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Germinability and vigor of partially-aged corn (*Zea mays* L.) seeds presoaked in Sampaguita (*Jasminum sambac* [L.] Aiton) flower extract and essential oil

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Abstract Corn seeds soaked in 3% concentration of flower extract resulted in higher percent germination and vigor compared to the other treatments for both varieties. The optimum concentration of essential oil needed to enhance germination and vigor was 1% and 2% for BPI Lagkitan and Sweet Corn, respectively. The 3% essential oil concentration was shown to be detrimental to partially-aged seeds. The use of essential oil somehow ensured pathogen-free seeds based on visual examination. Seed presoaking in Sampaguita flower extract or essential oil may be an alternative to chemical seed treatment in support of organic seed production.

Keywords: Artificially-aged seeds, Naturally-aged seeds, Seed quality, Seed treatment

Introduction

Rapid establishment of uniform and vigorous seedlings is a major factor contributing to a successful crop (Finch-Savage and Bassel, 2015). Hence, techniques like seed treatments are done to improve the performance of seed such that it will be vigorous enough to grow into a healthy plant. Seed treatment is any technique of subjecting seeds in a compound, a process, or various energy forms such as heat, radiation, or magnetism (Murphy, 2017). Seeds are treated to promote good seedling establishment, minimize yield loss, maintain and improve quality, and avoid the spread of harmful organisms such as seed-borne, soil inhabiting, and mobile organisms that attack plants later in their growth (Kiran *et al.*, 2014).

Pre-soaking of seeds is one method of seed treatment, wherein it alters the seed moisture content or hydrates the seed to a desired level in certain solutions like water, chemicals, and organic substances to allow imbibition which is needed by the seed to resume embryo growth, hence will hasten germination

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(Murphy, 2017). Some would include compounds, like herbicides, fungicides, organic solvents, plant extracts, etc. that are dissolved in water such that it would be also taken in or imbibed by the seed to attain a certain purpose (Finch-Savage and Bassel, 2015).

The effects of pre-soaking have been well studied. Pre-soaking of rice (*Oryza sativa*) seeds resulted in a lower time to 50% germination and mean germination time, higher germination percentage and index and higher energy of germination due to dormancy breakdown and increased metabolic activities (Basrasma *et al.*, 2005). In the study of Ahmad *et al.* (1998), it was indicated that wheat (*Triticum aestivum*) seed presoaking with drying also improved germination. Germination rate index (GRI) increased with soaking the seed before sowing but not beyond 12 hours. It is likely that the hydrolytic processes began during presoaking, and the resultant simple sugars that were released were utilized immediately for synthesis, upon germination. Subbaiah (1992) conducted a study on the effect of pre-soaking in organic solvents such on seed germination and seedling growth of cashew (*Anacardium occidentale*), stating that pre-soaking of cashew seeds in chloroform or acetone for two hours hastened and partially synchronized germination as well as field emergence. The organic solvents removed the waxy layer of the pericarp and thereby facilitated water imbibition and phenol exudation. Several plant extracts used as seed treatment could reduce seed-borne bacterial speck disease of tomato (*Pseudomonas syringae* pv. tomato) and are promising biological seed treatments (Karabuyuk and Aysan, 2018). Corn (*Zea mays*) seeds primed with chloride salts have alleviated the adverse effects of salt stress at germination stage (Ashraf and Rauf, 2001), while hydro-primed corn seeds have improved germination and seedling growth, germination index, seedling vigor index and length of seedling under both stress and non- stress conditions (Janmohammadi *et al.*, 2008).

