Germination of 'Red Lady' var. Carica papaya seeds treated with moringa leaf extract (MLE)

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Abstract

While papaya is known as one of the major fruits in export internationally, its germination, which can only be done through seeds taken fresh from the fruit, has reportedly been poor. This study aimed to determine and compare the effects of different concentrations of the crude aqueous moringa Leaf Extract (MLE) on the germination of the 'Red Lady' var. *Carica papaya* L. (papaya) seeds. The *Carica papaya* seeds were cleaned, dried, and treated with different concentrations of moringa leaf extract and then allowed to germinate for 10 days. The results showed that there is no significant difference between the germination percentage, rate of emergence, and germination index of seeds applied with MLE and those that were left untreated. Therefore, the results indicate the ineffectiveness of moringa leaf extract as a seed treatment in the improvement of the germination of 'Red Lady' var. *Carica papaya* L. seeds.

Introduction. - *Carica papaya* L., commonly known as Papaya, is a commercial fruit crop grown in tropical countries [1] and remains as the third most cultivated fruit in the world [2]. It has remained one of the major fruits produced in the Philippines together with mango, banana, and pineapple [3].

While there are many varieties of papayas, the 'Red Lady' variety is generally preferred due to its gynodioecious nature, long shelf life, immunity from the Papaya ringspot virus, and generally bigger mass. However, it is also known for its difficult germination and growth process [2]. Furthermore, its susceptibility to the damping-off disease at the nursery stage [4] propagates seed deterioration, making it difficult to be commercially produced at a large-scale level [5]. It can be said that these factors all contribute to the high cost of this variety [4].

The only way for commercial papayas to germinate is through seeds that come fresh from the fruit [1]. Papaya germination has reportedly been poor [6] because there is no uniformity in their germination and growth without pre-treatments [7]. The problems behind their difficulty in production are their poor germination, which is caused by seed deterioration observed early after harvest [8], seeds' sexual propagation, where only the females can produce fruit [9], and the seeds' outer seed coats or gelatinous sarcotesta, which can inhibit germination [7].

Furthermore, studies performed by Webster et al. [1] and Rodriguez et al. [6] have found that papaya is prone to seed dormancy, which is defined as the innate seed property determined by genetics with environmental influence partially mediated by abscisic acid and gibberellins through control of embryo growth and endosperm weakening [10], and is partially caused by the mechanical restraint of seed covering layers.

The first step for plant growth is germination, which has three phases. Phase I, imbibition, begins when the dry seed encounters water in the right environment. Here, the first visible sign of germination is the cracking of the seed coat [1]. Then, Phase II, where the water uptake plateaus because enough water has been absorbed to activate different kinds of metabolism [11] which increase metabolic activity [12], starts. Lastly, Phase III, or radicle emergence, normally occurs one to two days after the seed coat cracking [1].

To overcome seed dormancy, the growth hormones gibberellin and Abscisic Acid (ABA) can be used. Gibberellin can increase the growth potential of the embryo by weakening tissues surrounding the radicle [13]. Meanwhile, ABA, which can normally inhibit germination by weakening the seed coat, in controlled amounts, helps in the initiation of germination due to the initial tearing of the seed coat, leading to radicle protrusion [1].

To discover which factors affected the germination rate of papaya, researchers have turned to the exploration of pre-sowing methods such as seed scarification [6] and seed priming [14]. Moringa leaf extract (MLE), from the leaves of *Moringa oleifera* or Malunggay, has been considered by researchers as an effective treatment for the growth and germination of many plants, as it is a potential growth hormone and regulator that has been proven to contain growth hormones such as gibberellins and ABA [15], though ABA is in a significantly lower concentration as compared to gibberellins [16].

Furthermore, in the studies performed by Maishanu et al. [17], Yasmeen et al. [18], and Latif [16],

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the application of moringa leaf extract effectively improved seed germination, seedling vigor, growth, and productivity of crops such as cowpea, cereal forage, wheat, and bean plants. In the studies of Maishanu et al. [17], Abusuwar and Abohassan [15] and Latif et al. [16], both utilized the foliar spray method of MLE application. Yasmeen et al. [18] applied MLE on wheat plants through seed priming. While none of the aforementioned studies were done on dicotyledonous albuminous (seed type of papaya), Hedden [19] states that in dicotyledonous plants, GA-induced hydrolases weaken the endosperm, which would otherwise form a physical barrier to radicle emergence. In an effort by Yasmeen et al. [18] to explore the potential of moringa leaf extract as a priming agent for wheat (*Triticum aestivum* L.), the results showed that moringa leaf extract performed significantly better than the control and other priming agents, such as hydropriming and on-farm priming.

