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Comparative study of dormancy breaking efficiency in *Terminalia mantaly* seeds using microorganisms and conventional pre-treatment methods

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ABSTRACT

This study was carried out to comparatively look at the most efficient methods of dormancy breaking in *Terminalia mantaly* using microorganisms (*Lasiodiplodia theobromae*, *Candida* sp., and *Aspergillus* spp.), chilling, heating, 1% HCl, scarification, and Dil. H2SO4. Analysis of seed germination percentages (PGS) over 5-31 days revealed that *T. mantaly* seeds treated with 1% HCl exhibited the highest PGS (86.66%), followed by *L. theobromae* (66.66%), *Candida* sp. (60%), and untreated controls (46.66%). The treatment using *Aspergillus* spp. resulted in moderate PGS (40%), while scarification yielded the lowest PGS (13.33%). Notably, chilling, heat, and diluted sulfuric acid treatments did not achieve any seed germination within the 31-day period. *T. mantaly* seeds subjected to treatments with *L. theobromae*, *Candida* sp., and *Aspergillus* spp. demonstrated precocious germination (5-7 days after treatment). *L. theobromae* treatment exhibited the highest speed germination index (SGI) (2.91), followed by 1% HCl (2.62), and *Candida* sp. However, chilling, heating, and diluted sulfuric acid treatments recorded no germination and, consequently, no SGI. These results suggest that *T. mantaly* seeds treated with the microbial isolates and 1% HCl demonstrated enhanced dormancy breaking and germination, as reflected by higher PGS and SGI. This research contributes to our understanding of *T. mantaly* germination biology by highlighting the potential of microorganisms and hydrochloric acid in overcoming seed coat dormancy, particularly in species with hardened seeds. This opens new avenues for exploring their use in promoting successful germination and enhancing plant establishment.

Keywords: *Aspergillus spp.*, *Candida sp.*, Dormancy, *Lasiodiplodia theobromae*, Seed, *Terminalia mantaly*.

1. INTRODUCTION

The germination of seeds is one of the most critical stages of plants. Seeds tend to germinate when provided with favorable environmental conditions and factors necessary for germination such as; light, water, oxygen, and temperature (Taghvaei et al., 2022; Ankalaiah & Reddy, 2022a & 2022b; Rohini Latha, 2022). Nevertheless, some seeds fail to germinate even when the above favorable environmental conditions are provided, such seeds are said to be dormant (dormancy) (Guo et al., 2020; Luo et al., 2022). Not all seed dormancy has to do with the absence of favorable environmental conditions and factors some can be due to seed properties and inert conditions (Luo et al., 2022). Some seeds possess very hard seed coats which are impermeable to water as well as oxygen which inhibits/hinders the appreciable expansion of the embryo therefore leading to seed dormancy (Qaderi, 2023). Moreover, mature seeds with immature embryos have been reported to exhibit seed dormancy (Carruggio et al., 2021).

The presence of a hard seed coat is not often synonymous with dormancy, but it plays an important role in the defense against certain adversative external factors that might tend to cause mechanical injury to the embryo as well as prevent the embryo from drying out (Matilla, 2020). Certain environmental cues can be transmitted through the seed coat to the interior of the seed as well, thus, making the seed coat a transmission channel (Chen et al., 2023). The integrity of the seed coat surface is extremely important for seed quality and fitness during seed storage or germination, and diverse technologies are available for protecting and improving seed surface quality. *Terminalia mantaly* Perrier commonly referred to as Madagascar almond is a member of the *Combretaceae* family. *Terminalia mantaly* is a very popular tree in Nigeria, planted in almost every institutional setup and street because of its far-reaching branches that provide shade (Asomaning et al., 2021).