In this study, flower extract and essential oil of Sampaguita (*Jasminum sambac* [L.] Aiton) were used as pre-soaking treatment for two corn seed varieties (BPI Lagkitan and Sweet Corn). Sampaguita is a flowering shrub with small white fragrant flowers and is a species of jasmine. It belongs to the family Oleaceae and is believed to have originated from Himalayas but is now a native of Southwestern and Southern Asia. It is the Philippines' national flower and is considered as a symbol of honor and dignity (Sanchez *et al*., 2010). Sampaguita flowers are normally displayed and sold in churches for religious purposes, hence obtaining it would be easy. Sampaguita flower extract is said to contain active biochemical substances that have therapeutic and medicinal value and antioxidant potential and has displayed antimicrobial activity (Kunhachan *et al.*, 2012; Pandey and Tripathy, 2013). Its essential oils contain defense

compounds against insects and other animals and protect the plant by their antifungal and antibacterial properties (Shutes, 2018). Thus, these may be used to enhance the quality of seeds, as it can possibly protect seeds from insects and pathogens.

The general objective of the study was determined the effects of sampaguita flower extract and essential oil on the germination and vigor of two corn seed varieties (BPI Lagkitan and Sweet Corn). The specific objectives were determined the response of corn seeds to different concentrations of sampaguita flower extract and essential oil in the germination and vigor of corn and to compare effects of different concentrations of sampaguita flower extract and essential oil on the germination and vigor of corn seeds.

Materials and methods

The study was conducted at the Seed Science and Technology Laboratory, Institute of Crop Science, University of the Philippines Los Baños (UPLB), College, Laguna, Philippines. The experiment was laid out in completely randomized design (CRD) with eight replicates for germination test and four replicates for seedling emergence test. There were two pre-soaking treatments (Sampaguita flower extract and Sampaguita essential oil) with three levels of concentration (1%, 2%, and 3%) using two varieties of corn (BPI [Bureau of Plant Industry] Lagkitan and Sweet Corn). Untreated seeds served as control. To obtain partially-aged Lagkitan seeds, Accelerated Ageing Technique (AAT) was done by subjecting seeds to a temperature of 44℃ and 100% relative humidity for 168 hours or seven days which reduced the germination percentage from 98 to 81. Sweet Corn had an initial germination of 84%, which was already low, thus it did not undergo artificial aging.

Preparation of flower extracts and essentials oils

To obtain the flower extract, 100 g of Sampaguita flowers were collected, washed under running water for one minute and surface sterilized in 10% sodium hypochlorite (NaOCl) solution for five minutes, then rinsed with distilled water for one minute. Flowers were macerated and ground using mortar and pestle and 50 ml of distilled water were added. The solution was filtered using a strainer and cheese cloth. The filtrate served as stock solution and 1mL, 4mL, and 7.5mL of which were diluted to 150 mL distilled water, enough to satisfy a treatment level. The essential oil treatment was prepared by mixing 10 mL essential oil with 10 mL alcohol, which served as stock solution for further dilution in water to meet the desired concentration.

Presoaking treatments

The seeds were washed under running water for five minutes to remove dirt and other contaminants and surface sterilized in 10% NaOCl for five minutes, then rinsed in distilled water for another minute. Seeds were then presoaked in different treatments for eight hours at room temperature and blotted dry using clean paper towels.

Data collection

Percent germination (%). Fifty seeds per replicate were sown in moistened paper towels and placed equidistantly on top of paper. Adequate amount of distilled water was provided throughout the testing period to keep the paper towel moist. Counting of seedlings was done on the fourth (first count) and seventh (final count) day after sowing (DAS). The percent germination was determined using the formula:

$$Percent\ germination = \frac{no.of\ normal\ seedlings}{total\ number\ of\ seeds\ sown}\ x\ 100$$

Seed vigor

First Count. The first count was obtained by determining the total number of germinated seeds in the standard germination test set-up four DAS.

