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A Comparative Study on the Effects of Saline Water, Vinegar, and Milk on the Germination of Coriandrum sativum, Anethum graveolens, and Spinacia oleracea Seeds

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Abstract

Germination is considered to be an important stage in plants' development and is also extremely sensitive to various factors such as temperature, soil composition, oxygen levels and the fluid used to water. This study is focused on investigating the different effects of three different liquids used—saline water, vinegar, and milk—on the germination rates of *Coriandrum sativum*, *Anethum graveolens*, and *Spinacia oleracea* seeds. Seeds of each species were divided into four groups and watered on a daily basis with either distilled water (control), a saline solution, vinegar, or milk. Germination was then observed over a ten-day period, and the percentage of seeds successfully germinated in each group was carefully recorded.

The results showed that distilled water consistently produced the highest germination rates across all 3 species, with Spinacia oleracea displaying the fastest and most consistent emergence. Anethum graveolens also showed good performance in the control, while Coriandrum sativum demonstrated a slow yet steady germination pattern. In all treatments, vinegar drastically halted any germination with all seeds producing no sprouts during the observations, while saline water resulted in moderate delays and reduction in germination with spinach and dill being substantially affected. Likely osmotic stress contributed to this reduced germination. Milk appeared to disrupt germination due to high variability and slow germination rates. The high rates may be due to microbial activity and depletion of oxygen as the seed coats absorb moisture and respire. This study shows that water quality and chemical composition of the media used has a significant impact on seed germination and distilled water yielded the best results overall for germination while vinegar produced the most crippling effects for germination.

Keywords: Seed Germination; Abiotic Stress; Plant Physiology; Coriandrum sativum; Anethum graveolens; Spinacia oleracea

1. Introduction

Germination is a pivotal preparatory stage for the plant life cycle, where a seed moves from dormancy to active growth, and will determine whether a seedling will be successfully established and regulate its future health and agricultural yield (Bewley, 1997; Nonogaki, Bassel, & Bewley, 2010). The process of germination is regulated by both internal and external factors such as the seed's genetics, temperature, oxygen, soil quality, and the chemical nature of the liquid used in irrigation (Finch-Savage & Leubner-Metzger, 2006).

While most available literature on seed germination focuses on water, in natural environments plants are often exposed to other solutions. Of those solutions, saline water is arguably the most important to consider in areas affected by soil salinity, or when irrigation practices use poor-quality water. Saline water will limit osmotic potential, making it harder

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for seeds to absorb water, and the presence of high concentrations of ions like Na^+ or Cl^- can limit functionality of key enzymes and inhibit the required enzymatic function to accommodate germination (Al-Karaki, 2001; Munns & Tester, 2008). In fact, there is a strong evidence in support of salt stress leading to reduced germination rates, and reduced rate of seedling emergence for seed germination in a variety of species (Munns & Tester, 2008; Parihar, Singh, Singh, Singh, & Prasad, 2015).

Although vinegar is made up of mostly acetic acid, it is a known antimicrobial agent and food preservative; being near the low end of the pH scale can inhibit germination by damaging the cellular membranes and disrupting enzyme activity (Ferguson et al., 2003; MacDonald, 2004). Though vinegar may be used in experimental herbicide applications, fewer studies comparing its effects on edible plant seeds exist. While milk is a common ingredient in home gardening and traditional agriculture - due to its water, lipid, protein, and sugar content – it is also considered as an element that affects microbial populations in soil (Rao et al., 2007; Sharma & Banik, 2015). Some studies indicate that an amendment of organic material (milk, compost, etc.) adds microbial activity and soil fertility (Sharma & Banik, 2015), and other studies propose that too much organic matter can limit oxygen diffusion or support undesirable microbial populations, limiting germination ability (Rao et al., 2007).

