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EVALUATION OF CHEMICAL TREATMENTS ON *Echinochloa crus-galli* SEED DORMANCY AND PREMILINARY ALLELOPATHIC EFFECTS OF *Aegiceras corniculatum* MACERATES ON ITS GROWTH

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ABSTRACT

Echinochloa crus-galli is a potential species for allelopathic studies due to its characteristic advantages. However, the presence of seed dormancy impedes allelopathy research. Our laboratory study was carried out to evaluate the effects of several treatments, including gibberellic acid (GA3), 98% sulfuric acid, 1% sodium hypochlorite, and co-treatment (98% H_2SO_4 + GA_3) on the germination and vigor indices of *E. crus-galli* seeds. The results indicated that there was no effect on the seed germination and growth with GA_3 treatment, whereas 1% NaClO treatments was effective only with a 160-minute treatment, with a germination index of 42.50 ± 2.09, and a vigor index of 522.24 ± 23.97. The use of 98% H_2SO_4 prior to sowing significantly increased both parameters, with effective treatment time of 10 minutes and 20–40 minutes for seeds harvested at 12 months and 3-month respectively. These findings support the hypothesis that *E. crus-galli* seeds have a physical dormancy that delays germination. Furthermore, combined treatment of 98% H_2SO_4-GA_3 25 ppm significantly enhanced seed sprouting (73.12 ± 10.64) and vitality (550.13 ± 133.93), representing the most effective dormancy-breaking method. Based on our optimized protocol findings, we further investigated the allelopathic effect of *Aegiceras corniculatum* macerate on *E. crus-galli* growth, suggesting that this mangrove species could serve as a potential donor plant for allelopathic approaches in future *E. crus-galli* control strategies.

Keywords: *Aegiceras corniculatum*, allelopathy, *Echinochloa crus-galli*, germination index, seed dormancy, vigor index.

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INTRODUCTION

Allelopathy is a natural biological process in which biochemicals released from plants or microorganisms affect either inhibitory or stimulatory effects on the germination, growth, and survival of other organisms in natural communities (Rice, 1982; Einhellig, 1995). This interaction has been documented worldwide since the 1980s (Uludag et al., 2006) and has recently become such a noticeable topic. Allelochemicals released from several weed species often interfere with the growth and development of crops, adversely affecting the quality and productivity of agricultural products (Desai et al., 2016). Applications of allelopathy have been proven in forestry (Hashoum et al., 2017), crop production (Aslam et al., 2024), and weed control (Hussain et al., 2022; El-Sheikh et al., 2023). In weed management, the use of allelochemicals instead of synthetic herbicides and pesticides represents a remarkable contribution to sustainable agriculture (Cheng & Cheng, 2015).

Echinochloa crus-galli, known as cockspur grass or barnyard grass, belongs to the Poaceae family, and the genus *Echinochloa* commonly appears in almost all agricultural regions (Holm et al., 1997), especially in rice fields in Vietnam (Tan, 2000). This weed is estimated to reduce crop yields by 50% due to the uptake 80% of nitrogen sources near the roots (Chin, 2001; Nadeem et al., 2020). Management of *E. crus-galli* is challenging due to its similar morphologies and competitive growth with crops. Fortunately, this species has several properties of a model plant in allelopathic studies because of its advantages, such as high seed production (with an average of 100,000 seeds per plant (Norris, 1992)); small seeds, worldwide distribution; and similar growth characteristics to rice. However, the germination features of this grass that are obstacles to allelopathic research, such as asynchronous seed maturation and a dormancy state being present. Seed dormancy is a stage that allows a species to adapt to unfavourable conditions, regulated by several factors such as light, temperature, dry storage duration and genetics (Bentsink & Koornneef, 2008). According to Shabbir et al. (2019), *E. crus-galli* seeds possess innate dormancy for 12–14 months after dispersal. Therefore, breaking dormancy and increasing the *E. crus-galli* germination are required for allelopathy research. Several chemicals have been tested for dormancy removal and germination promotion, including GA_3, H_2SO_4, and KNO_3 (Zhou et al., 2016).

