CLUSTER TWO

Seed Priming & Plant Growth

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Studies under this cluster aim to further improve the future of the agricultural industry.

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Germination of *Oryza sativa* L. NSIC Rc222 under sodium chloride (NaCl) stress hydroprimed at various time durations

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Abstract

Soil salinity decreases plant productivity and induces slow growth especially under very high levels ranging from 9 dS/m to 15 dS/m. To overcome the negative effects of soil salinity, hydropriming or soaking of seeds in distilled water is used as a seed priming technique to activate various metabolic processes in the seed which can promote growth under stress. The study evaluated the germination of *Oryza sativa* L. NSIC Rc222 for seven days under 15 dS/m salt stress hydroprimed at 12 h, 24 h, and 48 h, with unprimed seeds as the control. Germinated seeds were counted every day, and seedling height was measured to compute for the final germination percentage (FGP), speed of germination (SG) and seedling vigor index (SVI). Results show that hydropriming the seeds for 48 h resulted in the highest SG and SVI values while no significant differences were recorded among the FGP of all treatments. Increasing hydropriming duration improves the germination parameters of *Oryza sativa* L. NSIC Rc222 seeds under sodium chloride (NaCl) stress. In conclusion, hydropriming is effective in mitigating the negative effects of saline stress on SVI and SG but it has no effect on germination viability.

Introduction. - One major environmental constraint faced in agriculture is soil salinity, wherein it induces slow growth among plants and subsequently decreases crop yield. Soil salinity is the amount of dissolved salts in the soil which occur as ions, particularly sodium chloride ions. Under increased soil salinity, the osmotic potential in the soil decreases due to the direct effects of ion toxicity which leads to the inhibition of the plant's ability to take up water from the soil [1]. This results in the reduction of several germination parameters such as final germination percentage (FGP) and speed of germination (SG), yielding less vigorous seedlings [2].

The germination and early seedling growth of *Oryza sativa* L. can be negatively affected by increasing salt concentrations [3]. About 48 million hectares of land in humid regions of South and Southeast Asia are fit for *Oryza sativa* L. production but are constrained by soil salinity due to the surface intrusion of saline water from the flooding of rivers and streams during tidal fluctuations and typhoon surges, high actual surface evaporation on dry periods, usage of salinized groundwater for irrigation, and accumulated fertilizer residues [4].

Among the seed priming techniques, hydropriming is the most economical and accessible option in germinating seeds under a variety of stress conditions [5]. Hydropriming is done by soaking seeds in distilled water for a specified number of hours before sowing. It was reported by Sher et al. [6] that hydropriming may improve the stand establishment, seedling vigor, and productivity of field crops under optimal and suboptimal conditions.

Khafagy et al. [7] evaluated the effects of hydropriming on the germination of different *Oryza sativa* L. rice varieties under normal and saline conditions, while Prasad et al. [8] studied rice seedling vigor. Farooq et al. [9] looked at rice hydropriming optimization, but its effect particularly on the NSIC Rc222 variety has not yet been studied. In these three studies mentioned, rice germination was significantly improved which suggests that hydropriming might also be a useful seed priming technique for the Rc222 variety.

The NSIC Rc222 variety of rice is classified by the International Rice Research Institute (IRRI) in the study of Mondal and Borromeo [10] as salt-sensitive rice, susceptible to soils of very high salinity level (9.0-15 dS/m). The evaluation of the germination of *Oryza sativa* L. NSIC Rc222 under salt stress hydroprimed at various time durations is significant to identify more convenient seed treatment methods which can improve its germination performance.

This study evaluated the germination of *Oryza sativa* L. NSIC RC222 under sodium chloride (NaCl) stress hydroprimed for 12 h, 24 h and 48 h. The specific aims are:

(i) evaluate the germination of *Oryza sativa* L. NSIC Rc222 subjected to saline stress hydroprimed for various time durations (0 h, 12 h, 24 h, and 48 h) using the following parameters:

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a. Final germination percentage (FGP),
b. Speed of germination (SG), and
c. Seedling vigor index (SVI);

(ii) evaluate if there is a significant difference among the treatments using Analysis of Variance (ANOVA) at $\alpha = 0.05$ and Least Significant Difference (LSD) using Microsoft Excel Software for Windows 10.

Methods. - *Oryza sativa* L. NSIC Rc222 seeds were hydroprimed by soaking in distilled water for 12, 24, and 48 hours following a randomized complete block design while the control group was left unprimed. Seeds were air-dried for two (2) hours and were subjected to 15 dS/m saline solution to simulate salt stress and to 0 dS/m as control in their corresponding petri dishes. These were left to germinate for seven (7) days inside a temperature-controlled incubator [8]. Germinated seeds were counted every 24 hours and all the germination parameters were measured at the end of seven (7) days. Data analysis was then carried out.

Acquisition of Seeds. Certified *Oryza sativa L.* NSIC RC222 seeds were acquired from the Department of Agriculture - Western Visayas Integrated Agricultural Research Center. The seeds were stored in an airtight container at room temperature until ready for use.

Construction of the Incubator. An incubator with dimensions 0.6m x 0.45 m x 0.45 m was constructed (Figure 1) and covered with glass on top. A heat lamp was attached to a temperature controller set to 29 ± 0.2 degrees Celsius and four (4) LED tubes are fixed on top of the incubator at a 12-hour light and 12-hour dark photoperiodic cycle with the light intensity maintained at 4000 lux during the light cycle [11].