Based on this information, there have been few experimental studies directly comparing the effects of three liquids - saline water, vinegar, and milk - on seed germination across multiple species under controlled conditions. Further, few studies have attempted such comparisons using accessible, real-world materials that could be implemented into education opportunities. However, the opportunity to conduct such experiments offer great hands-on science opportunities and inquiry-based learning experiences. Students can observe and interpret possible biological changes from environmental variables or impacts of seed germination (Hofstein & Lunetta, 2004; Nonogaki et al., 2010).

The main goal of the project is to test the effects of saline water, vinegar, and milk on the germination of three of the most commonly consumed edible plants: Coriandrum sativum (coriander), Anethum graveolens (dill), and Spinacia oleracea (spinach). In addition to measuring the effects of different treatments on germination rates, this study will add to our knowledge of plant responses to environmental stressors, while also supporting scientific thinking in classroom and informal learning environments (Hofstein & Lunetta, 2004).

2. Materials and Methods

2.1. Seed Selection and Botanic Description

The experiment used three commonly cultivated plant species, selected based on differences in seed size, germination behavior, and economic value. The species were:

- Spinacia oleracea (Spinach): Spinach germinates relatively quickly if properly managed, but it is sensitive to erratic temperatures and moisture. Spinach seeds are medium in size (about 4–5 mm), round to triangular, and are normally dark brown in color. Germination of seeds requires constant moisture and moderate temperatures with germination normally occurring within 5–10 days if grown in the right conditions.
- Coriandrum sativum (Coriander): Annual species that grows quickly; seeds are relatively large compared to celery seed (approximately 3 mm), seeds are spherical, brown or light brown. Germinates quickly when conditions are favorable.
- Anethum graveolens (Dill): Annual aromatic herb; seed size is medium (approximately 3 mm), seeds are flat and pale brown, and light weight. Dill has moderate-seed germination velocity; not tolerant to excess moisture.

All seeds used were sourced from certified horticultural suppliers to provide genetic purity, ability to germinate, and uniformity in the seed characteristics. All seeds were stored in the recommended cool, dry area prior to the experiments, to maintain dormancy and prevent germination until the germination trials were conducted.

(Source: Original)

Figure 1 Seeds prepared for germination

2.2. Experimental Design and Conditions

The experimental design was a 3 × 4 factorial design with three plant species and four different types of liquid treatments for a total of twelve different experimental conditions. The research was conducted indoors to completely eliminate external variation through wind, insects, rain, or temperature.

Each unit of the experiment consisted of:

- One transparent plastic container (all were the same volume: 150 mL)
- A sterile cotton pad as a non-nutritive germination substrate
- Five seeds of the same species on one cotton medium

In total we had 12 containers:

3 plant species × 4 liquid types=12 individual labelled containers

All containers were placed in a straight line on a flat, sunlit window sill under indirect natural daylight for approximately 10 hours a day. This was done to allow for similar distribution of light across all treatments and to eliminate fluctuations by using one natural light source.

The ambient indoor temperature was between 22°C and 26°C, using a digital thermometer which was placed near the containers to monitor temperature. Relative humidity was a factor controlled by using transparent plastic wrap with small perforations that encapsulated the containers. This limited evaporation while allowing air exchange.

2.3. Water Treatment Types and Preparation

Four different liquids were chosen to reproduce average life conditions and test the effects of liquid on seed germination. All liquids were prepared fresh, on-site each day, and applied at room temperature and through a sterile plastic syringe.

Distilled Water (Control): Ordinary potable water drawn from your household faucet. This water was used as a control group in order to evaluate the action of the treatment groups against.

Saline Water: A salt solution was created by dissolving 1 teaspoon of table salt (NaCl, approximately 5 grams) into 250 mL of distilled water. The solution was stirred until dissolved. This concentration was created to replicate mildly saline soil or irrigation water.

Vinegar: Commercially available vinegar (white vinegar, 5% acetic acid) was used without any dilution. Vinegar will create an acidic environment and was used to assess the effects of low pH on seed germination.