Plant parts have been used as the sources of allelochemicals due to the substantial quantities of bioactive compounds secreted in their life cycle (Desai et al., 2016), especially mangrove plants as halophytes, containing an enriched source of specialised metabolites (Dhaou et al., 2022). *Aegiceras corniculatum*, also called Black Mangrove, River Mangrove, or Khalsi, belonging to the Myrsinaceae family, is distributed in coastal areas or mangrove forests, believed to possess several potential allelochemicals.

Therefore, this study aims to (i) investigate the seed dormancy dynamics and the most promising treatment for breaking *E. crus-galli* dormancy, and (ii) evaluate the allelopathic effects of *A. corniculatum* macerates on the growth and vigor of *E. crus-galli* seedlings. We expect that (i) the dormancy of *E. crus-galli* seeds will naturally break after a particular storage time as well as its dormancy could be overcome in a short treatment time by various chemicals, and (ii) the macerates from *A. corniculatum* fruits inhibit the growth of target species' seedlings.

MATERIALS AND METHODS

Sample collection and preparation

Only plump and mature *E. crus-galli* seeds with greyish brown color and exhibiting full structural characteristics, were harvested in Quynh Phu district, Thai Binh province, on 14th October, 2018. The study was carried out in the laboratory of the Faculty of Biology, Hanoi National University of Education, Vietnam. The seeds were dried at 40 ºC for three days to remove excess water before storage. *A. corniculatum* fruits were collected in the Xuan Thuy National Park, Nam Dinh province, on 15th September, 2018. The fruit part was reported to contain the highest total phenolic content, considered as potential allelochemicals (Mohapatra & Basak, 2021) with nearly equal concentrations in pericarps and hypocotyls (Xiang et al., 2010). In our study, we used *A. corniculatum* pericarps, which are the post-reproductive products of crypto-vivipary, potentially releasing allelopathic compounds into the surrounding environment during decomposition. The macerates were prepared by soaking 10 g of pericarps in 10 mL of distilled water (1:1 w/v) for 24 hours in the dark at room temperature (25 ºC). The supernatants were collected after centrifugation and subsequently stored in -20 ºC until use for allelopathic assessment and total phenolic content analysis.

Determination of the dynamics in dormancy of *Echinochloa crus-galli* seeds

The 0-month harvested seeds were used to investigate seed dormancy state according to time. 98% sulfuric acid (H_2SO_4) 20-minute treatment were utilized with a repetition of every 30 days.

Pre-treatments of *Echinochloa crus-galli* seeds

E. crus-galli seeds were soaked for 24h in distilled water as a control applied for comparison to all treatments. Subsequently, 100 seeds in each replicate were germinated by adding 20 mL of distilled water in the Petri-dishes. Seeds from different storage periods were soaked in various chemical treatments gibberellic acid **(**GA_3), 1% sodium hypochlorite (NaClO), 98% sulfuric acid (H_2SO_4), and 98% sulfuric acid (H_2SO_4) and 98% sulfuric acid **(**GA_3) co-treatment in triplicate to determine modes of seed dormancy - physiological or physical types. The papers in the Petri-dishes were maintained moist throughout the experiments by adding 2 mL of distilled water every day. The hierarchy overview of various treatments is shown in Figure 1.