It is widely used and generally accepted because of its fast-growing rate. Once the seeds of *T. mantaly* (which is already very thick) are allowed to dry, they exhibit a certain level of seed dormancy, this impedes quick and synchronous germination due to seed coat dormancy which constitutes a serious constraint in the propagation of the plant (Tchuente-Tchuenmogne et al., 2017). Information on the breaking of dormancy in *T. mantaly* using common conventional methods such as; boiling of seeds, cold water soaking, nipping of seeds, and soaking in sulphuric acid (H2SO4) has been documented by. Some of the methods have been widely used on other seeds. Nonetheless, the use of microorganisms in the breaking of mechanical seed coat dormancy has been scantily reported for other seeds, but little or no reports have been made on the use of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Candida* spp. (Robin) Berkhout., and *Aspergillus* spp. Fresenius. Thus, justifying the uniqueness of this research. Thus, this work aimed to evaluate the effect of different treatments on the germination promotion of *Terminalia mantaly* seeds under laboratory conditions.

2. MATERIALS AND METHODS

Terminalia mantaly seeds (matured, ungerminated, and viable) were washed in distilled and sterilized water and submitted to ten different treatments (Table 1). Following inoculation, seeds were individually placed on moistened germination paper within plastic trays. Two sheets of moistened filter paper, sterilized with distilled water, were used per tray. Trays were then placed in a germination chamber maintained at 25±2°C with a 12-hour light photoperiod. A completely randomized experimental design was employed, with five replicates consisting of 40 seeds each, totaling 2000 seeds across the experiment.

Table 1 Treatments used in this research under laboratory conditions

Codes	Treatments	References
T1	Untreated seeds (control).	Luna et al., 2019
T2	Seeds were immersed in suspensions of *Aspergillus* sp.1 at a concentration of 106 conidia mL-1 for 24 hours.	Campos et al., 2020
T3	Seeds were immersed in suspensions of *Lasiodiplodia theobromae* at a concentration of 106 conidia mL-1 for 24 hours.	Campos et al., 2020
T4	Seeds were immersed in suspensions of *Candida* sp. at a concentration of 106 conidia mL-1 for 24 hours.	Campos et al., 2020
T5	Seeds were chilled inside a refrigerator (of 4 ºC) and left for 24 hours.	Calderon-Flores et al., 2021
T6	Seeds were placed in the oven (60ºC) for 25 min. The seeds were removed	Luna et al., 2019

	and immersed in cold distilled water, and air dried before planting.	
T7	Seeds were soaked in 1% HCl (Hydrochloric acid) for 15 min. The seeds were removed, rinsed with distilled water, and allowed to air dry before planting.	Rostami and Shasavar, 2009
T8	Seeds were scarified mechanically opposite the micropyle to expose the embryo.	Rostami and Shasavar, 2009
T9	Seeds immersed in suspensions of *Aspergillus* sp.2 at a concentration of 106 conidia mL-1 for 24 hours.	Campos et al., 2020
T10	Seeds were soaked in diluted sulphuric acid for 20 min. The seeds were removed, rinsed with distilled water, and allowed to air dry before planting.	Rostami and Shasavar, 2009

Assessments were on 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 17, and 31 days after treatment (DAT). The percentage of germinated seeds (PGS) was estimated by the following formula: PGS (%) = (NGS/TNS) × 100, where: NGS represents the number of germinated seeds; TNS represents the total number of seeds (Yousof and Ibrahim, 2013). The speed germination index (SGI) was calculated as described by Yousof and Ibrahim, (2013) using the following formula:

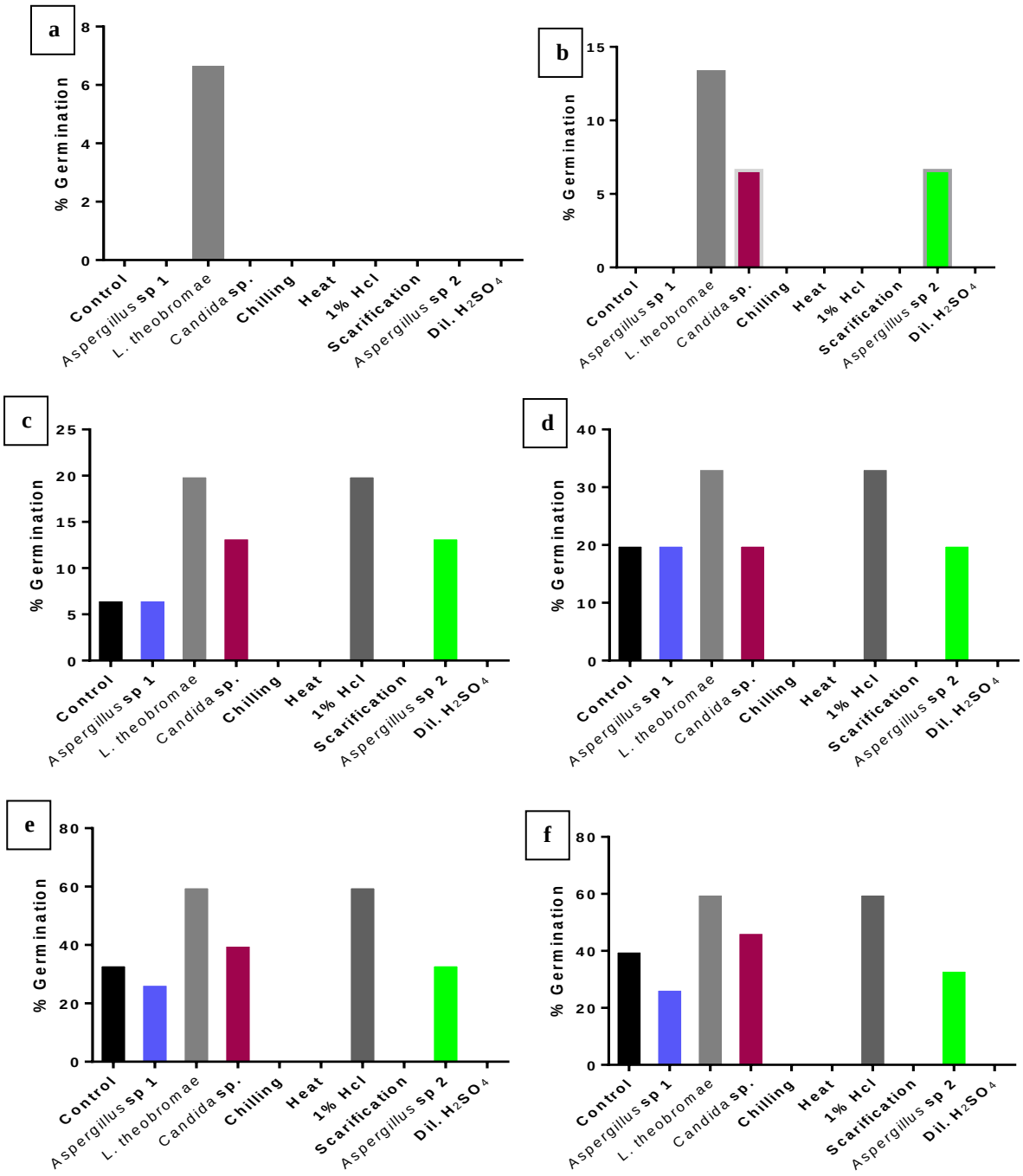
$$\frac{Number\ of\ seeds\ germinated}{Days\ of\ first\ count} + - - - + \frac{Number\ of\ seeds\ germinated}{Days\ of\ final\ count}$$

Statistical analysis utilized mean values from each replicate (n=5). One-way analysis of variance (ANOVA) was performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). Before ANOVA, assumptions of homogeneity of variances and normality were confirmed through Levene's test and Shapiro-Wilk test, respectively. Duncan's multiple range test (DMRT) was then employed to identify significant differences between treatment means. A significance level of $\alpha = 0.05$ ($P \leq 0.05$) was applied for all statistical tests.