Vigor index. Vigor index was determined through the speed of germination test, which has also been obtained from the standard germination test set-up. Seedlings with 2 mm radicle protrusion were considered germinated. Counting was done daily one DAS and daily thereafter until seven DAS. The vigor index was computed using the formula:

$$\text{Vigor index} = (n/1) + (n/2) + (n/3) + \ldots + (n/7)$$

Where, n = number of germinated seedlings

Seedling growth rate test (root and shoot length, and biomass). Seedling growth rate test was done by randomly selecting 10 seedlings from the standard germination test. The root and shoot length (cm) of the selected seedlings were measured using a ruler. After the measurement, the seeds (endosperm) were removed, and the seedlings were put in a coin envelope. The seedlings were then oven-dried for 72 hours at 70 ℃ and were weighed to determine its biomass in grams.

Seedling emergence. To assess the seedling emergence, four replicates were used, such that 50 seeds were sown per replicate in unsterilized soil in a black polyethylene pot. Adequate amount of water was supplied to the soil throughout the testing period. The number of seedlings that have emerged was noted eight DAS. The seedling emergence percentage was computed using the formula:

$$\% \text{ Seedling emergence} = \frac{no.of\ normal\ seedlings\ emerged}{total\ number\ of\ seeds\ sown}\ x\ 100$$

Data analysis

The data was analyzed using the Analysis of Variance (ANOVA) of STAR 2.0.1 software developed by the International Rice Research Institute (IRRI). It was used to determine whether the flower-based treatments, with varying concentrations, have a significant effect on the germination and vigor of corn seeds. In addition, the mean comparison was done and interpreted using the Least Significant Difference (LSD) test at 5% level of significance.

Results

Presoaking of sweet corn seeds in Sampaguita flower extract and essential oil

Presoaked seeds in 3% flower extract resulted to higher percent germination (%G) compared to other treatments (Table 1). Meanwhile, seeds soaked in 2% essential oil concentration yielded higher %G compared to other essential oil treatments. The 1% and 2% flower extract and 2% essential oil treatments were not significantly different from each other but were significantly higher than the control in terms of %G. Seeds soaked in 1% essential oil had lower %G than the control. The 3% essential oil treatment was shown to have the lowest %G among all treatments. This implies that an essential oil concentrationhigher than 2% can be disadvantageous to sweet corn seeds.

Table 1. Percent germination of sweet corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Percent Germination[1]
Control		83.5c
Flower Extract	1%	87.5b
	2%	88.5b
	3%	90.5a
Essential Oil	1%	75.5d
	2%	85.25b
	3%	31.5e

[1] Means with the same letter are not significantly different. CV (%) = 7.58

The first count (FC) is the number of normal seedlings that have emerged o four days after sowing and is an indication of the level of vigor of seeds. The presoaked seeds in flower extract had significantly higher number of normal seedlings compared to untreated seeds (Table 2). The 1% and 2% flower extract treatments were not significantly different from the 2% essential oil treatment in terms of FC. Meanwhile the 2% essential oil treatment was not significantly different from the control. The 1% essential oil treatment had significantly lower FC than the control while 3% essential oil treatment had the lowest FC among all treatments.

Table 2. First count of sweet corn seeds presoaked in different presoaking treatments

Presoaking Treatment	Concentration	First Count[1]
Control		41c
Flower Extract	1%	43ab
	2%	44ab
	3%	45a
Essential Oil	1%	37d
	2%	42bc
	3%	15e

[1] Means with the same letter are not significantly different. CV (%) = 7.15

In terms of the vigor index (VI), presoaking treatments using flower extracts regardless of concentration were not significantly different from each other but were higher than the control and the essential oil treatments (Table 3). This means that flower extract treatments were effective in increasing the VI of sweet corn seeds. Vigor index of seeds presoaked in essential oils except for the 2% concentration were even lower than the control which indicates that essential oil treatments are not at all effective in increasing VI.