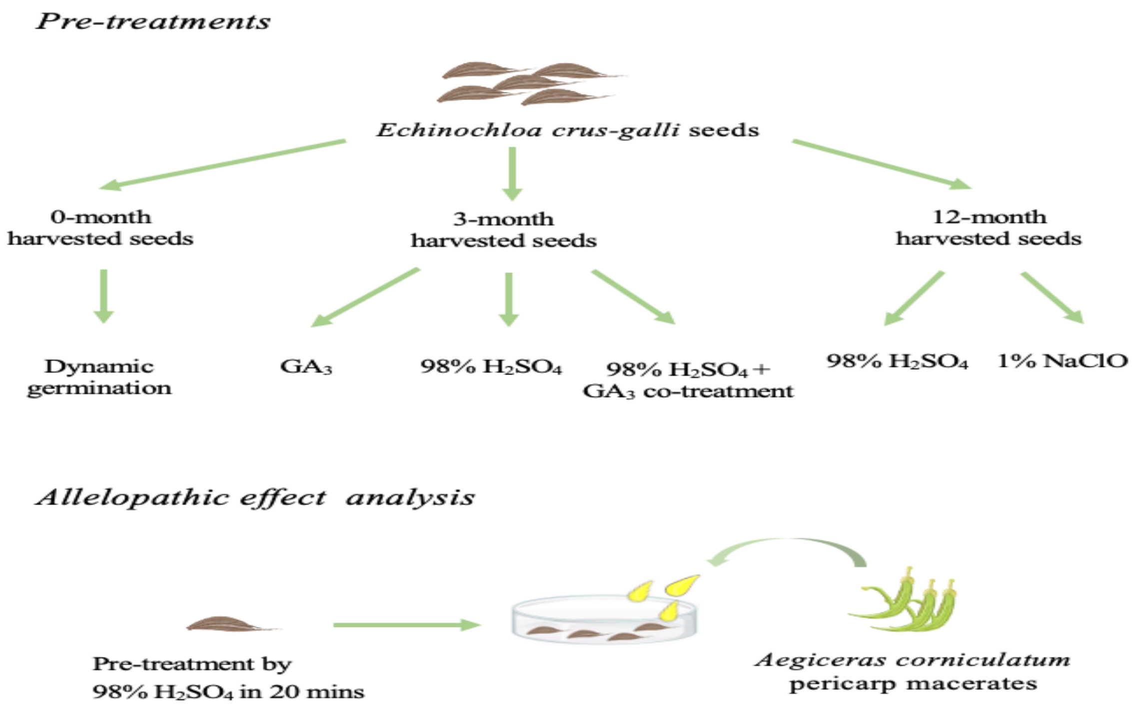

Figure 1. Hierarchy diagram of experimentations

- Treatment 1**.** Gibberellic acid **(**GA_3): The 3-month harvested seeds for gibberellic acid (GA_3) treatment were immersed in 1.5 mL at different concentrations of GA_3 at 25, 50 and

100 ppm for 24 h before germinating in the Petri-dishes.

- Treatment 2. 98% sulfuric acid (H_2SO_4): The volume of 10 mL of concentrated sulfuric acid was used for 10, 20, 40 and 80 minutes for 3-month and 12-month harvested seeds. The seeds were rinsed off three times with distilled water before a period of 24 h-soaking in 10 mL water to germinate.

- Treatment 3. 1% sodium hypochlorite (NaClO): 1% NaClO in various time courses (20, 40, 80 min, 160 min) was applied. The treated seeds were then transferred to a beaker of 10 mL of distilled water to wash off the used chemical. After that, the seeds were sown on the Petri-dishes containing moistened papers.

- Treatment 4. 98% H_2SO_4 and GA_3 co-treatment: This trial was carried out by soaking the seeds in H_2SO_4 for 20 min, and the acid was then removed by soaking in distilled water soaked for 24 h in 2 mL GA_3 (25, 50, 100 ppm) and sown on the Petri-dishes.

The germinated seed numbers and seedling length were recorded within 6 days to calculate the germination index (GI) and vigor index (VI).

The germination and vigor indices of the seedlings were calculated according to the formula of AOSA (1983).

The germination index (GI):

$$GI = \frac{\text{No. of germinated seed}(s)}{\text{Day of first count}} + ... + \frac{\text{No. of germinated seed}(s)}{\text{Day of final count}}$$

The vigor index (VI):

VI = [seedling length (cm) × germination percentage]

Where: Seedling length (cm) = Shoot length (cm) + Root length (cm)

Both the seedling length and germination percentage were calculated in the data of the last recorded day (the 6^{th} day of germination).

Bioassay

One-hundred *E. crus-galli* seeds in each replicate were treated with concentrated sulfuric acid (H2SO4) for 20 minutes. After pre-treatment, the seeds were soaked in 2 mL of *A. corniculatum* macerates for 24 h, and were then allowed to germinate in the Petri-dishes. The seeds were irrigated with 2 mL of macerates everyday within 6 days. The germination and vigor indexes were recorded to assess the allelopathic potentials of the macerates.