Figure 1. Incubator setup.
Left: top view, with petri dishes containing RC222 seeds.
Right: front view.

Preparation of Seeds. The seeds were surface-sterilized following the procedures used by Khafagy et al. [7]. Seeds were soaked in 1.0% (v/v) sodium hypochlorite solution for three minutes. The residual chlorine was washed out using distilled water. Seeds were divided into four sub-samples and assigned to treatment groups with a total of six replicates each using randomized complete block design. One petri dish containing 25 seeds was used per replicate and each was labeled according to the treatment group and replicate number.

Seed Hydropriming. The seeds were hydroprimed by soaking in a beaker filled with distilled water for 12, 24, and 48 hours, with a ratio of six seeds per ten mL of water. The seeds were subsequently air-dried for two hours.

Preparation of Growing Media and Sowing of Seeds. Two layers of Whatman No, 1 filter paper were placed in each of the 24 petri dishes (9 cm diameter) to serve as the growing medium for the seeds. The 25 seeds were sown in a 1:8:16 circular fashion and stored in the growing media to germinate for seven days.

Simulation of Saline Stress. The 15 dS/m saline solution was prepared by dissolving 9.6 grams of NaCl in one liter of distilled water. A PASCO conductivity probe with ± 10% accuracy was used to measure the salinity in a stepwise manner. Three replicates of each treatment were treated with 10 mL of the 15 dS/m saline solution while the remaining three replicates were given 10 mL of distilled water for control.

Counting of Germinated Seeds and Measurement of Seedling Height. The number of germinated seeds were counted every day for seven days while the seedling height was measured at the end of the germination period. Ten (10) seedlings were randomly selected from each replicate for the measurement of the seedling height using a vernier caliper [7]. The mean of the selected seedling heights per replicate was then calculated.

Data Analysis. The germination parameters, namely the final germination percentage (FGP), speed of germination (SG), and seedling vigor index (SVI) of the germinated seedlings for each treatment group were compared using One-Way Analysis of Variance (ANOVA). If the One-Way ANOVA result is significant, then Fisher's Least Significant Difference (LSD) was used as the post-hoc test for multiple comparisons at $\alpha = 0.05$. All data analyses were done using Microsoft Excel.

$$FGP = \frac{Number\ of\ germinated\ seeds}{Number\ of\ total\ seeds} \times 100\%$$

$$SVI = FGP \times seedling\ length\ (root\ +\ shoot)$$

$$SG = \frac{Number\ of\ germinated\ seeds}{Days\ of\ first\ count} + \ldots\ldots + \frac{Number\ of\ germinated\ seeds}{Days\ of\ final\ count}$$

Safety Procedure. Sodium chloride is an eye irritant, thus wearing external protective clothing such as gloves, eye, and face protection were practiced. Eye irritations were mitigated by washing the eyes thoroughly with water. There were also risks associated with the use of sodium hypochlorite such as skin irritation and serious eye damage. Throughout the experiment, the chemicals were handled with care and external protective clothing was always worn. Direct contact with the chemical was mitigated by thorough washing.

Results and Discussion. - The study aimed to evaluate the germination of *Oryza sativa* L., NSIC Rc222 under NaCl stress hydroprimed for various time durations.

Final Germination Percentage. The highest FGP mean among Rc222 seeds subjected to 15 dS/m saline stress was found in 48 h hydropriming duration with 94.67% mean FGP, followed by 12 h and 24 h while the lowest was found in the unprimed

seeds with 82.67% mean FGP (Figure 2). It must be noted that the mean FGP of the unprimed seeds was lower than 85%, which indicates a significant breakdown in its processes as the seeds were adversely affected by salt stress. Meanwhile, in the 0 dS/m treatment, the 24 h hydropriming treatment yielded the highest FGP mean with 96.00%, followed by 48 h and the lowest in the 12 h treatment and unprimed seeds with both an FGP mean of 93.33%.

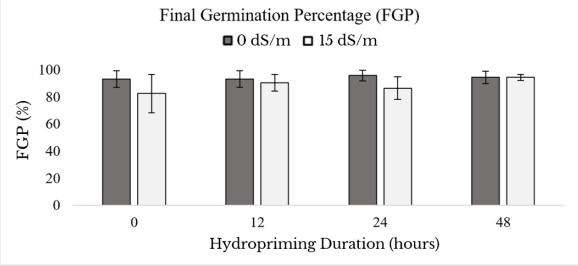

Figure 2. Final Germination Percentage (FGP) between control and saline-stressed conditions (mean values, n=3 per condition).

There is no significant difference among the mean FGP values of all treatment groups in both 0 dS/m and 15 dS/m saline level with a p-value of 0.05 (Table 1). It was observed that the seeds hydroprimed at longer durations and subjected to saline stress exhibited maximum germination in the fourth or fifth day, while most of the unprimed seeds germinated by the sixth and seventh day. At the end of seven days, however, most seeds from all treatment groups have already germinated. Hydropriming, in general, and the various soaking durations did not significantly affect the FGP of Rc222 seeds grown in 0 dS/m (no saline) and 15 dS/m saline treatments.

Table 1. Multiple mean comparisons for the FGP values among all treatment groups (n=3 per condition).