Table 3. Vigor index of sweet corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Vigor Index[1]
Control		57.65b
Flower Extract	1%	66.43a
	2%	65.72a
	3%	68.03a
Essential Oil	1%	48.89c
	2%	57.80b
	3%	13.69d

[1] Means with the same letter are not significantly different. CV (%) = 8.05

The 2% and 3% flower extract treatments yielded the highest root and shoot length (Table 4 and 5). Both treatments produced longer roots and shoots compared to the other treatments including the control. The root length of seedlings from the 1% flower extract treatment was not significantly different from the 2% essential oil treatment and the control. In terms of shoot length, 1% flower extract treatment and the 2% essential oil treatment were not significantly different from each other but were significantly higher than the control., The 1% essential oil treatment produced shorter roots than the control. Meanwhile, the 3% essential oil treatment had the shortest roots and shoots, indicating that there was root and shoot growth inhibition.

Table 4. Root length of sweet corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Root Length (cm)[1]
Control		13.95b
Flower Extract	1%	14.55b
	2%	15.69a
	3%	16.13a
Essential Oil	1%	10.18c
	2%	12.29b
	3%	1.38d

[1] Means with the same letter are not significantly different. CV (%) = 10.57

Table 5. Shoot length of sweet corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Shoot Length (cm)[1]
Control		9.41c
Flower Extract	1%	11.23ab
	2%	12.01a
	3%	12.03a
Essential Oil	1%	8.70c
	2%	10.11b
	3%	2.11d

[1] Means with the same letter are not significantly different. CV (%) = 11.97

The presoaked sweet corn seeds in 3% flower extract had the highest seedling biomass or dry weight (Table 6) and was significantly different from the other treatments. The 1% flower extract treatment significantly increased seedling biomass compared to control, 2% flower extract and 2% essential oil treatments. The 1% essential oil concentration was not significantly different from the control. Meanwhile, the 3% essential oil concentration had

significantly reduced the seedling biomass as it is much lower compared to the control.

Table 6. Seedling biomass of sweet corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Seedling Biomass (g)[1]
Control		0.40cd
Flower Extract	1%	0.47b
	2%	0.45bc
	3%	0.52a
Essential Oil	1%	0.38d
	2%	0.44bc
	3%	0.11e

[1] Means with the same letter are not significantly different. CV (%) = 11.15

The presoaked sweet corn seeds to 3% flower extract was significantly different with the control. However, it was not significantly different with the other flower extract treatments and the 2% essential oil treatment (Table 7). Moreover, presoaking the sweet corn seeds in flower extract treatments and 2% essential oil treatment improved the emergence of seedlings in an unsterilized soil or in the field. The 1% essential oil treatment was not significantly different with the control, thus have the same potential with the control. Meanwhile, the 3% essential oil concentration had significantly lessened the number of emerged seedlings in the unsterilized soil compared to control.

Table 7. Seedling emergence of sweet corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Seedling Emergence (%)[1]
Control		79b
Flower Extract	1%	83.5ab
	2%	81.5ab
	3%	89.5a
Essential Oil	1%	78b
	2%	84ab
	3%	65c

[1] Means with the same letter are not significantly different. CV (%) = 8.52

Presoaking of Lagkitan seeds in Sampaguita flower extract and essential oil

The presoaked partially-aged Lagkitan seeds in the flower extract treatments had significantly higher %G than the control, but were not significantly different with the essential oil treatments. Thus, the flower extract

treatments were effective in increasing %G of Lagkitan corn seeds. The essential oil treatments were not significantly different with the control. It indicates that the presoaked Lagkitan corn seeds in essential oil treatments had the same germination potential with the untreated seeds. Thus, sampaguita essential oil was not effective in increasing the %G of Lagkitan seeds.

Table 8. Percent germination of Lagkitan corn seeds subjected to different presoaking treatments

Presoaking Treatment	Percent Germination[1]
Control	80.5b
Flower Extract	88a
Essential Oil	83.25ab

[1] Means with the same letter are not significantly different. CV (%) = 8.15

The first count of seeds soaked in 3% flower extract and 1% essential oil were not significantly different from each other but were significantly different from the control, thus these treatments produced more vigorous seedlings than the control and other treatments (Table 9). Meanwhile, the control was not significantly different from the 2% and 3% essential oil concentration in terms of the number of normal seedlings that germinated on the fourth day after sowing.