Biochemical analysis

The total phenolic content in *A. corniculatum* pericarp macerates was determined according to the Folin-Ciocalteu procedure (Waterhouse, 2002). Folin-Ciocalteu reagent, gallic acid, and Na_2CO_3 were purchased from Sigma- Aldrich Co. Calibration curve was developed as follow 0, 20, 40, 60, 80 and 100 µg/mL aqueous gallic acid. Absorption at 750 nm was measured using Biotex Epoch 2, USA. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of sample.

Statistical analysis

Mean and standard deviation (SD) were calculated and expressed as Mean ± SD. The data was analysed following biological statistic by Excels and SPSS 20.0. Significant differences between control and treatments were analysed at $P \leq 0.05$.

RESULTS

Dynamics of *Echinochloa crus-galli* seed dormancy

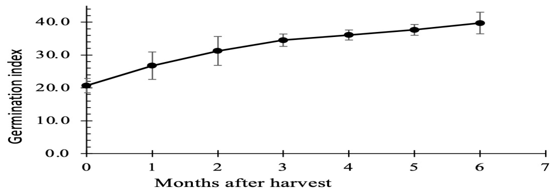

Figure 2. Dormancy dynamics of *Echinochloa crus-galli* seeds as a function of time expressed in months (n = 3)

This experiment was carried out to test if the dormancy-breaking of *E.crus-galli* seeds increases monthly based on its germination index. Apparently, Figure 2 revealed an upward trend of germination ability, a two-fold increase after 6 months of harvesting compared to the beginning, from 20.70 ± 2.18 to 39.76 ± 3.29, respectively.

Pre-treatments of *Echinochloa crus-galli* seeds

Effect of gibberellic acid (GA_3) treatment

GA_3 was used as a stimulus that can evoke *E. crus-galli* seed physiological dormancy. However, the result showed no significant difference between various concentrations and control treatment either in germination (P = 0.033) or vigority (P = 0.271) of 3-month harvested seeds (Table 1). The germination index and vigor index of *E. crus-galli* 3-month harvested seeds treated with GA_3 were found to be from 0.76 to 1.56 and 14.27 to 35.08, respectively.

Table 1. Effects of gibberellic acid (GA_3) on the germination index (GI) and vigor index (VI) of *Echinochloa crus-galli* 3-month harvested seeds

Treatments	Germination index (GI)	Vigor index (VI)
Control	0.40 ± 0.26	12.93 ± 11.97
GA_3 25 ppm	1.56 ± 0.63	32.28 ± 16.45
GA_3 50 ppm	1.29 ± 0.15	35.08 ± 13.76
GA_3 100 ppm	0.76 ± 0.42	14.27 ± 9.06

Notes: Data was presented as mean ±SD (n = 3). No significant difference (ANOVA, at P = 0.05) in the same column.

Effect of 1% sodium hypochlorite (NaClO) and 98% sulfuric acid (H_2SO_4) treatments

The aim of this investigation was to evaluate the effectiveness of various chemical compounds in alleviating physical seed dormancy. Two parameters, germination index and vigor index, were recorded in Figure 3.