Since poor germination techniques are a threat to the production of papayas, researchers continually explore new ways to increase germination rates. The results of this study could yield valuable knowledge that may be useful to the agricultural field, as it would determine whether moringa leaf extract, an inexpensive and organic treatment, has positive effects on the germination of papaya. This study may also address the high cost of commercial papayas, especially that of the ‘Red Lady’ variety, should its effects result in an enhanced yield of papayas.

It is evident from earlier studies that moringa leaf extract contains hormones such as gibberellins and ABA that may be beneficial to germination, but there seems to be a lack of published literature focused on the effects of MLE as a potential growth hormone on the germination of seed types like that of *Carica papaya,* which is dicotyledonous albuminous. Thus, this study aims to test whether the application of varying concentrations of crude aqueous moringa leaf extract would enhance the germination index, rate of emergence, and germination percentage of the ‘Red Lady’ papaya variety seeds. Its specific objectives are:

(i) To determine the final germination percentage, rate of emergence, and germination index of seeds for each concentration (no MLE, 2 mL MLE: 9 mL water, 2 mL MLE: 19 mL water, 2 mL MLE:29 mL water);
(ii) To determine the germination index every two days for each concentration, and

(iii) To determine if there are significant differences between the parameters of each treatment group

Methods. - *Moringa oleifera* leaves were collected and used for the making of moringa leaf extract. The *Carica papaya* var. “Red Lady” seeds were extracted, cleaned, washed, tested for viability, dried, and treated with varying concentrations of MLE then allowed to germinate and observed for 10 days. During the designated data gathering period, the germination parameters were measured for the computation of raw data and statistical analysis.

Preparation of Carica papaya seeds. *Carica papaya* “Red Lady” var. seeds were manually extracted from the fruit, rubbed in between two rough cloths, and cleaned using distilled water to remove the sarcotesta and mucilage [6]. The viability was done using the floating technique [20] and then left to air dry for the next 12 hours. There were four treatments in each location (T1, T2, T3, C) with five replicates each with 15 seeds allocated to each replicate.

Preparation of crude moringa leaf extract. Manually collected *Moringa oleifera* leaves were cleaned using distilled water, then gently dried [21,22], and weighed to 300 g Following the method by Abusuwar and Abohassan [15], 300 g of *Moringa oleifera* leaves were placed in a conventional electric mixer together with 300 mL of distilled water in a (1:1 by volume) ratio and passed through a strainer to separate the juice from the residue. The juice was collected and diluted to form different concentrations of MLE. Because the juice was already in a 1:1 juice to water ratio, the different concentrations were made as follows:

T1: 2 mL of juice mixed with 9 mL of distilled water (1:10 by volume);
T2: 2 mL of juice mixed with 19 mL of distilled water (1:20 by volume), and
T3: 2 mL of juice mixed with 29 mL of distilled water (1:30 by volume).

Preparation of Seed Set-up. Once dry, the seeds were immersed in 100 mL of room temperature distilled water for 6 hours and then washed using distilled water to remove any residue. The seeds were then placed on a folded paper towel with a distance of at least 2 times the diameter of the seeds between each other and moistened using a syringe with the prepared concentrations of MLE which is 2.5 times the weight of the paper towel used for 24 hours.

Germination of Seeds. Following the methods of Severiano et al. [23], the seeds were positioned vertically and evenly distributed in 20 sets of 15 seeds per location with a distance of at least 2 times the diameter of the seeds between each other on a paper towel moistened with distilled water that is 2.5 times the weight of the paper towel, folded in half twice then packed in labeled resealable plastic bags. The *Carica papaya* seeds were allowed to germinate for 10 days and watered every other day.

Measurement of Plant Growth Parameters. During the measurement of parameters, the paper towels were carefully unfolded. The parameters used in this study were final germination percentage (GP), rate of emergence (RE), and germination index (GI). The final germination percentage (GP) was calculated following the formula of Al-Ansari and Ksiksi [24]:

$$GP = \frac{Number\ of\ total\ germinated\ seeds}{Total\ number\ of\ seeds\ tested} \times 100$$

The rate of emergence (RE) was calculated following the formula of Dayeswari et al. [25]:

$$Rate\ of\ emergence = \frac{X_1}{Y_1} + \left(X_2 - \frac{X_1}{Y_2}\right) + \ldots + \left(X_n - \frac{X_{n-1}}{Y_n}\right)$$

wherein X_1 - number of seeds germinated on first count; X_2 - number of seeds germinated on second count; X_2 - number of seeds germinated on the nth day; Y_1 - number of days from sowing to first count; Y_2 - number of seeds germinated on second count; Y_n - number of seeds germinated on the nth day.