Table 9. First count of Lagkitan corn seeds presoaked in different presoaking treatments

Presoaking Treatment	Concentration	First Count[1]
Control		35d
Flower Extract	1%	44ab
	2%	44ab
	3%	46a
Essential Oil	1%	45a
	2%	42bc
	3%	40c

[1] Means with the same letter are not significantly different. CV (%) = 8.03

The presoaked Lagkitan seeds in 3% flower extract had the highest VI and were significantly different from the control and other treatments (Table 10). However, this treatment was not significantly different with the 2% flower extract and 1% essential oil treatments. All the presoaking treatments have yielded significantly higher VI than the untreated seeds. Thus, artificially aged Lagkitan corn seeds were more vigorous when presoaking treatments were done, especially in 3% flower extract treatment.

Table 10. Vigor index of Lagkitan corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Vigor Index[1]
Control		60.76e
Flower Extract	1%	84.97b
	2%	87.59ab
	3%	91.78a
Essential Oil	1%	87.89ab
	2%	75.70c
	3%	68.94d

[1] Means with the same letter are not significantly different. CV (%) = 8.31

The presoaked Lagkitan seeds to 3% flower extract and 1% essential oil treatment yielded the longest root length among all the treatments (Table 11). However, these treatments were not significantly different with the control, thus these treatments had the same root growth potential as the control. The 1% flower extract treatment had also the same potential as the untreated seeds. The control had significantly higher mean root length compared to 2% flower extract, 2% and 3% essential oil treatments, thus it was able to grow longer roots than the said presoaking treatments. The 3% essential oil treatment had inhibited root growth because it gave the lowest root length among all the treatments.

Table 11. Root length of Lagkitan corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Root Length (cm)[1]
Control		16ab
Flower Extract	1%	14.8bc
	2%	14.4c
	3%	16.8a
Essential Oil	1%	16.2a
	2%	14.5c
	3%	7.9d

[1] Means with the same letter are not significantly different. CV (%) = 9.34

The presoaked Lagkitan corn seeds to 3% flower extract have significantly higher shoot length compared to other treatments and were significantly different with the other treatments including the control (Table 12). Thus, this treatment was effective in increasing the shoot length or promoting longer shoot growth. All the remaining treatments except the 3% essential oil treatment were not significantly different from each other. Thus,

these presoaking treatments have the same shoot growth potential as the control and have not significantly increased the shoot length. Meanwhile, the 3% essential oil treatment had somehow inhibited shoot growth because it had the lowest shoot length among the treatments.

Table 12. Shoot length of Lagkitan corn subjected to different presoaking treatments

Presoaking Treatment	Concentration	Shoot Length (cm)[1]
Control		10.8b
Flower Extract	1%	11.5b
	2%	11.3b
	3%	13.4a
Essential Oil	1%	11.1b
	2%	10.8b
	3%	6.8c

[1] Means with the same letter are not significantly different. CV (%) = 8.01

The presoaked artificially aged Lagkitan corn seeds to 3% flower extract yielded the highest seedling biomass or dry weight (Table 13). However, it was not significantly different from the control, 1% and 2% flower extract treatments. Thus, the flower extract treatments had not significantly increased the biomass and have the same potential with the untreated seeds. The 1% and 2% flower extract treatments were also not significantly different with the 1% essential oil treatments. Meanwhile, the 2% and 3% essential oil treatments have significantly lower biomass compared to control, since both have lower shoot length than control.

Table 13. Seedling biomass of Lagkitan corn seeds subjected to different presoaking treatments

Presoaking Treatment	Concentration	Seedling Biomass (g)[1]
Control		0.78ab
Flower Extract	1%	0.78ab
	2%	0.78ab
	3%	0.83a
Essential Oil	1%	0.74b
	2%	0.66c
	3%	0.37d

[1] Means with the same letter are not significantly different. CV (%) = 9.34

The partially-aged Lagkitan corn seeds presoaked in flower extract regardless of concentration had a higher percent seedling emergence than the

essential oil treatments. However, both the flower extract and essential oil treatments were not significantly different from the control.