Milk: Full-fat cow's milk (3.2% fat) was used as a treatment. Milk was not boiled or diluted, and was applied as is. Milk contributes organic material, proteins, and natural sugars to the medium, and was included to accept for any bio stimulant or microbial response.

Each container was given precisely 5 mL of the assigned liquid treatment daily with a sterile 10 mL syringe. The volume was chosen to achieve consistent hydration of the cotton medium without oversaturation or waterlogging.

(Source: Original)

Figure 2 Distilled Water

(Source: Original)

Figure 3 Saline Water

(Source: Original)

Figure 4 Vinegar

(Source: Original)

Figure 5 Milk

Sterile syringes were used for each type of liquid to avoid cross-contamination. Used syringes were rinsed with distilled water and stored clean and dry.

2.4. Substrate and Germination Medium

A soilless approach to germination was used in this study to eliminate variability as a result of soil composition, nutrient availability in soil, moisture and soil texture, and soil microbiome. Rather than soil, each container component was lined with one layer sterile medical-grade cotton pad that was moistened with the treatment being employed.

The cotton pads acted as:

- A moisture-retentive base for germination
- A visible surface for daily observation
- A neutral medium without nutrients, therefore the only variable affecting seed germination used was the type of liquid being used for treatment Each pad was monitored each day for the presence of mold or discoloration and any semblance of microbial growth especially in containers treated with either milk or vinegar.

2.5. Seed Planting Procedure

Seeds were handled using sanitized tweezers to avoid contamination. Each container received five seeds from the assigned plant species which were evenly distributed on the cotton surface. The seeds were pressed gently into the cotton to promote contact without embedding them.

No seed pre-treatment (soaking or scarification) occurred to preserve the natural germination behavior of seeds and maintain consistent starting conditions across treatments.

The containers were labeled with:

- Species name (abbreviated)
- Treatment label (e.g., W for water, S for saline, V for vinegar, M for milk)
- Date when experiment began

2.6. Environmental Measures and Standardization

All containers were placed equidistantly in a row on a windowsill to limit variance in light exposure, and to further reduce potential micro-environmental variability, on subsequent days we rotated every container to a new location. For example, the container on the far-left side would be moved to the far-right side and so on.

A digital thermometer and humidity meters were placed near the experimental containers and the room temperature and humidity were recorded at regular intervals.

Plastic lids or food-grade plastic wrap was placed on each container, loosely, to maintain a humid microclimate and provide air exchange, which is critical for aerobic respiration during germination.

2.7. Observation Schedule and Visual Monitoring

The experiment was run for 7 consecutive days. The observations were made daily, each morning between 09:00 AM and 10:00 AM.

The observations followed these protocols:

- The container lid or wrap was removed carefully, so as not to disturb the seeds.
- Visible root (radicle) or shoot (plumule) emergence was then checked on the cotton pad.
- Mold, fungal growth, or any other signs of seed rot were recorded.
- The number of seeds showing visible germination was recorded.

Cotton moisture level was evaluated and, only if the pads appeared dry, an additional 1–2 mL of liquid was added.

All results were obtained without the use of external nutrients, growth hormones, or mechanical stimuli; the only varying variable was the liquid used per container.

3. Results and discussion

3.1. Results regarding the influence of different types of water on the germination of spinach seeds.

In table 1, data are centralized regarding the average number of germinated spinach seeds for the experimental model water types.

Table 1 The average number of germinated spinach seeds for the experimental model water types over the 7-day period.

Spinach - average total number of germinated seeds							
Type of Water/ Days	1	2	3	4	5	6	7
Distilled Water	0	2	3	2	3	5	7
Vinegar	0	0	0	0	0	1	1
Saline Water	0	0	0	1	1	2	3
Milk	0	0	0	0	1	1	2

From the centralized data in Trip 1 of Table 1 on the germination of spinach (Spinacia oleracea) seeds with different liquids, it was observed that of the 10 seeds planted in each container, those watered with distilled water had the most regular and earlier germination activity than the other treatments. Distilled water led to at least 3 seeds germinating by Day 3 and 7 seeds by Day 7, indicating good conditions provided by distilled water and fully warranting its quality as a control treatment.