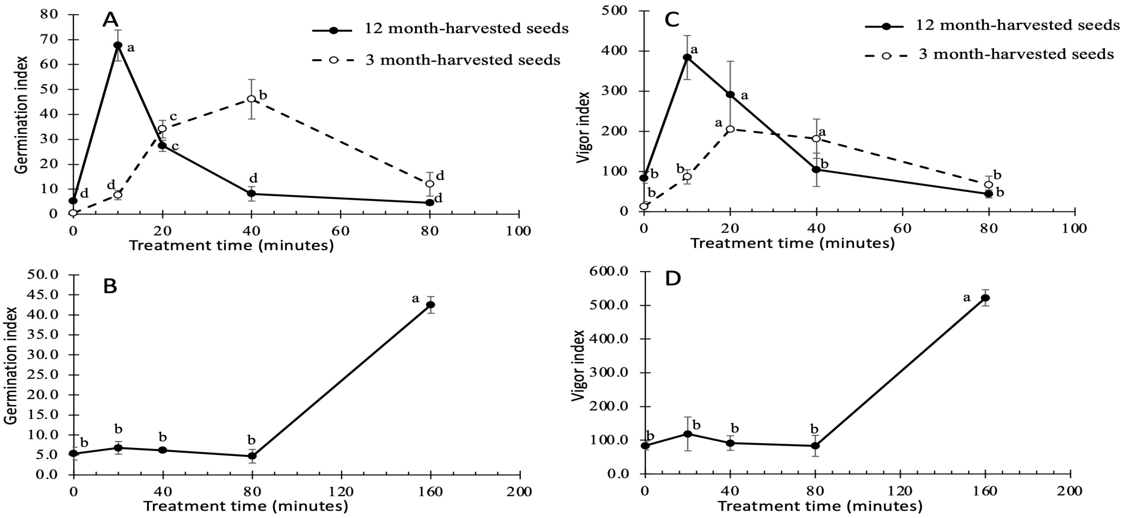

Figure 3. Gemination (A) and vigor index (B) of seeds treated with H_2SO_4 for different time treatments, and gemination index (C) and vigor index (D) of seeds treated with 1% NaClO for different time treatments. (Error bars represent standard deviation. The different lettersshow the significant difference by the Turkey test (P < 0.01, n = 3)

Concentrated H_2SO_4 was used to break the dormancy in both 3-month and 12-month *E. crus-galli* harvested seeds. There were significant differences in both parameters of seeds treated with 98% H_2SO_4 for different durations (Figs. 3A, 3B). The germination index of 12-month harvested seeds treated with H_2SO_4 for 10 minutes was the highest, at 67.64

± 6.21, followed by 20-minute treatment, which was threefold lower, both showed significantly different compared to other treatments; while the highest values of 3-month harvested seeds were 46.09 ± 7.93 for 40-minute treatment, and the treatment of 20 minutes ranked after, at 34.11 ± 3.52. The vigor index followed a similar pattern to that of germination in both types of harvested seeds. Beyond these treatment times, the germination capacity and vigor sharply reduced, as shown in the graphs. Thus, for 12-month harvested seeds, 98% H_2SO_4 treatment for 10 min was the most effective, yielding the highest values in both parameters. The 3-month harvested seeds required longer immersion times to achieve the optimal dormancy-breaking, at 20 or 40 minutes; however, 20-minute treatment was suggested because of its favourable vigor index and standard deviation.

1% NaClO was applied to 12-month harvested seeds. There was no difference in both germination and vigor indices of seeds treated with 1% NaClO for 0 to 80 minutes; only the 160-minute treatment showed significantly higher values of germination (42.50 ± 2.09) and vigor indices (522.24 ± 23.97), respectively (Figs. 3C, 3D). Compared to 98% H_2SO_4 treatment, 1% NaClO acted as a less effective factor due to prolonged exposure to promote dormancy-breaking.

Effect of 98% H_2SO_4 and GA_3 co-treatment

The experiment was designed to determine whether GA_3 had an effect on germination after soaking in H_2SO_4 for 20 minutes. Figures 4A, 4B indicated that H_2SO_4 20 min combined with GA_3 25 ppm was the most effective, with a germination index of 73.12 ± 10.64 and vigor index of 550.13 ± 133.93, while the other treatments were not significantly different, or even had lower effects compared to the control. In comparison to GA_3 treatments, both parameters of seeds co-treated by H_2SO_4-GA_3 were several folds higher than those treated with GA_3 alone. Even compared with 98% H_2SO_4 treatment for 3-month seeds (Fig. 3), the co-treatment still showed its greatest effectiveness in dormancy-breaking.