Table 14. Seedling emergence of Lagkitan corn seeds subjected to different presoaking treatments

Presoaking Treatment	Seedling Emergence (%)[1]
Control	81ab
Flower Extract	85a
Essential Oil	78b

[1] Means with the same letter are not significantly different. CV (%) = 7.93

Discussion

The 3% flower extract treatment was most effective in increasing the germination and vigor of partially-aged Sweet Corn and Lagkitan seeds. Compared to other treatments, 3% flower extract yielded the highest values in all parameters tested. Generally, the flower extract treatments increased the germinability and vigor of the partially-aged Sweet Corn and Lagkitan seeds. Moreover, it was observed that higher concentrations of flower extract resulted in higher seed germination and vigor.

Sampaguita flower extract contains phytoconstituents such as glycosides, saponins, flavonoids, terpenoids alkaloids, phenols, tannins, saponins, coumarins, anthocyanins, quinones, betacyanins, phytosterols, carbohydrates and proteins (Suaputra *et al.*, 2021). The improvement in seed quality of presoaked partially-aged seeds in Sampaguita flower extract may be due to the presence of antioxidants (Kunhachan *et al.*, 2012; Wu *et al.*, 2021; Wei *et al.*, 2007). Antioxidants are molecules that can neutralize reactive radicals that become less active by accepting or donating electrons to prevent oxidative stress (Asaduzzaman and Asao, 2018).

In partially-aged seeds, presoaking in Sampaguita flower extract may have enhanced the seed's defense mechanism in protecting the cell membrane from damage brought about by reactive oxygen species. The enhanced seed germination possibly was achieved due to accumulation of antioxidants and the improvement of cell membrane integrity (Siri *et al.*, 2013). In addition, the substances present in the flower extract were able to somehow lessen, if not eliminate mold growth on corn seeds based in visual examination. The higher the concentration of the extract, the lesser the appearance of mold on seeds.

In the essential oil treatments, the 2% concentration managed to increase the germinability of partially-aged Sweet Corn seeds but not the vigor. The 1%

and 3% essential oil concentrations, however reduced the germination and vigor of Sweet Corn seeds. For partially-aged Lagkitan seeds, 1% essential oil concentration was more favorable in increasing germination and vigor than the higher concentrations. This was because germination tend to increase in an optimal essential oil concentration, which was 2% in the case Sweet Corn and 1% in the case of Lagkitan seeds, and reduced or inhibited at lower and higher concentrations (Barkai-Golan, 2001). The 3% essential oil treatment greatly reduced germination of Sweet Corn and Lagkitan seeds. It is because it contains phenol, an active component of essential oil, which at higher concentration affects the cell membrane, thus, altering permeability of the membranes, transport systems, electron transport, and energy production (Ismaiel and Pierson, 2000). Phenolic compounds were known to interfere with the production of the membrane potential, which is important for the membrane-bound ATPase to generate ATP from ADP, which is important in the vegetative growth of plants (Rico-Munoz, 2007). All essential oil treatments had not incurred mold growth on seeds, though some seeds were decayed. This was due to sampaguita essential oil has main constituents of linalool, benzyl acetate, and benzyl benzoate which expressed antibacterial and antifungal properties (Ahmed *et al.,* 2016).

Based on the results of this study, it is recommended to use Sampaguita flower extract as presoaking treatment since it generally improved the germination and vigor of Lagkitan and Sweet Corn seeds. It is also recommended to use the ideal essential oil concentration as seed presoaking treatment, if it is to minimize or eliminate fungal growth on Lagkitan and Sweet Corn seeds. The utilization of flowers extracts or essential oils as presoaking treatment may serve as an alternative to chemical treatments in support of organic seed production.

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