All milk-inoculated replicates exhibited a delayed and less-sequential germination process, likely a result of the increased viscosity and decreased gaseous exchange of the substrate as a result of feeding substrates that contain milk. All milk inoculated seeds exhibited no germination until Day 5 with only 2 seeds germinating by Day 7. The delayed germination rate and reduced frequency of germination coupled with evidence of microbial growth may infer inhibitory effects or a reduced pH due to the potential for lactic acid.

Vinegar-treated containers had the least amount of total germination due to the acidity, (approximately pH of 2-3) likely stopping seed metabolism and the radicle flush. In all three replicates, only one germination occurred for the entire 7 days of observation, clearly showing the negative effects vinegar had on spinach seed viability and enzymatic activation.

For saline water treatment (NaCl, ~2% w/v; 5g per 250 ml of distilled water) data averaged in between W1 and W2. Spinach germination took longer than the distilled water group, on average showing its first signs on day 4. On average 3 seeds germinated per container by day 7. Overall, some germination appeared but slower than the control group, occurring less uniformly. Salinity increased osmotic stress therefore decreasing water uptake by the seed which delayed metabolically activation.

Furthermore, any excess ionic toxicity from sodium ions may have affected cell functions which limited germination even more.

Considering all these trends, it appears distilled water provided the best germination for spinach seeds while milk provided some moderate limitations predominantly by limiting oxygen and inducing microbial growth. The vinegar had the greatest suppressive effect because of the strong acidity, which paused germination during the initial development of germination. Saline water also slowed the germination, but the greatest impact is that the germination rate and the reduced uniformity was due to osmotic stress and salt accumulation. All results correspond with established findings that extreme pH and salinity perform adversely on seed germination and early seedling vigor.

3.2. Results regarding the influence of different types of water on the germination of dill seeds.

In table 2, data are centralized regarding the average number of germinated dill seeds for the experimental model water types.

Table 2 The average number of germinated dill seeds for the experimental model water types over the 7-day period.

Dill - average total number of germinated seeds							
Type of Water/ Days	1	2	3	4	5	6	7
Distilled Water	0	2	3	3	4	6	8
Vinegar	0	0	0	0	0	0	0
Saline Water	0	0	0	1	1	3	4
Milk	0	0	0	0	1	1	2

Based on the summarized data presented in Table 2, dill seeds that were watered using distilled water demonstrated the most active rate of germination relative to the other treatments tested. Out of ten seeds per container, there were two germinated seeds by Day 2, which increased to eight germinated seeds by Day 7, indicating appropriate balance of water and adequate oxygenation. The appearance of the radicle extending beyond the seed coat and the evenness of the seedlings indicated that acid washed and distilled water created the appropriate physiological environment for germination.

In contrast, the saline water set exhibited stunted, delayed, and limited germination, consistent with the osmotic stress imparted upon the seeds by the 2% NaCl solution. Germination was not noted until Day 4, and few seeds across the replicates had germinated (not exceeding 4 seeds) by the seventh day. The seedlings noted to emerge also displayed stunted elongation and uneven cotyledon growth. These patterns support literature reporting salt-influenced inhibition of water uptake and metabolism early in seed development.

Milk-treated dill seeds had negligible germination. Only 2 seeds germinated on day 7, and the germination process started later than all other treatments; typically, first emergence occurred on Day 5. Contributing to the slower germination process may be the decreased aeration and the fermentation of bacteria to produce lactic acid in the medium which can inhibit enzyme activation and hydration of tissues, thus creating a hostile environment for germination.

The most inhibited group was that treated with vinegar (5% acetic acid). Germination did not occur in any of the replicates or for all 7 days of observations. The high acidity likely disrupted cellular homeostasis, compromised seed coats, and inhibited metabolic processes required for radicle emergence, indicating complete inhibition which is evidence of phytotoxicity in early stages of seed use.

