and their interaction significantly influenced germination percentage, growth rate, vigor index, root length, fresh weight, and dry weight of seedlings. The optimal response was obtained with distilled water and 25 percent extract, achieving the highest germination percentage (90%), maximum germination potential (96.67%), and growth rate (9.42% day⁻¹). Low extract concentrations (0–25%) with distilled water are recommended as the most effective combination to improve the physiological quality of kopay chili seeds through priming.

INTRODUCTION

Chili (*Capsicum annum* L.) is a leading horticultural commodity in Indonesia with significant potential as an export product. However, during the period 2018–2022, chili exports consistently remained lower than imports. The lowest export volume was recorded in 2019 at only 7.04 tons, while the highest was in 2022, with an increase reaching 48.5 tons (Manurung, 2021). Enhancing chili productivity can be achieved by improving seed viability and vigor (Afandiyah & Purnamaningsih, 2020); Sutariati et al. (2021)). Seed vigor serves as a key indicator for evaluating germination uniformity and rate, with high viability and vigor values reflecting superior seed quality (Sari et al., 2022).

Seed priming is a pre-sowing treatment in which seeds undergo controlled hydration. This technique, which involves a regulated process of hydration followed by drying, has been shown to increase the rate of germination (Rhaman et al., 2020). Types of seed priming include hydropriming, osmopriming, nutrient priming, chemical priming, biopriming, priming with plant growth regulators (PGRs), and priming with plant extracts. Several studies using plant extracts in seed priming have reported improvements in germination performance (Maurya et al., 2022).

One type of plant extract that can be used in seed priming is derived from ferns. *Davallia denticulata* is known to contain triterpenoids,

steroids, flavonoids, saponins, and tannins. Several studies on seed priming using plant extracts have demonstrated positive effects on seed germination. The study conducted by Manuswamy et al. (2019) reported that showed that seed priming of chili (*Capsicum annum* L.) with 10 percent moringa (*Moringa oleifera*) leaf extract enhanced germination and vigor index. The study by Muthuselvan et al. (2019) reported that seed priming of maize with 1 percent noni (*Morinda tinctoria* L.) leaf extract increased germination, germination rate, and dry weight.

In addition to the type of priming material and extract concentration, which can influence seed germination, the solvent used in the extraction process also affects seed priming efficacy. Commonly used polar solvents in extraction include water, methanol, ethanol, butanol, and acetic acid (Leksono et al., 2018). Each solvent has a distinct dielectric constant or degree of polarity, where a higher dielectric constant indicates a more polar solvent. Distilled water, methanol, ethanol, and acetone have dielectric constants of 80, 33, 24, and 21, respectively (Khotimah et al., 2018). Ethanol can dissolve compounds ranging from moderately polar to polar, with phenolic compounds being one of the classes of substances soluble in ethanol (Suhendra et al., 2019). Methanol is capable of extracting bioactive compounds such as phenolics, alkaloids, flavonoids, tannins, and saponins (Akasia et al., 2021). Butanol possesses polar

characteristics and can attract polar groups such as flavonoids, tannins, and phenolic compounds, which are readily soluble in n-butanol due to its polarity (Reetz and König, 2021). The aim of this study was to determine the effect of chili seed priming using *Davallia denticulata* extract obtained with different types of solvents.

METHODS

Time and Location of the Study

This study was conducted from November 2023 to February 2024 at the Plant Physiology Laboratory and Greenhouse, Department of Biology, Universitas Andalas, Padang, West Sumatra, Indonesia (latitude: -0.914518, longitude: 100.459526).

Experimental Design

The experiment employed a factorial Completely Randomized Design (CRD) with two factors. The first factor was the type of solvent: a1 = distilled water, a2 = methanol, a3 = ethanol, and a4 = butanol. The second factor was the concentration of fern extract: b0 = 0 mL/L, b1 = 25 mL/L, b2 = 50 mL/L, and b3 = 100 mL/L. The combination of these two factors resulted in 16 treatment combinations with three replications, yielding a total of 48 experimental units.

Collection of *Davallia denticulata* Leaves

Leaves were collected from the Biological Research and Education Forest and the surrounding area of Andalas University campus. The collected leaves were placed in

collection bags, then washed under running water.

Preparation of Extract

Extraction with Distilled Water

Plant extraction using the maceration technique with distilled water began with the preparation of plant material, which was first cleaned and then ground to obtain 10 g of fine material. The ground material was placed in 100 mL of distilled water and left to stand overnight. The extract was then filtered using filter paper to separate the plant simplicia. Subsequently, the extract was heated in an oven at 60°C for approximately 1 hour to evaporate the solvent, leaving the desired active compounds (Rahmawati *et al*, 2010).