Saline Concentration	Final Germination Percentage (%)				
	0 h	12 h	24 h	48 h	p-value
0 dS/m	93.33	93.33	96.00	94.67	ns
15 dS/m	82.67	90.67	86.67	94.67	ns

ns = not significantly different at $p \leq 0.05$

Hydropriming seemed to have affected the germination of *Oryza sativa L.* only at the earlier stages, especially among seeds hydroprimed at longer durations. This could be due to the different biological mechanisms triggered by hydropriming such as the release of enzymes that produce soluble food nutrients which enabled the seeds to germinate upon sowing [8]. After some time, however, seeds hydroprimed at shorter durations germinated as well due to the optimum light and temperature conditions present inside the incubator [12]. These conditions eventually allowed the seeds from all treatment groups to grow to a minimum of 2 mm radicle length by the end of seven days. Due to this, the FGP values among all treatment groups in the study were almost similar in both normal and saline conditions, and thus, no significant difference was recorded.

Speed of Germination. The highest SG mean recorded for the 15 dS/m saline treatments was with 48 h hydropriming with SG mean of 34.8, followed by 24 h, 12 h, and the lowest from unprimed seeds with 7.69 (Figure 3). Similar results were observed among the seeds subjected to 0 dS/m, wherein the highest SG mean was recorded in 48 h hydropriming with mean of 43.37, followed by 24 h, 12 h, and the lowest from the unprimed seeds with SG mean of 21.6.

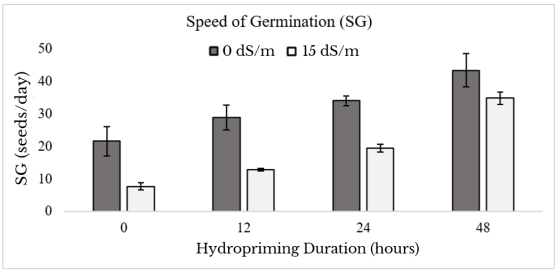

Figure 3. Speed of Germination (SG) between control and saline-stressed conditions (mean values, n=3 per condition)

Hydropriming significantly increased the speed of germination of Rc222 seeds, wherein under both 0 dS/m and 15 dS/m saline treatments, 48 h hydropriming obtained the highest SG values with 34.80 seeds/day and 43.37 seeds/day, respectively (Table 2). It was observed that the seeds hydroprimed at these durations germinated the earliest upon sowing while the unprimed seeds and 12 h hydropriming have only started to germinate on the third day. Under 0 dS/m, the SG values are comparable between the unprimed seeds and the 12 h hydropriming, as well as between 12 h and 24 h hydropriming treatments. Subjecting saline stress significantly decreased the mean SG values among Rc222 seeds but hydropriming at particularly longer durations mitigated these negative effects.

Table 2. Multiple mean comparisons for the SG values among all treatment groups (n=3 per condition).

Saline Concentration	Speed of Germination (seeds/day)				
	0 h	12 h	24 h	48 h	p-value
0 dS/m	21.56[b]	28.93[a b]	33.98[a]	43.37	<0.001*
15 dS/m	7.69	12.91	19.42	34.80	<0.001*

* Significantly different at $p \leq 0.05$
[z]In a row per saline concentration, means with the same letters are not significantly different at 5% level of significance.

Hydropriming *Oryza sativa L.* NSIC Rc222 seeds mitigated the effects of salt stress because the speed of germination (SG) increased as hydropriming duration was increased. The faster speed of germination (SG) in longer soaking durations was caused by biological and physiological processes such as the acceleration of the emergence phase and

multiplication of radical cells which limit the exposure of the seeds to the stressful conditions in the environment [13]. Findings were similar in studies conducted on *Triticum aestivum L.* (wheat) by Basra et al. [14] and *Momordica charantia* (gourd) seeds by Adhikari et al. [15] wherein increasing the duration of hydropriming up to 48 hours significantly improved the duration of germination. Hydropriming seeds enabled the completion of metabolic activities prior to planting which reduced the time for the seed to germinate [16].

Seedling Vigor Index. Among the seeds subjected to 15 dS/m saline stress, SVI increased with longer durations of hydropriming, wherein the 48 h hydropriming yielded the highest SVI with 7320.20, followed by the 24 h, 12 h, and the lowest in the unprimed seeds with 2453.00 (Figure 4). The seeds grown in 0 dS/m also yielded an increasing SVI with longer hydropriming durations except for the 48 h hydropriming which obtained a lower SVI of 8912.16 than the 24 h hydropriming which yielded an SVI mean of 10348.47.

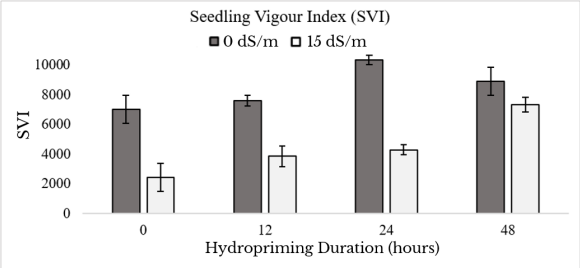

Figure 4. Seedling Vigor Index (SVI) in control and saline conditions (mean values, n=3 per condition).