3.3. Results regarding the influence of different types of water on the germination of coriander seeds.

In table 3, data are centralized regarding the average number of germinated coriander seeds for the experimental model water types.

Table 3 The average number of germinated coriander seeds for the experimental model water types over the 7-day period.

Coriander - average total number of germinated seeds							
Type of Water/ Days	1	2	3	4	5	6	7
Distilled Water	0	3	3	4	5	6	7
Vinegar	0	0	0	0	0	0	1
Saline Water	0	0	0	1	2	3	3
Milk	0	0	0	0	1	2	2

Based on the summary data in Table 3, the coriander seeds watered with distilled water and saw the most germination in a comparative context, or control, group overall. Germination was present beginning as early as day 2 and at an average of 3 seeds germinated. Combining this data, by Day 7, there was 7 seeds that germinated. This indicated that distilled water provided adequate hydration, consistent pH, as well as effective allowances in terms of oxygen levels (aw). Seedlings were uniform and healthy with well-developed radicles and cotyledons. These observations demonstrated the application of neutral pH water was sufficient in supporting early physiological processes.

Saline water (NaCl 2%) provided inhibitory effects of a moderate nature. Gauging when germination began, seedlings were defined at Day 4, and finished as 3 out of 10 germinated officially at Day 7. Seedlings as a whole were neither suppressed nor reduced. Seedling growth was nevertheless stunted, as evidenced by shorter radicles and pale, fragile hypocotyls combinations. The symptoms would be consistent with osmotic stress and ionic toxicity limiting nutrient loads and cell expansion.

Milk-treated coriander seeds showed delayed and inhibited germination of the seeds. In typical germination, germination was only observable, usually by day 5; however, by day 7, only 2 seeds per container had germinated. The slow-rate and limited number of germinated seeds are likely attributed to low aeration, pore clogging of the substrate, and likely caused for bacterial (lactic acid) fermentation, all of which impact the enzymatic reactions and water uptake needed for germination.

The group with their seeds treated with vinegar (around 5% acetic acid) experienced total inhibition of germination. In fact, in total replication, one seeds germination was noted with generalized day (day 7). The acidic environment likely led to the denaturation of proteins, damage to seed membranes, and cessation of metabolism. Therefore, vinegar is very phytotoxic to coriander seed germination.

4. Conclusion

Distilled water produced the earliest and most consistent germination among the three seeds (spinach, dill, and coriander). When using distilled water, the oxygen content ensured optimal moisture, pH levels were neutral and adequate for healthy radicle growth (and even fungal inhibition) from the seed with regards to moisture (H2O), oxygen gas (O2), and radicle extension of the seed and seedlings development.

The vinegar treatment appeared to be strongly inhibitory. No seeds of any species germinated in this condition, likely from the high acidity of vinegar (pH ~2-3) damaging the cell membranes and suppressing enzyme activity.

Salty water (2% NaCl) negatively impacted germination across all three species, delaying germination initiation as well as the total number of seeds that germinated, likely because these conditions of osmotic imbalance and lashed Na+ and Cl- accumulated in tissue inhibited the uptake of water and inhibited metabolic function in seedling.

The type of milk treatment also inhibited germination of seed, although spinach and dill were primarily inhibited. The viscosity of Milk as well as microbial growth of yeast could have affected available oxygen and disrupted germination conditions.

Though, the different species responded differently. Among the three species, dill was the most resistant to salt stress and spinach did the best in distilled water. Coriander was more salt stress-sensitive overall, but could not repeat the results of dill. However, it performed better than spinach in milk, despite demonstrating poorer results comparatively to dill in milk.

The study confirms that not every plant genotype reacts identically to chemical or environmental stress, and each has a specific germination response pattern. Distilled water remains the most favorable for seed germination in all cases studied.

Based on the results obtained, distilled water is recommended as the optimal irrigation liquid to support healthy seed germination and early seedling development.

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