The germination index (GI) was calculated with the formula:

$$GI = (10 \times N_1) + (9 \times N_2) + ... + (2 \times N_9) + (1 \times N_{10})$$

wherein N_1, N_2, ..., N_{10} represents the number of germinated seeds on the first, second and subsequent days until the 10th day and the multipliers (e.g. 10, 9, etc.) are weights given to the days of the germination.

Data Analysis. The means and the standard deviation of each growth parameter in the concentration groups were computed. One-way Analysis of Variance (ANOVA) at a 95% confidence level was used to analyze the data.

Safety Procedure. To prevent injuries caused by the materials and equipment used, the researchers used protective equipment such as gloves, masks, and lab gowns. All leftover materials have been disposed of properly and to prevent the risk of acquiring the COVID-19 virus, meetings among researchers were kept to a minimum.

Results and Discussion. - *Carica papaya* L. seeds treated with the most diluted treatment (1:30) germinated the most, with an average germination percentage of 35.55%.

Table 1. Effects of MLE on the germination of 'Red Lady' var. *Carica papaya* L. seeds.

Treatment	Germination Percentage	Rate of Emergence	Germination Index
C (no MLE)	35.11% ± 37.41	22.67 ± 30.53	84.67 ± 120.85
T1 (1:10)	35.11% ± 38.99	22.99 ± 31.48	84.54 ± 123.27
T2 (1:20)	32.00% ± 32.39	20.38 ± 25.36	71.07 ± 96.38
T3 (1:30)	35.55% ± 29.78	20.31 ± 24.77	69.93 ± 97.08

They were then followed by the seeds in the most concentrated treatment (1:10) and the control group (water only), with an average germination percentage of 35.11%. The least number of germinated seeds were found in the second most concentrated treatment (1:20), which has an average germination percentage of 32.00%. The germination percentage shows how many seeds are viable in a population or sample [26].

Meanwhile, seeds were fastest to germinate in the most concentrated treatment, followed by the control group, the second-most concentrated treatment, and lastly, the most diluted treatment. Their rates of emergence were 22.99, 22.67, 20.38, and 20.31, respectively. The rate of emergence indicates the speed of seed germination [25].

On the other hand, the control group had the highest germination index (84.67), followed by the most concentrated treatment (84.53), second-most concentrated treatment (71.07), and most diluted treatment (69.93). According to Javaid et al. (2018), the germination index indicates differences between each concentration, as it measures the percentage and speed of germination in each group.

There was no significant difference between the effects of each treatment on the germination of the seeds.

Our study has discovered that moringa leaf extract (MLE) does not affect the final germination percentage of *Carica papaya* L. seeds. This might be because of the concentrations of MLE that have been used for seed treatment. In this study, we used 1:10, 1:20, and 1:30 MLE, which may be concentrations that are neither high nor low enough to affect the final germination percentage of *Carica papaya* L. seeds. Mona et al. [27] found that increasing concentrations of MLE reduced the germination percentage of *Vicia faba* seeds as compared to the control treatment. This may be due to the presence of allelopathic compounds in MLE, which increases along with the concentration of the said extract [15]. According to Oyerinde et al. [28], allelopathic compounds tend to inhibit germination, specifically affecting radicle growth, which is one of the most important indicators of germination. Meanwhile, Nouman et al. [29] found that 1:30 MLE was most effective in improving the final germination percentages of *C. ciliaris, P. antidotale*, and *E. crusgalli* seeds, as compared to the other treatments. Thus, it can be theorized that the concentration of seed treatment plays a huge role in improving the germination parameters of plants.

Furthermore, the differences in the environmental conditions between each of the set-ups could have caused the researchers' varying results with respect to the final germination percentages of the *Carica papaya* L. seeds, leading to no statistically significant differences in the overall results. There is a study by da Silva et al. [30] which found that exogenous gibberellins inhibit the germination of *Coffea arabica* seeds, most likely due to how it avoids germination in full sunlight. Although *Carica papaya* seeds need full sunlight to germinate [31], this highly suggests that the process of germination largely depends on the environmental conditions around the seeds, such as the amount of light they receive, which was different for each location in this study.