Extraction with 70 percent Ethanol and 70 percent Methanol

Plant extraction using the maceration technique with 70 percent ethanol and 70 percent methanol began with the preparation of plant material, which was cleaned with water. The cleaned material was then ground to obtain 10 g of fine plant tissue. Subsequently, the ground material was mixed with 100 mL of 70 percent ethanol or 70 percent methanol and left to stand overnight, a process referred to as maceration. After maceration, the extract was filtered using filter paper to separate it from the plant residue. The filtrate was then concentrated by heating in an oven at 40°C to evaporate the solvent, yielding the desired active compounds (El-ghfar *et al.*, 2016)

Extraction with n-Butanol

Plant extraction using the maceration technique with n-butanol began with the preparation of plant material, which was cleaned with water. The cleaned material was then ground to obtain 10 g of fine plant tissue. Subsequently, the ground material was mixed with 200 mL of n-butanol and left to stand overnight, a process referred to as maceration. After maceration, the extract was filtered using filter paper to separate it from the plant residue. The filtrate was then concentrated by heating in an oven at 40°C to evaporate the solvent, leaving the desired active compounds (Phongtongpasuk and Poadang, 2014).

Application of Extract

Kopay chili seeds of uniform size were selected. The seeds were then soaked in *Davallia denticulata* extract for 3 hours according to the treatment factors.

Observation Parameters

The parameters observed in this study included germination percentage (%), growth rate (% day⁻¹), maximum germination potential (%), vigor index (%), root length (cm),

seedling freshweight (g), and seedling dry weight (g).

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA). When significant differences were detected, Duncan's New Multiple Range Test (DNMRT) at a 5 percent significance level was performed as a post hoc test.

RESULTS AND DISCUSSION

The results of the analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) conducted on chili (*Capsicum annuum* L.) seed priming with *Davallia denticulata* extract obtained using different types of solvents showed significant effects on chili seed germination. The findings are presented as follows.

Effect of Solvent Type

Table 1 shows that the type of solvent significantly affected germination percentage, growth rate, vigor index, root length, and seedling fresh weight, but had no significant effect on maximum germination potential and seedling dry weight.

Table 1. Effect of solvent type on germination percentage, maximum germination potential, growth rate, vigor index, root length, seedling fresh weight, and seedling dry weight

Treatment	Germination (%)	Maximum Germination Potential (%)	Growth Rate (% day ⁻¹)	Vigor Index (%)	Root Length (cm)	Seedling Fresh Weight (g)	Seedling Dry Weight (g)
a1 (Aquades)	85.83 b	94.17 a	9.37 b	32.22 c	1.55 b	0.24 ab	0.041 a
a2 (Metanol)	78.33 b	94.17 a	8.95 b	30.84 bc	1.51 b	0.26 b	0.043 a
a3 (Etanol)	77.50 b	90.83 a	8.69 ab	30.42 b	1.35 ab	0.25 b	0.043 a
a4 (Butanol)	65.00 a	93.33 a	8.11 a	27.96 a	1.23 a	0.22 a	0.041 a

From Table 1, it can be seen that the use of distilled water as a solvent increased the germination percentage of kopay chili seeds (85.83%), growth rate (9.37% day⁻¹), vigor index (32.22%), and root length (1.55 cm), which were significantly different from those obtained with butanol as a solvent, which resulted in a germination percentage of 65.00 percent, growth rate of 8.11 day⁻¹, vigor index of 27.96 percent, and root length of 1.23 cm. In contrast, butanol produced the lowest germination percentage, maximum germination potential, growth rate, and vigor index compared to other solvents (distilled water, methanol, and ethanol). Each solvent has distinct characteristics and varying tendencies in extracting plant metabolites. Distilled water may have produced the best germination performance because it can extract compounds that support the germination of kopay chili seeds.

The type of solvent affects the concentration of active compounds in the extract, as active compounds will dissolve more readily when their polarity is similar to that of the solvent (Sasadara & Wiranata, 2022). According to Pote *et al.* (2020), one factor influencing the success of the extraction process is the solvent used, as compounds dissolve according to their polarity. Methanol and ethanol are polar solvents capable of extracting a greater amount of secondary metabolites compared to ethyl acetate and n-hexane, which are semi-polar and non-polar solvents, respectively.

Terpenoids are among the compounds extracted by all types of solvents used. Terpenoids play a role in stimulating the activity of gibberellin hormones, which influence cell division (Suwirmen *et al.*, 2022). Gibberellins can stimulate the activity of hydrolytic enzymes, particularly α -amylase in seeds, which facilitates starch hydrolysis and accelerates seed germination (Bajafitri and Barunawati, 2018). This is consistent with the findings of Fauzi *et al.* (2019), who reported that the application of gibberellins increased the germination percentage of *Sonchus arvensis* seeds. Similarly, Triyadi *et al.* (2023) demonstrated that different priming media significantly affected germination speed, vigor index, and root length of soybean seeds. Higher seed growth rates are associated with greater seed vigor.