Seeds hydroprimed for longer durations and subjected to saline stress have visibly grown higher seedling heights in a shorter period, which explains the increasing SVI values except for the 24 h hydropriming which obtained a significantly higher SVI compared to the 48 h hydropriming under 0 dS/m (Table 3). The seeds subjected to 15 dS/m salt stress exhibited the highest SVI with 48 h hydropriming, where the mean value was found to be significantly higher compared to the 24 h, 12 h hydropriming treatments and the unprimed seeds.

Table 3. Multiple mean comparisons for the SVI values among all treatment groups (n=3 per condition).

Saline Concen-tration	Seedling Vigor Index				
	0 h	12 h	24 h	48 h	p-value
0 dS/m	7019[b]	7617[ab]	10348	8912[a]	0.002*
15 dS/m	2453	3860[a]	4293[a]	7320	<0.001*

* Significantly different at $p \leq 0.05$
[Z]In a row per saline concentration, means with the same letters are not significantly different at 5% level of significance.

Under 0 dS/m treatment, 24 h hydropriming resulted in a higher SVI than 48 h hydropriming treatment. This correlates with a previous study by Kumar et al. [17] that determined 24 h as the maximum length of time for which *Oryza sativa L.* seeds should be soaked, as exceeding it could cause seed deterioration. Although imbibition is vital for the re-constitution of biomembranes, activation of enzymes, mobilization of storage compounds, and protein synthesis in the seed, it can also cause imbibition damage, particularly when water is taken up rapidly [7]. Meanwhile, the SVI values obtained for the 15 dS/m saline treatment in the current study were similar to Khafagy et al. [7] where the highest SVI was with *Oryza sativa L.* seeds hydroprimed for 48 h compared to 12 h, 24 h, and 36 h. This suggests that hydropriming for longer durations increases the SVI especially when the conditions such as saline stress deem seed repair to be necessary.

Limitations. In the preparation of the 15 dS/m saline solution, table salt was used which may have possibly contained other additives aside from the 97% NaCl that could induce negative effects on the germination parameters of the seeds. Moreover, there might have been some discrepancies in the computation of the SG values due to the inconsistencies in the schedule of counting of germinated seeds as caused by the conflicting schedules of the researchers. Lastly, in the measurement of the seedling height using vernier caliper, the roots and leaves were straightened out by hand and laid flat on a piece of paper. Due to the risk of breaking the seedling, the roots may not have been straightened out entirely and uniformly, which rendered the measurement of the seedling height and the computation of the SVI to be less precise.

Conclusion. - Hydropriming was concluded to not have any significant effect on the final germination percentage but can effectively accelerate the growth and significantly improve the seedling vigor of Rc222 seeds subject to 15 dS/m salt stress. Moreover, 48 h hydropriming is found to be the most effective soaking duration in alleviating the effects of saline stress among *Oryza sativa L.* NSIC Rc222 seeds.

Recommendations. - It is recommended that *Oryza sativa* L. NSIC Rc222 (rice) seeds should be hydroprimed for 48 hours to mitigate the negative effects on its germination. Furthermore, it is recommended that a uniform schedule for data collection will be imposed, especially for the counting of germinated seeds. Use of digital software such as ImageJ may also be considered for a more precise measurement of the seedling height. Lastly, it is recommended that technical grade sodium chloride salt shall be used in making the saline solution to minimize the presence of other compounds that may also affect the germination of the *Oryza sativa* L. NSIC Rc222 seeds.

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Growth of *Momordica charantia* (bitter gourd) amended by *Chanos chanos* (bangus) and *Oreochromis niloticus* (tilapia) fish offal fertilizer

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Abstract

Inorganic fertilizers have been widely used in enhancing the growth of crops. However, excessive use of inorganic fertilizers can have adverse environmental effects. Thus, there is a need to find alternative ways and one is by using fish offal from aquaculture by-products as fertilizers. Fish offal are organic fertilizers known to contain a healthy balance of significant plant nutrients. But only few studies were done on plant growth effects using fish offal from different fish species. Here, we compared the number of leaves, area, and growth rate of *M. charantia*, an important medicinal vegetable crop, in soil amended with fish offal of *C. chanos* and *O. niloticus* at 7.5% (v/v) concentration. Results showed that there is no significant difference on the growth of *M. charantia* between the fish offal of *C. chanos* and *O. niloticus*. Therefore, fish offal fertilizers from either fish species enhanced the growth of *M. charantia* plant providing a more cost-efficient and environment-friendly way of growing *M. charantia*.

Introduction. - Inorganic fertilizers have been the staple way of enhancing soil fertility because they are quick-release fertilizers, making the nutrients immediately available for the crop. However, inorganic fertilizers can cause leaching and excess algal growth before the nutrients are actually taken up by the plant, consequently damaging the aquatic ecosystem [1]. Therefore, there is a need to find alternative sources of fertilizer, and one potential source is the fish offal that comes from culturing fish.

Organic fertilizers improve soil fertility, soil structure, water retention capacity, physical and chemical properties, soil pH, microbial activity, and crop yield [2]. Organic fertilizers are also environment-friendly as stated by Akande et. al (2004) in Ahmad et al. [2]. Agricultural wastes, livestock wastes (manure), domestic and industrial wastes (compost), and fishery/aquaculture wastes like fish offals are commonly used organic fertilizers [2, 3].