3. RESULTS

Statistical analysis revealed a highly significant difference ($P < 0.01$) between treatments, sampling moments, and their interactions for the percentage of germinated seeds. The results from the germination studies carried out for 31 days showed that, at 5 DAT (days after treatment), *T. mantaly* seeds inoculated with *L. theobromae* recorded 6.66% germination (Figure 1a), while there was no germination recorded for other treatments. At 6 DAT, the highest PGS (percentage of germinated seeds) was obtained for *T. mantaly* seeds treated separately with *L. theobromae* (13.33%), Candida sp. (6.60%), and *Aspergillus* sp. 2 (6.66%) (Figure 1b). It is worth noting that *T. mantaly* seeds treated with *L. theobromae* and 1% HCl showed the highest PGS at 7, 8, and 9 days after treatment, as the percentages ranged from 20% (7 DAT) to 60% (9 DAT) (Figures 1c, d and e). At 10 DAT, only seeds inoculated with Candida sp. experienced an increase in percentage germination to 46.60% (Figure 1f). At 11 DAT, the highest percentages of germinated seeds were recorded on the seeds inoculated with *L. theobromae* (66.66%) and *Candida* sp. (60%) (Figure 1g).

According to (Figures 1h, i, j, k, and l), the highest PGS was recorded on the *T. mantaly* seeds treated with 1% HCl (80-86.66% at 12 and 31 DAT, respectively) followed by *L. theobromae* (63.35-66.66% at 12 and 31 DAT, respectively). 1% HCl treatment recorded the highest % germination with 86.66%, followed by *L. theobromae* treatment (66.66%), *Candida* sp. (60%), untreated seeds (46.66%), *Aspergillus* spp. (40%), and scarification (13.33%). However, chilling, heat, and Dil.H2SO4 treatments recorded no germination. The different measurements carried out on the seeds revealed that the highest speed germination index (SGI) was obtained on the *T. mantaly* seeds treated with *L. theobromae* (2.91), followed by 1% HCl (2.62), and *Candida* sp. (2.32). Whereas, chilling, heat, and Dil.H2SO4 treatments recorded no germination, hence; no SGI has been registered (Table 2).


a
% Germination
0
2
4
6
8
Control
Aspergillus sp 1
L. theobromae
Candida sp.
Chilling
Heat
1% Hcl
Scarification
Aspergillus sp 2
Dil. H2SO4
b
% Germination
0
5
10
15
Control
Aspergillus sp 1
L. theobromae
Candida sp.
Chilling
Heat
1% Hcl
Scarification
Aspergillus sp 2
Dil. H2SO4
c
% Germination
0
5
10
15
20
25
Control
Aspergillus sp 1
L. theobromae
Candida sp.
Chilling
Heat
1% Hcl
Scarification
Aspergillus sp 2
Dil. H2SO4
d
% Germination
0
10
20
30
40
Control
Aspergillus sp 1
L. theobromae
Candida sp.
Chilling
Heat
1% Hcl
Scarification
Aspergillus sp 2
Dil. H2SO4
e
% Germination
0
20
40
60
80
Control
Aspergillus sp 1
L. theobromae
Candida sp.
Chilling
Heat
1% Hcl
Scarification
Aspergillus sp 2
Dil. H2SO4
f
% Germination
0
20
40
60
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Control
Aspergillus sp 1
L. theobromae
Candida sp.
Chilling
Heat
1% Hcl
Scarification
Aspergillus sp 2
Dil. H2SO4