Effect of Extract Concentration

Table 2 shows that the application of *D. denticulata* extract concentration significantly affected germination percentage, growth rate, vigor index, root length, and seedling dry weight, whereas maximum germination potential and seedling fresh weight were not significantly affected.

From Table 2, it can be observed that a *D. denticulata* extract concentration of 0 mL/L resulted in the highest germination percentage (83.33%), growth rate (9.65% day⁻¹), vigor index (33.10%), and root length (1.59 cm). This indicates that the 0 mL/L concentration provided better results compared to the 100 percent

Table 2 Effect of extract concentration on germination percentage, maximum germination potential, growth rate, vigor index, root length, seedling fresh weight, and seedling dry weight

Treatment	Germination (%)	Maximum Germination Potential (%)	Growth Rate (% day ⁻¹)	Vigor Index (%)	Root Length (cm)	Seedling Fresh Weight (g)	Seedling Dry Weight (g)
b0 (0)	83.33 b	93.33 a	9.65 b	33.10 b	1.59 b	0.24 a	0.040 a
b1 (25)	78.33 ab	95.83 a	8.64 a	29.74 a	1.38 ab	0.25 a	0.042 ab
b2 (50)	75.83 ab	94.17 a	8.56 a	29.16 a	1.42 ab	0.24 a	0.042 ab
b3 (100)	69.17 a	89.17 a	8.27 a	29.43 a	1.23 a	0.24 a	0.044b

Note: Values followed by the same letter within the same treatment and variable indicate no significant difference according to Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

concentration. The application of biostimulants at low concentrations also tended to enhance germination in kopay chili. Concentrations of 25 percent and 50 percent showed germination potential with germination percentages of 78.83 percent and 75.83 percent, respectively. This trend was also reflected in the root length parameter, with values of 1.38 cm and 1.42 cm, respectively. The 100 percent concentration yielded the lowest germination performance compared to the lower concentrations (0%, 25%, and 50%). This suggests that excessively high concentrations of *D. denticulata* biostimulant were not able to induce germination in kopay chili seeds. Appropriate concentrations can promote germination, whereas excessively high concentrations may interfere with the germination process. According to Adnan *et al.* (2017), optimal concentrations produce favorable seed responses; concentrations that are too low may not cause significant changes, while excessively high concentrations may result in a decline in performance. The findings of Fadhilah *et al.* (2022) indicated that mung bean extract treatments with varying concentrations did not significantly affect maximum germination potential. In another

study, maize seed priming with 1 percent noni (*Morinda tinctoria* L.) leaf extract was most effective in increasing germination percentage, root length, and seedling dry weight (Muthuselvan *et al.*, 2019).

The application of *D. denticulata* extract concentration did not produce favorable effects on chili seed germination. This is presumably because the high concentrations used (25%, 50%, and 100%) allowed dissolved compounds to interfere with the germination process. According to Lubis *et al.* (2018), excessively high extract concentrations can disrupt cellular functions and inhibit seed germination, whereas concentrations that are too low have no effect, highlighting the importance of applying an optimal concentration. High concentrations of allelochemicals can cause the degradation of cell wall enzymes, thereby impairing enzyme activity and function. Enzymatic inhibition in seeds reduces the growth energy during germination, leading to a decline in the germination process (Respatie *et al.*, 2018). This finding is consistent with the study by Janusauskaite (2023), which reported that increasing sunflower extract concentration reduced pea germination. Similarly,

Patanè *et al.* (2023) demonstrated that increasing concentrations of Cannabis sativa L. extract delayed wheat seed germination.

Effect of the Interaction Between Solvent Type and Extract Concentration

Based on Table 3, the interaction between solvent type and *D. denticulata* extract concentration affected germination percentage, maximum germination potential, growth rate, vigor index, root length, seedling fresh weight, and seedling dry weight of chili seeds.

From Table 3, it can be observed that there was an interaction between the two factors—solvent type and extract concentration—on various physiological parameters of the seedlings. Treatments using distilled water

with concentrations of 0 percent (a0b0) and 25 percent (a0b1) tended to yield better results, particularly in growth rate, germination percentage, and maximum germination potential. The growth rates in a0b0 and a0b1 were high and not significantly different from other top-performing treatments, with values of 9.65 percent day⁻¹ and 9.42 percent day⁻¹, respectively. Germination percentages of 83.33 percent and 90.00 percent, along with maximum germination potentials of 93.33 percent and 96.67 percent, also demonstrated very good performance. This indicates that the interaction between distilled water as a solvent and low extract concentrations (0–25%) creates physiological conditions that optimally support the germination process.