Soil fertilization using fish waste compost was reported to cause an increase in leaf yield of *L. sativa L.* It caused a significant increase of nitrogen, phosphorus, potassium, sodium, calcium, and magnesium in leaves of the plant [4]. Also, fermented fish waste was found to enrich the soil nutrients required for plant growth and favorably influenced the conducting functions of xylem and phloem vessels [5]. Results of the study conducted by Lema and Degebassa [1] has shown that fertilizers made from fish offal can provide readily absorbed nutrients required for growth and yield production of tomato and onion [1]. Different fish offals have been tested for their properties in enhancing the growth of plants. In addition, the liquid fish silage of *Nemipterus japonicus*, at 5.0%-10.0% has a great effect on the growth of *Brassica rapa subsp. chinensis* in terms of its height, leaf number, leaf area, and fresh weight [3]. Therefore, fish offals help plants grow efficiently by providing the nutrients that are needed for growth such as nitrogen, phosphorus, and potassium.

The test plant, *Momordica charantia L* belongs to the family of Cucurbitaceae and is commonly known as Ampalaya in the Philippines. It is a widespread vegetable crop grown in Asia and in other parts of the world. It is usually grown as an annual crop, but it can also be considered as a perennial crop in mild and frost-free winter areas. *M. charantia* is best sown from October to February as it is its ideal planting season because cool weather is better for production [6]. These conditions are required for the germination of *M. charantia* seeds. *M. charantia* has been suggested to be an economically important crop as it has shown dietary advantages as the immature fruit contains vitamin A and vitamin C [7] [6]. *M. charantia* was chosen as a test plant because it can be cultivated throughout the year. *M. charantia* has been widely utilized in traditional medicine for many treatments due to the phytochemicals present in the herb that have been identified to exhibit medicinal activities such as antibiotic and antidiabetic which makes it one of the most nutritious plants [8]. It was noticed that the vegetative growth and herbage yield of *M. charantia* was significantly enhanced by the application of different organic fertilizers [8].

Fish offal fertilizers are organic fertilizers made from by-products of the fish industry which are known to contain significant quantities of nitrogen as well as a healthy balance of all 18 nutrients such as

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amino acids which can be significant for crops growth. According to the study of Lema and Degebassa [1], it was known that plants rapidly respond to and grow vigorously when they are regularly fertilized with fish fertilizers. Also, fish offal such as heads, guts, and fins are suitable for agricultural use since it has high contents of nutrients, such as N, P, and Ca [9]. Because of this, composting initiatives using fish waste have been carried out in various parts of the world in search of alternative and viable techniques for transforming fish waste into useful agricultural products [10]. A study conducted by Widyastuti [11] has shown that applying fish waste fertilizer of fermented *C. chanos* has a significant effect on the growth of plants. A study also showed that fish wastes of *O. niloticus* boosted the production of *Solanum lycopersicum L.*, and *Allium cepa* [1]. Therefore, fish offals of *C. chanos* and *O. niloticus* are excellent sources of nutrition for soils and plants.

It has already been studied that fish offal from *C. chanos* and *O. niloticus* are effective fertilizers in improving the growth of plants, but the comparative effectiveness of these fish offal fertilizers in a specific concentration on the growth of a plant has not been determined yet.

Studies have only looked at the comparison of the effects of fish offal fertilizer to inorganic fertilizer and few studies have been done on comparing the effects of fish offal fertilizer sourced from the culture of different fish species. Our research will therefore compare the effects of fish offal fertilizers from *C. chanos* and *O. niloticus* in a specific concentration on the growth of *M. charantia*. This research will also test if *C. chanos* will have a more significant impact on the plant growth of *M. charantia* than the fish offal of *O. niloticus*. *M. charantia* will be used as the test plant of the research as it is native to the Philippines which is one of the parameters of the study. The research will also be exploring the amount of concentration that will be given to the test plant which is 7.5%. The research specifically aims to determine which fish offal fertilizer in a specific concentration is a better alternative to inorganic fertilizers for the optimal and maximum growth of a plant like *M. charantia*.

The study chose the fish species *C. chanos* and *O. niloticus* because they are the second and first most important farmed fish in the Philippines respectively [12]. The significance of this study is to reduce the use of inorganic fertilizers which are harmful to the environment. This study was chosen to be able to propose a better fish offal as an alternative fertilizer to inorganic fertilizer.

This study aims to determine the comparative growth of *Momordica charantia* amended with the fish offals of *Chanos chanos* and the fish offals of *Oreochromis niloticus* in a specific concentration. effects of *C. chanos* and *O. niloticus* fish offals as fertilizers on the growth of *M. charantia* in a specific concentration. It specifically aims to:

(i) To count the number of fully expanded leaves of *M. charantia* after the introduction of the treatment groups, *C. chanos* (B), *O. niloticus* (T), and the negative control group (C), tap water, on the 21st day after planting to determine the average number of fully expanded leaves;

(ii) To measure the height of *M. charantia* from the ground level to the leaf base of the highest fully expanded leaf after the introduction of the treatment groups, B, T, and C, on the 5th, 9th, 13th, 17th, and 21st day after planting to calculate growth rate in cm/day.

(iii) To determine the largest fully expanded leaf per sample per treatment per replicate of *M. charantia* to calculate the leaf area in cm^2; and

(iv) To determine the difference in the growth of *M. charantia* when compared among treatment groups (B, T, and C) and within treatment groups.