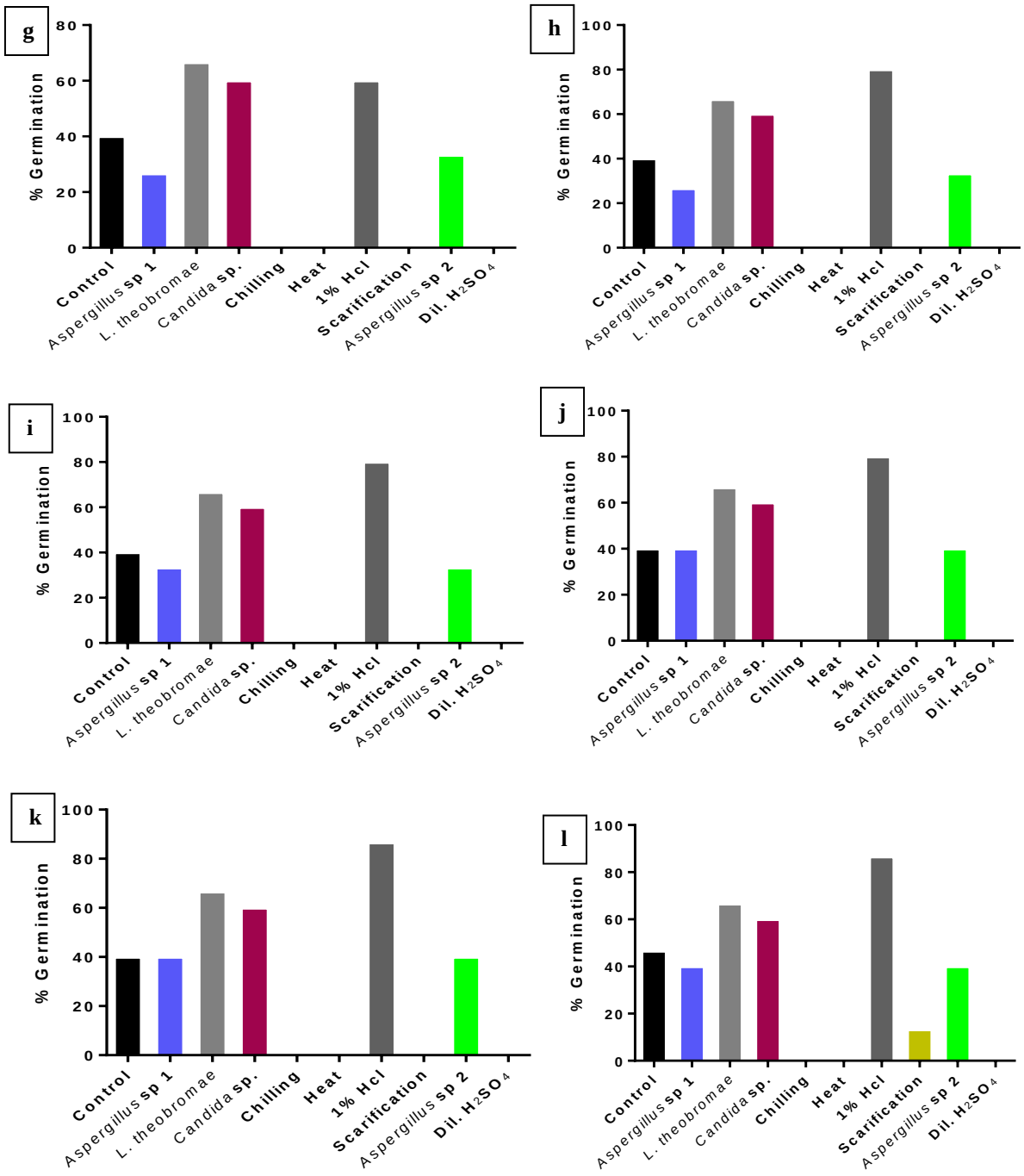

Figure 1 Effect of ten treatments on the percentage of germinated seeds after 5 (a), 6 (b), 7 (c), 8 (d), 9 (e), 10 (f), 11 (g), 12 (h), 13 (i), 15 (j), 17 (k), and 31 (l) days after treatment. Data are the average of 40 seeds per replicate (with 5 replicates)

Table 2 Effects of biological, chemical, and scarification pretreatment methods on the speed germination index (SGI)

Treatments	SGI
Control	1.70
Aspergillus spp. 1	1.26
L. theobromae	2.91
Candida spp.	2.32

Chilling	0.00
Heat	0.00
1% HCl	2.62
Scarification	0.12
Aspergillus spp. 2	1.26
Dil.H2SO4	0.00