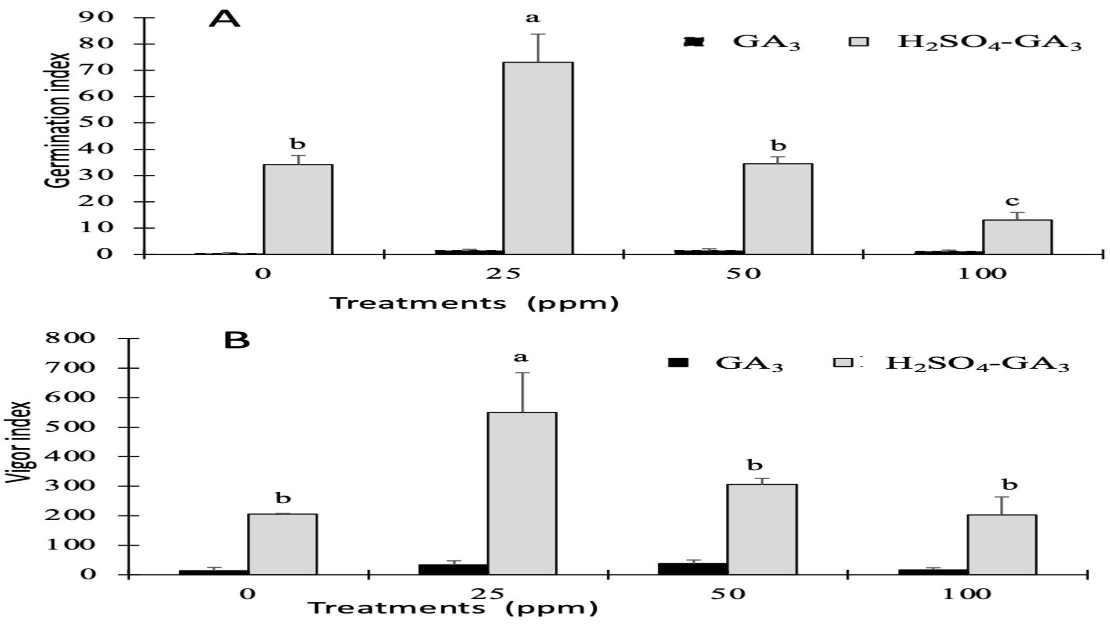

Figure 4. Gemination (A) and vigor index (B) of seed treated with GA_3 and a combination of 98% H_2SO_4 and GA_3 for various concentrations. Error bars represent standard deviation. The different letters show the significant differences by Turkey test ($P < 0.01$, $n = 3$)

Allelopathic effect of *Aegiceras corniculatum* on *Echinochloa crus-galli* seeds

This experiment aimed to test the allelopathic effect of the source species, *A. corniculatum*, on the target species, *E. crus-galli*. The results are shown in Table 2.

The fruit pericarp of *A. corniculatum* significantly inhibited germination compared with the control. While the germination index in the control treatment was 34.36 ± 0.43, it was lower in the fruit pericarp, at 20.28 ± 3.02. There was no significant difference in vigor index between treatments with or without maceration. These results indicated that *A. corniculatum* effectively inhibited the germination potential of *E. crus-galli* seeds.

Table 2. Allelopathic effect of *Aegiceras corniculatum* on germination and vigor indices of *Echinochloa crus-galli* seeds

	Germination index (GI)	Vigor index (VI)
Control	34.36 ± 0.43^a	196.71 ± 6.60^a
Fruit pericarp	20.28 ± 3.02^b	173.9 ± 23.00^a

Notes: Data was presented as mean ±SD (n = 3). Means with the same letter show no significant difference at p=0.05 in the same column (T-test).

Total phenolic content of *Aegiceras corniculatum* macerates

Total phenolic content of *A. corniculatum* macerate was analysed, as phenolic compounds are considered specialised metabolites with potential allelopathic effect. The phenolic content in *A. corniculatum* fruit pericarps was relatively low, at 0.05 ± 0.02 (mg GAE/g sample FW).

DISCUSSION

Dormancy is a period during which germination, growth, and development of organisms are delayed. Most plants develop one or more types of dormancies to adapt to adverse environmental conditions. In allelopathy research, seed dormancy is a main obstacle; therefore, dormancy must be broken to obtain high seed germination rates. Dormancy might result from physiological mechanisms, or water impermeable of the seeds (Emongor et al., 2004). *E. crus-galli* seeds also exhibit *a* dormancy state (Miyahara, 1974). According to Shabbir et al. (2019) fresh *E. crus-galli* seeds naturally take 12-14 months to break dormancy. Indeed, our results in seed dormancy dynamics indicated that *E. crus-galli* seeds innately reduce dormancy over time, and seed coat structure changes, evidenced by monthly increases in germination and vigor indices.