Methods. - The experimental study was conducted for a period of 21 days to compare the effects of *C. chanos* and *O. niloticus* fish offals as fertilizers on the growth of *M. charantia* at 7.5% v/v concentration. The fish offals of *C. chanos* and the fish offals of *O. niloticus* were acquired from the local fish markets. The offals were then boiled, settled overnight, and strained using a muslin cloth. Then, the strained fish offals were placed evenly in aluminum pans for sun-drying up to a period of 11 days. The dried fish offals were ground using a mortar and pestle and were added and mixed with previously homogenized soil. After which, twenty seeds of *M. charantia* were planted and ten seeds were randomly chosen for the measurement of plant height, the number of leaves, and leaf area. One-way ANOVA was used for the statistical analysis.

Experimental set-up. The study utilized three blocks with three pots per block in each of the researchers' backyards. The placement of the samples was determined using the randomized complete sampling block design (RCBD) and through an electronic random generator.

Twenty seeds were planted in each pot. Then, the researchers conducted a fishbowl random sampling to identify ten seeds from the total number of seeds sprouted in each pot to be used as test plants for the data analysis. Researchers' backyards were chosen as the study sites because they were the places that were most suitable in the work unit's field of study, which is agriculture. Backyard is the place where the plants can get enough sunlight and is the place that is not frequently disturbed by people. A plant house with the dimension of 421.16 cm x 343.35 cm x 200 cm was built with garden nets in the backyards of the researchers for the plants to grow and be protected against pests and insects. A soil analysis test using the jar test method was carried out by the researchers. The results of the soil analysis indicated that the researchers obtained silt loam soil, sandy loam soil, and pure loam soil.

Materials and Equipment. For the preparation of fish offal fertilizers, *C. chanos* and *O. niloticus*, were obtained from local markets. Distilled water, aluminum foil, and aluminum pans were obtained from grocery stores and mortar and pestle from local hardware stores. Muslin cloth was bought from a

local textile store and cooking barrels were obtained from the respective homes of the researchers.

For planting, *M. charantia* seeds were obtained from Pacifica Agrivet Supplies. Watering cans and pots were bought from local agricultural and hardware stores while soil and tap water were obtained from the researchers' houses.

For measurements, vernier calipers and kitchen scales were bought from local hardware stores.

Data Gathering and Analysis. The data gathering was done for 45 days and involved the preparation of the fish offal fertilizers, planting of *M. charantia* seeds, and the measurements of the identified parameters such as the number of leaves, growth rate, and leaf area.

Preparation of fish offal fertilizers. One kg of *C. chanos* and *O. niloticus* fish offals, specifically the fins, liver, intestine, heart, kidney, and stomach, were separated and used as fertilizer following the method of Lema & Deghebassa [1]. The identified fish offals were then cut into smaller pieces. After that, 222 ml of distilled water was boiled until it reached a temperature of 100 degrees Celsius, and the cut fish offals were added. The fish offals were cooked for 17 minutes and were stirred every three minutes [13]. The resulting mixture was left overnight to allow the solids to settle. After 12 hours, the liquid components were separated using a muslin cloth and disposed. The solid components were sundried for 11 hours every day from six in the morning until four in the afternoon for 12 days. After every drying process, the aluminum foil pan was covered with an aluminum foil sheet and stored at room temperature [1]. The dried fish offal were crushed using a mortar and pestle to obtain an amorphous and flaky fertilizer.

Incorporation of the dried fish offal fertilizers. The dried fish offals, measured at five parts, were directly mixed with 67 parts of soil to achieve a 7.5% volume-to-volume concentration [14] in the sack using a trowel and placed in a pot [15].

Planting of Momordica charantia seeds. Twenty seeds of *M. charantia* were sown evenly in each pot with the dimensions of 44.45 cm x 20.32 cm x 14.00 cm. The pots in each block were placed five cm apart and each block separated with a 30 cm distance. The seeds were sown one cm deep and placed four cm apart in length and 6.5 cm apart in width for each seed. For watering, *M. charantia* were watered at around four to five in the afternoon everyday using one L of tap water in each pot [6].

Measurements. The number of leaves of *M. charantia* were manually gathered by counting the total number of fully expanded leaves at the end of the experiment at Day 21.

The height of *M. charantia*, was measured using a vernier caliper from the ground level to the leaf base of the highest fully expanded leaf and was manually recorded by the researchers on the 5th, 9th, 13th, 17th, and 21st day after planting for computations.

The growth rate of *M. charantia* was determined using the following formula,

$$\left(\frac{final\ height - initial\ height}{n}\right)$$

where n is the number of days between the final day and the initial day of determination [16].

The leaf area of *M. charantia* was computed using the Counting Grid Squares (CGS) method, using the formula: Leaf Area = (NGS × OGA), where NGS is the number of grid squares inside the leaf outline and OGA is the area of a single square grid [17]. The data was gathered after the 21st day of planting for computations.

Data Analysis. The number of leaves, growth rate and leaf area were computed by the researchers using their respective formulas. Standard deviation was also calculated based on the number of replicate samples. The statistical analysis of the study was done using the Jamovi Version 2.2.2.0 software. One-way analysis of variance (ANOVA) was used to compare the means and the least significant difference (LSD) was used to separate the means at significance level $p<0.05$. A normality test was also performed to ensure that the data, specifically for the leaf area, was normally distributed despite its small sample size.