Another key finding of this experiment is that moringa leaf extract (MLE) does not affect the rate of emergence of *Carica papaya* L. seeds. This is in contrast to the results of Basra et al. [32], which stated that 1:30 MLE was the optimal concentration to increase the emergence rate of hybrid *Zea mays* seeds, which corresponded with the findings of Yasmeen et al. [18] in their study on *Triticum aestivum* L. seeds. One of the possible reasons might be because the data gathering process of 10 days was too short. Normally, it takes *Carica papaya* L. seeds two to three weeks to successfully germinate when the sarcotesta is removed [33], which was done in this study. This may also have been due to the lack of uniformity in the germination of *Carica papaya* L. seeds [7], which the MLE may have failed to overcome. Furthermore, the environmental conditions, especially the light, heat, and humidity, during the preliminary tests and the actual data gathering period may have been different, which may explain the difference in outcomes.

Moreover, unfavorable conditions may lead to seed dormancy and significantly lower the rate of seedling emergence. To overcome seed dormancy, gibberellins increase the growth potential of the embryo by weakening the tissues surrounding the radicle [13], while ABA inhibits germination by weakening the seed coat. GA, on the other hand, stimulates seed germination and breaks seed dormancy by increasing the growth potential of the embryo and by inducing hydrolytic enzymes. A study by Vishal and Kumar [34] also claims that GA and ABA act antagonistically in mediating plant development and thus it is imperative that optimal levels of GA and ABA must be attained in order to maintain favorable conditions (lower ABA and higher GA). Furthermore, seeds with a low level of ABA produced during their development require a proportionately low amount of GA to germinate, whereas those with a higher concentration of ABA produced during seed development require a higher amount of GA to germinate [35]. The external conditions also play a significant role in the concentrations of ABA and GA, with unfavorable conditions resulting in higher ABA levels and favorable conditions resulting in lower ABA levels. In that regard, Vishal and Kumar [34] state that favorable conditions aid in GA biosynthesis which in turn successfully counters the inhibitory effect of ABA. This implies that the environmental conditions under which the experiment was performed were unfavorable, and may be a possible factor in delayed germination in some locations.

The last key finding in this study is that moringa leaf extract does not affect the germination index of *Carica papaya* L. seeds. However, MLE improved the germination index of *Capsicum annuum* L. seeds [36], *Triticum aestivum* L. [18], *C. ciliaris*, *P. antidotale*, and *E. crusgalli* seeds [29]. Because the overall final germination percentage and rate of emergence in this study did not have any statistically significant difference, the overall germination index that was measured also resulted in the same outcome.

Limitations. Other types of germination parameters were considered but ultimately discarded due to the researchers' lack of access to proper laboratory equipment. This also caused the researchers to be unable to chemically analyze the moringa leaf extract in-depth, which may be a major factor as the ideal concentration of MLE must be calculated based on the preexisting exogenous and indogenous gibberellins and ABA in both the seeds and extract.

Moreover, the study was only able to use seeds taken from hermaphroditic fruits of the Red Lady variety. Due to lack of manpower and monetary restrictions, the work unit was only able to use a total of 900 seeds. Only seeds located in the middle part of the fruit were gathered, as advised by the experts from the Department of Agriculture.

Conclusion. - Statistical analysis using one-way ANOVA at a 95% confidence interval found that there was no significant difference between the effects of each treatment on the germination of 'Red Lady' var. *Carica papaya* seeds. Therefore, the application of varying concentrations of the crude aqueous moringa leaf extract (MLE) did not enhance the germination index, rate of emergence, and germination percentage of *Carica papaya* seeds of the 'Red Lady' variety.

Recommendations. - Future studies are recommended to experiment in one laboratory setting with the necessary equipment available and use materials other than paper towels which may be able to provide a more in-depth chemical analysis of the effects of moringa leaf extract on the germination of 'Red Lady' var. *Carica papaya* seeds. It is also recommended to extend the allotted germination time for the seeds to a minimum of two weeks. Furthermore, this study used seeds taken from hermaphroditic 'Red Lady' var. *Carica papaya* fruits. It is possible that the seeds taken from female fruits of the same variety may yield different results. It may also be beneficial to analyze the endogenous and exogenous Gibberellin and ABA concentrations in both seeds and extract in order to manipulate the MLE concentrations accordingly with the optimal balance for germination taken into consideration.

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