Table 3. Effect of solvent type and *D. denticulata* extract concentration on germination percentage, maximum germination potential, growth rate, vigor index, root length, seedling fresh weight, and seedling dry weight of kopay chili seeds

Treatment	Germination (%)	Maximum Germination Potential (%)	Growth Rate (% day ⁻¹)	Vigor Index (%)	Root Length (cm)	Seedling Fresh Weight (g)	Seedling Dry Weight (g)
bo	83.33 c	93.33 ab	9.65 d	33.10 f	1.59 ab	0.24 ab	0.040 ab
a1b1	90.00 c	96.67 ab	9.42 d	32.64 ef	1.56 ab	0.23 ab	0.038 a
a1b2	86.67 c	93.33 ab	9.12 cd	31.28 def	1.55 ab	0.24 ab	0.048 c
a1b3	83.33 c	93.33 ab	9.29 d	31.87 def	1.48 ab	0.25 ab	0.040 ab
a2b0	83.33 c	93.33 ab	9.65 d	33.10 f	1.59 ab	0.24 ab	0.040 ab
a2b1	73.33 bc	96.67 ab	8.62 abcd	29.57 cde	1.64 b	0.28 b	0.043 abc
a2b2	70.00 bc	93.33 ab	8.56 abcd	29.00 bcd	1.60 ab	0.28 b	0.048 c
a2b3	86.67 c	93.33 ab	8.95 bcd	31.68 def	1.19 ab	0.25 ab	0.040 ab
a3b0	83.33 c	93.33 ab	9.65 d	33.10 f	1.59 ab	0.24 ab	0.040 ab
a3b1	76.67 bc	93.33 ab	8.37 abcd	28.91 bcd	1.16 ab	0.26 b	0.042 ab
a3b2	90.00 c	93.33 ab	9.10 cd	30.20 cdef	1.52 ab	0.25 ab	0.042 ab
a3b3	60.00 ab	83.33 a	7.62 abc	29.47 cde	1.13 ab	0.25 ab	0.045 bc
a4b0	83.33 c	93.33 ab	9.65 d	33.10 f	1.59 ab	0.24 ab	0.040 ab
a4b1	73.33 bc	93.33 ab	8.13 abcd	27.86 bc	1.16 ab	0.24 ab	0.045 bc
a4b2	56.67 ab	93.33 ab	7.44 ab	26.18 ab	1.02 a	0.20 a	0.040 ab
a4b3	46.67 a	93.33 ab	7.21 a	24.70 a	1.12 ab	0.20 a	0.042 ab

Note: Values followed by the same letter within the same treatment and variable indicate no significant difference according to Duncan's New Multiple Range Test (DNMRT) at the 5% significance level.

The use of distilled water is known to selectively and gently extract secondary metabolites, particularly polar compounds, compared to organic solvents such as ethanol, methanol, and butanol. This allows hydrophilic bioactive compounds to be available in sufficient quantities to stimulate germination without causing stress or toxicity to the seeds. In contrast, high concentrations of organic solvents (a2b3, a3b3, and a4b3) tended to reduce nearly all parameters, with germination percentages of only 46.67–60.00 percent, low growth rates (around 7.2–7.6% day⁻¹), and low vigor indices. This suggests that certain solvent types and high concentrations can interact negatively with seed physiological processes during the early germination phase.

Thus, the interaction between distilled water and low extract concentrations (0–25%) represents the most effective treatment combination for stimulating germination. This finding highlights that, in the application of bio-stimulants, not only the type of active compound but also the solvent type and application concentration must be considered. During germination, seeds still possess sufficient food reserves and active basic metabolic systems, so excessive external stimulation can disrupt their physiological balance. Seeds have natural mechanisms that regulate germination, including the availability of germination-promoting hormones. Endogenously, seeds contain gibberellin (GA_f), which plays a key role in germination. Other hormones

such as auxins and abscisic acid (ABA) also play roles in regulating germination and dormancy in seeds (Shu *et al.*, 2016).

CONCLUSION AND RECOMMENDATIONS

The type of solvent (distilled water, methanol, ethanol, and butanol), extract concentration (0%, 25%, 50%, and 100%), and their interaction were shown to influence the germination process of kopay chili seeds. Distilled water (a1) consistently produced the most optimal results across nearly all physiological parameters, including germination percentage (85.83%), growth rate (9.37% day⁻¹), and vigor index (32.22%). Treatments with low concentrations (b0 and b1) performed better than those with higher concentrations, with b0 (0%) yielding the highest growth rate and vigor index. The combination of distilled water with a 25 percent extract concentration (a1b1) was the best treatment, producing a germination percentage of 90.00 percent, maximum germination potential of 96.67 percent, and growth rate of 9.42 percent day⁻¹. Therefore, the use of distilled water with a low biostimulant concentration (0–25%) is recommended for priming kopay chili seeds.

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