Safety Procedure. Personal protective equipment (PPE) such as gardening gloves and aprons were used in order to protect the researchers while performing the experiments when working with hazards.

Proper waste disposal was practiced. Excess fish offals were properly identified before they were disposed of in the food wastes bin.

Results and Discussion. - This section is divided into 3 components. The first component shows the effect of the application of the dried fish offals of *C. chanos* and *O. niloticus* and the non-fertilized control on the number of leaves of the *M. charantia* plant. The second component shows the effect of the application of fish offals on the leaf area of the *M. charantia* plant. Lastly, the third component compares the effect of the *C. chanos* and *O. niloticus* dried fish offals as fertilizers and the control group on the growth rate of the *M. charantia* plant.

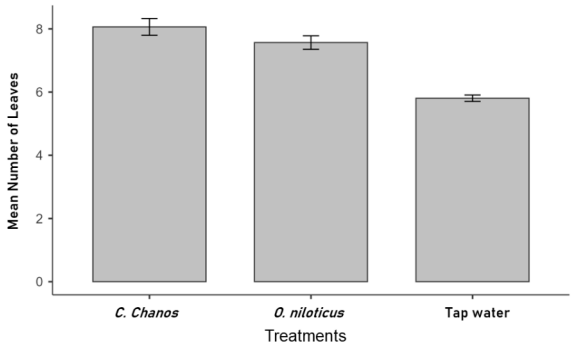

Figure 1. This figure shows the mean number of leaves of *M. charantia* amended by *C. chanos and O. niloticus* offal fertilizer.

Effects on the number of leaves. The mean number of leaves of the treatments fertilized with *C. chanos* offal has the highest mean value among all the treatments at 8.06 as shown in Fig. 1. It is followed by the treatments containing *O. niloticus* which measures an average mean number of 7.57. Lastly, the control group, tap water, has the lowest mean value of 5.81.

The mean number of leaves varied among treatment groups. This is probably due to the difference in the amount of nitrogen (N) present in the soil. According to [15], the amount of available N is directly proportional to the number of leaves to be produced by the plant. The available organic nitrogen present in the fish fertilizer enhanced the number of leaves and branches of the plant [1]. At $p<0.001$, there was a significant difference between the two (2) treatment groups and the control group. This significant difference observed between the two treatment groups and the control group is supported by the study done by Hamaiel et al. [8]. They found that the high nitrogen concentration present in the soil improved the physical condition of the soil, providing enough energy for microbial activity thereby increasing the availability and uptake of nutrients. However, no significant difference was observed between the two (2) treatment groups, *C. chanos*, and *O. niloticus* offal fertilizers indicating that both treatments acted the same in terms of producing leaf numbers of *M. charantia* plant. It is to be noted that only fully expanded leaves were manually counted by the researchers.

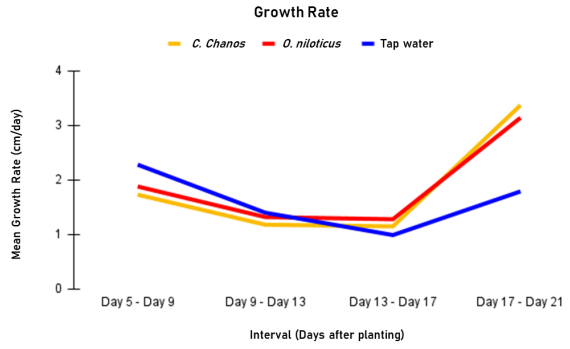

Figure 2. This figure shows the mean growth rate of *M. charantia* amended by *C. chanos* and *O. niloticus* offal fertilizer.

Growth rate of M. charantia. The growth rates based on plant height were measured at different time intervals (Day 5 - Day 9, Day 9 - Day 13, Day 13 - Day 17, and Day 17 - Day 21). Fig. 2 shows the changes in the growth rates per treatment group at different intervals. At Day 5 to Day 9, the control group, tap water, (2.28 cm/day) has the highest mean growth rate as compared to *O. niloticus* (1.88 cm/day) and *C. chanos* (1.73 cm/day). At Day 9 to Day 13, the tap water (1.40 cm/day) still exhibited the highest mean growth rate, followed by the *O. niloticus* (1.32 cm/day) and lastly, the *C. chanos* (1.18 cm/day). At Day 13 to Day 17, *O. niloticus* (1.28 cm/day) had the highest mean growth rate and was followed by the *C. chanos* (1.15 cm/day) while the tap water control measured the lowest growth rate at 0.99 cm/day. Lastly, at Day 17 to Day 21, *C. chanos* (3.37 cm/day) produced the highest mean growth rate as compared to the *O. niloticus* (3.14 cm/day) and tap water (1.79 cm/day). This time interval also recorded the fastest mean growth rate in all the treatments indicating the plants reached the exponential growth stage.

Day 0 to Day 4 measurements were not included in the data gathering as this time point was allocated for the *M. charantia* plants to grow.