GA_3 treatments were used to determine whether *E. crus-galli* seeds exhibit physiological dormancy, while NaClO and H_2SO_4 treatments were used to overcome physical dormancy and promote water absorption. GA_3 treatments did not affect germination, suggesting this species does not exhibit physiological dormancy or that a physical barrier prevents GA_3 absorption in newly harvested seeds. Besides, our hypothesis was that NaClO might physically soften the seed coats and promote water absorption (Khanh et al., 2008); however, germination ability and the vigor index only increased after 160 minutes of immersion (Fig. 3). Concentrated H_2SO_4 proved to be an effective factor, requiring only 10–40 minutes to significantly increase germination ability and vigor, indicating that concentrated H_2SO_4 played a role in breaking the seed coat and then promoted the ability of water absorption. A similar result was observed in a study by Bichoff et al. (2018), using 98% H_2SO_4 for 20 min showed the most efficient to overcome *Leucaena leucocephala* seed dormancy, resulting in higher values of first germination count, percentage, speed index and average time of germination. Acid scarification (soaking of seeds in H_2SO_4 for 60 and 80 minutes and in HCl for 12 h and 15 h) was efficient at breaking dormancy and promoting germination (Haider Ali et al., 2011). H_2SO_4-GA_3 co-treatments were tested to determine if GA_3 could stimulate seedling growth and root elongation after the seed coats were scarified by H_2SO_4. As expected, germination and vigor index were significantly higher than the single GA_3 or H_2SO_4 treatments. This implies that *E. crus-galli* seeds possess physical dormancy related to coat structures that reduce the water permeability. In our research, some measurement variability were observed, and the sample size or the number of replicates needs to be increased to improve reliability. Further studies comparing the germination ability of *E. crus-galli* under natural and various storage conditions would be of interest.

In our study, the total phenolic content of *A. corniculatum* macerates was found to be low because distilled water was used as a solvent. A small proportion of phenolic compounds are water-soluble and released by water leaching (Kuiters, 1990). Some studies reported the amounts of phenolic compounds with high levels, but using methanol solvent in other parts of mangrove plants in Sundarbans, India (Banerjee et al., 2008) and in Vietnam, indicating mangrove extracts had the effect of α-amylase inhibition and growth inhibition on *Raphanus sativus* (Tan & Thuy, 2015; Mai & Tan, 2017). Despite the low phenolic content, our results highlighted the allopathic effect of *A. corniculatum* fruit-pericarp macerate on *E. crus-galli* seed germination. A similar observation was reported in Dhaou et al. (2022) study with the allelopathic activity of the leaf part, suggesting that this species can be considered as a potential source for allelopathy studies.

CONCLUSION

E. crus-galli seed dormancy-breaking naturally occurs in more than 6 months, as the germination and vigor indexes increased over time. In this study, the fact that there was no significant effect of GA_3 on these indexes of harvested seeds, while there was a significant effect of NaClO and H_2SO_4 on the indexes, indicates that its dormancy, at the very least, was regulated by physical mechanisms. For physical dormancy testing, no effect was observed in 1% NaClO treatments to breaking dormancy of *E. crus-galli* 12-month harvested seeds, except at 160 minutes. Moreover, treating *E. crus-galli* seeds pre-sowing by 98% H_2SO_4 for 20–40 minutes for 3-month harvested seeds and 10 min for 1-year harvested seeds showed a significant increase in germination ability and vitality. Co-treatment of 98% H_2SO_4 and GA_3 performed an outstanding result in both germination and vigor indexes of 3-month harvested seeds, with the highest values belonging to H_2SO_4-GA_3 at 25 ppm, reaching 73.12 ± 10.64 and 550.13 ± 133.93, respectively. Accordingly, 98% H_2SO_4 and GA_3 co-treatment was considered as an optimal treatment for the removal of *E. crus-galli* seed dormancy. Allelopathic potential of *A. corniculatum* macerate on *E. crus*-galli seed germination was observed, with a significant reduction of germination capacity. Further studies are necessary to identify the insight allelochemicals from *A. corniculatum* macerate inhibited target species, and to elucidate their modes of action. Mangrove species contain a high diversity of specilised metabolites that are important for ecological aspects, particularly in allelopathy. Further investigations are encouraged to gain a better understanding of the effects of mangrove macerates on specific species, which could support forestry and crop management in prospect.

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