4. DISCUSSION

This study demonstrated seed coat dormancy in *T. mantaly*, evidenced by the relatively low germination percentage observed in the control group. However, inoculation with specific microorganisms significantly affected germination dynamics. *T. mantaly* seeds treated with *L. theobromae*, *Candida* sp., and *Aspergillus* spp. exhibited early germination (5-7 days after planting), aligning with findings by (Delgado-Sánchez et al., 2010). They reported that microorganisms like *Phoma* sp., *Trichoderma koningii*, and *Penicillium chrysogenum* facilitated seed coat dormancy breaking in *Opuntia streptacantha*, potentially through enzymatic degradation of the seed testa. Supporting the findings of this study, demonstrated that *Rhizopus* sp. aseptically promotes seed coat dormancy break in *Thelocactus hexaedrophorus*, a *Chihuahuan* Desert cactus. Similarly, Olvera-Carrillo et al., (2009) observed fungal hyphae penetrating the funicular envelope of exhumed *O. tomentosa* seeds after seven months, facilitating germination.

However, they noted that germination resulted in weak embryos with limited growth potential. These studies suggest that specific microorganisms, particularly fungi, can mechanically overcome seed coat dormancy by degrading the pericarp (seed wall) through enzymatic or physical means. The effectiveness of each scarification technique depends on the specific seed species and its dormancy mechanisms. Finding the optimal treatment duration and intensity is crucial for successful germination without compromising seed viability. Ongoing research focuses on developing precise and efficient scarification methods for diverse plant species, considering factors like seed coat morphology, embryo sensitivity, and potential negative impacts (Rostami and Shasavar, 2009). Several chemical agents can be employed to scarify seed coats, promoting water and gas exchange for efficient germination.

Sulfuric acid remains the most widely used approach due to its effectiveness in both concentrated and industrial forms. Other chemicals like sodium hypochlorite and hydrogen peroxide have also exhibited potential for scarification. Therefore, while chemical scarification offers efficient dormancy-breaking potential, its limitations, including safety concerns, additional processing steps, and the risk of reduced germination, currently restrict its commercial feasibility. Research efforts are directed toward developing safer and more precise chemical treatments, optimizing protocols for specific seed species, and exploring alternative scarification methods to overcome these limitations and expand the potential of chemical scarification in seed propagation (Campos et al., 2020).

Mechanically scarified seeds exhibited low germination, potentially due to delayed planting after treatment, making them susceptible to pathogens, or damage to the embryo from soil microorganisms or wounding during the process (Aminu, 2012). Diluted sulfuric acid treatment likely led to complete dormancy break and embryo injury due to prolonged exposure (Aliero, 2004). Failure to germinate after chilling suggests cold stratification is not an obligatory requirement for *T. mantaly* seeds, aligning with observations of embryo damage by after cold treatment. Among all treatments, *T. mantaly* seeds treated with 1% HCl demonstrated the highest germination percentage. This finding corroborates with Goddard et al., (2009), who reported enhanced germination and viability of Benghal dayflower seeds pretreated with HCl.

5. CONCLUSION

This study demonstrates that *T. mantaly* seeds treated with specific microbial isolates and 1% HCl exhibited significantly higher germination percentage (PGS) and speed germination index (SGI) compared to controls. Inoculation with *L. theobromae*, *Candida* sp., and *Aspergillus* spp. likely facilitated dormancy break through enzymatic degradation of the seed testa, specifically targeting water-impermeable components. HCl treatment effectively weakened and breached the hard seed coat, enabling water and oxygen imbibition, critical prerequisites for germination. These findings contribute to our understanding of germination biology in *Terminalia* species. Notably, it highlights the potential of specific microorganisms and 1% HCl as effective, non-invasive methods for overcoming dormancy in plants with hard seed coats. This opens avenues for further research on microbial diversity and optimized protocols for promoting robust germination and seedling establishment in such species.

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Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study.

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Data and materials availability

All data associated with this study are present in the paper.

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