Variation in the mean growth rates among treatment groups at different time intervals was observed. It is to be noted that the plant height was used as an indicator for the growth rate parameter. At $p<0.01$, there was a significant difference between the *O. niloticus* treatment group and the control group. This can be attributed to an increase in available organic nitrogen present in the soil as fish offal fertilizers were applied [14]. Furthermore, nitrogen nutrition is directly linked to the development of plants [17]. However, no significant difference was observed between the two (2) treatment groups, *C. chanos* and *O. niloticus*, offal fertilizers, implying that both offal treatments provided similar amounts of nitrogen to the soil.

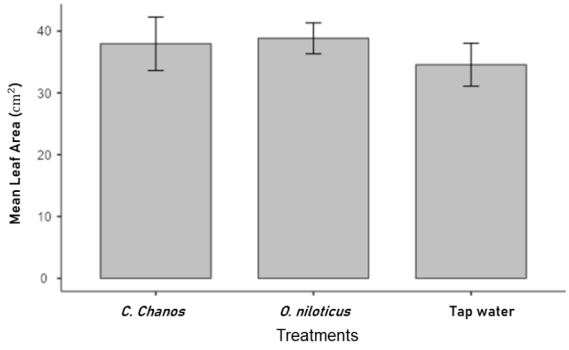

Figure 3. This figure shows the mean leaf area of *M. charantia* amended by *C. chanos and O. niloticus* offal fertilizer.

This figure shows that the leaf area of the treatments containing *C. chanos*, 37.9 cm^2, has the highest mean leaf area. Treatments containing *O. niloticus* measured at 38.8 cm^2 come after. The lowest mean was yielded by the control group, tap water, which was measured at 34.6 cm^2.

Comparison of the leaf area. The area of the largest leaves from each treatment was compared using the CGS method (Fig. 3). *M. charantia* plants grown on *O. niloticus* offal fertilizer were found to have the highest mean leaf area at 38.8 cm^2. Treatments containing *C. chanos* measured at 37.9 cm^2 come after. The lowest mean leaf area was yielded by the control group, tap water, which was measured at 34.6 cm^2.

The mean values of the leaf area among the treatment group varied. The results can be supported by the claim of Rachman and Suwars (1990) which was stated by Karim et. al [15] that the application of nitrogen increases leaf dimensions (length and width of leaf) of the plant. At $p>0.05$, no significant difference was observed between the treatment

groups and the control group. However, the Counting Grid Squares (CGS) method which is a direct and manual method of computing for leaf area was done by the researchers.

The mean values of the leaf area among the treatments varied. These results can be supported by the claim of Rachman and Suwars (1990) mentioned in Karim et. al [15] that the application of nitrogen, in our case the nitrogen-rich fish offal, increases leaf dimensions (length and width of leaf) of the plant. There is no significant difference observed between the treatment groups and the control group ($p<0.05$). However, the Counting Grid Squares (CGS) method, a direct and manual method of measuring leaf area, was the only method employed in this study. Other methods such as the use of ImageJ software may produce more accurate measurements.

Table 1. This table shows the comparison between the effects of *C. chanos*, and *O. niloticus* with the effects of the negative control on the growth rate, leaf number, and number of leaves of the *M. charantia*.

Treatment	Number of leaves	Growth rate	Leaf area
C. chanos	8.06*	1.85	37.9
O. niloticus	7.57*	1.91*	38.8
Tap Water	5.81	1.61	34.6

n.s = no significant differences ($p>0.05$) observed with the control group
* = significant differences ($p<0.05$) observed with the control group

In general, *O. niloticus* fish offal fertilizer has a more significant effect on the growth rate of *M. charantia* when compared to *C. chanos*. For the number of leaves, no significant difference was observed between the two treatment groups. However, compared to the control group, *C. chanos* and *O. niloticus* fish offal fertilizers have significant effect on increasing the number of leaves of *M. charantia*. Lastly, in terms of the leaf area, no significant difference was observed among the two fish offal fertilizers and the control group.

Conclusion. - The One-way ANOVA analysis showed that there is a significant difference on the overall growth parameters of *M. charantia* between the control and the fish offal fertilizers of *C. chanos* and *O. niloticus*. These results indicate the efficacy of fish offal as organic soil fertilizer. However, *C. chanos* and *O. niloticus* showed no significant difference when compared between each other on the overall growth parameters of *M. charantia*. This implies that the application of either fish offal fertilizer enhanced the growth of *M. charantia* plant. Thus, the application of *C. chanos* and *O. niloticus* offal fertilizers would have similar effects on the growth of juvenile *M. charantia* plants. However, further analysis on the use of fish offal as organic fertilizers should be done as phytotoxicity and nitrogen overload may occur.

The results of the study may serve as one of the bases for field trials of *C. chanos* and *O. niloticus* offal fertilizers on ampalaya. Similar effects on the growth of other crops is not certain because of the findings of this study.

Recommendations. - For the number of leaves, the AV-K method utilizing leaf nodes can be used to calculate the total number of leaves. When the plant sample used in the study is a vine, stem length can be measured instead of plant height. Moreover, software such as ImageJ can be used for the measurement of the leaf area for a more precise result. The soil to be used in the study should be tested for nutrient availability. Lastly, the effects of *C. chanos* and *O. niloticus* offal fertilizers on other crops may be known using the methods of this study.

Acknowledgment. - We would like to thank the Department of Agriculture - Region VI for communicating with us regarding our queries and concerns about the acquisition of our test plant, *Momordica charantia,* prior to our data gathering period.

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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:

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

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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