

Evaluation of pregermination treatments as potential promoters of germination and development in tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Objective: To evaluate diverse pregermination treatments aimed at accelerating, synchronizing, and enhancing seed germination, as well as promoting the development of vigorous tomato seedlings, by assessing the efficacy of hydropriming methods, chemical compounds, plant extracts, and bioprimering strategies.

Design/Methodology/Approach: Germination rate, velocity, and temporal dynamics were determined using variables such as total germination, maximum daily germination, germination interval, and time to 50 % germination (T_{50}). Seedling vigor was assessed through measurements of stem and root length, in addition to dry matter accumulation. An analysis of variance (ANOVA) was conducted under a completely randomized design for each evaluated variable.

Results: Several treatments significantly enhanced the germination potential of tomato seeds and effectively improved seedling morphological attributes. Nevertheless, none of the treatments succeeded in modifying the temporal dynamics of the germination process.

Study Limitations/Implications: Future research should focus on evaluating the most relevant findings of this study under productive conditions and in relation to resistance against environmental stressors.

Findings/Conclusions: Hydropriming and bioprimering with understudied *Bacillus* and *Pseudomonas* species exerted positive effects on germination and seedling vigor indicators, respectively. Moreover, garlic, onion, and chili extracts demonstrated favorable effects on both germination and seedling development parameters.

Keywords: priming, bioprimering, plant extracts, germination, development.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important horticultural crop worldwide. It is cultivated on nearly five million hectares and exceeds 190 million tons in annual global production (FAOSTAT, 2023), with approximately 3.5 million tons produced in Mexico (SIAP, 2023). Tomato is also among the most widely used ingredients in gastronomy, as it constitutes a rich source of lycopenes, vitamin C, and phenolic compounds with notable antioxidant properties that are relevant to human health (Rodríguez *et al.*, 2024).

Seed germination efficiency and, consequently, production levels may be constrained by factors such as seed-coat hardness, natural aging, endogenous inhibitors, and unfavorable environmental conditions (Lamichhane *et al.*, 2018). Within this framework, the production of vigorous and uniform seedlings is a decisive determinant of productive success, as it directly influences survival, vegetative growth, and final yield. This scenario has fostered the development of pregermination treatments based on the controlled manipulation of physical, chemical, and biological factors to optimize germination and enhance seedling vigor. Among the most widely implemented strategies are priming treatments, including distinct variants such as hydropriming, chemical priming with solutes, and biopriming, all of which can improve germination performance and subsequent development (Dhal *et al.*, 2022). More recently, plant extracts rich in flavonoids, organosulfur compounds, and carotenoids have also been explored; however, substantial gaps remain regarding their allelopathic properties and underlying modes of action (Da Silva Carvalho *et al.*, 2021).

Systematic research on pregermination treatments is essential for developing technologies that optimize tomato seedling production by improving germination, vigor, disease resistance, and crop establishment. Elucidating the mechanisms associated with each treatment enables the selection of the most effective strategies under diverse production conditions. Accordingly, this study aimed to evaluate different pregermination treatments on tomato germination and the development of vigorous seedlings using priming techniques and assays with plant extracts, with the purpose of identifying the most effective strategies.

MATERIALS AND METHODS

The experiments were conducted between January 2024 and June 2025 at the facilities of Colegio de Postgraduados, Montecillo Campus, Texcoco, State of Mexico. Saladette tomato seeds, cultivar Río Grande (2020 lot), marketed by “<http://www.lasemilleria.com>”, were used.

Pregermination Treatments

Hydropriming

Hydropriming was evaluated for its effects on germination. This treatment involves seed hydration for a defined period without allowing radicle protrusion, followed by drying until the initial dry weight is reattained (Pandey *et al.*, 2022). In the present study, 100 seeds (0.22 g) were hydrated for 24 h, dried for 1 h using a fan, and then stored. Seed weight was monitored periodically until it matched the initial dry weight. The duration of this treatment and that used in the chemical-compound experiment was established based on the water-uptake curve, which indicated that seeds required 27 h to germinate.

Plant extracts

Germination

Aqueous extracts were prepared from fresh samples of five plant species: garlic (*Allium sativum* L.), onion (*Allium cepa* L.), habanero chili (*Capsicum chinense* Jacq.), ginger (*Zingiber officinale* Roscoe), and aloe vera (*Aloe vera* L.), at concentrations of 15, 25, 50, and 75 % in

16 mL volumes. Water and mortars were pre-cooled to 16 °C and -18 °C, respectively. For each plant material, 0.21 g was weighed and macerated for 3-4 min (garlic, chili, onion) or 5-6 min (ginger, aloe vera), gradually adding water. Each extract was adjusted to the required concentration according to Table 1.

Development

Based on germination tests, two of the most promising extracts were selected: 25% chili pepper extract (CH25) and 50% onion extract (O50). Three treatments were established: seeds soaked in CH25, seeds soaked in O50, and a control soaked in distilled water (I). An additional untreated control (T) was also included. For each treatment, 20 seeds were soaked for 24 h in 10 mL of the corresponding extract, and the control was soaked in distilled water (I).

Chemical compounds

Five chemical compounds were selected based on their prevalence in the seed conditioning literature to evaluate their effects on germination: acetylsalicylic acid ($C_9H_8O_4$ or ASA) at concentrations of 0.06 and 0.12%, potassium nitrate (KNO_3) at 1 and 3%, copper sulfate ($CuSO_4$) at 1 and 2%, calcium chloride ($CaCl_2$) at 2 and 3%, and sodium chloride ($NaCl$) at 0.5 and 1%. Initially, the concentrations matched those reported in the literature, and based on the results, the concentration was adjusted for the second

Table 1. Volumes of plant extracts and water needed to obtain the concentrations of the experiment based on a total volume of 16 mL.

Required Concentration (%)	Extract Volume (mL)	Water Volume (mL)
15	2.4	13.6
25	4	12
50	8	8
75	12	4

Table 2. Amounts of substance needed to prepare the solutions for the experiment based on a total volume of 16 mL.

Chemical substance	Solution concentration (%)	Amount of substance (g)
ASA	0.06	0.01
	0.12	0.02
KNO_3	1	0.16
	3	0.48
$CuSO_4$	1	0.16
	2	0.32
$CaCl_2$	2	0.32
	3	0.48
NaCl	0.5	0.08
	1	0.16

stage of the experiment. The treatment consisted of soaking the seeds in the chemical compounds in solution for 24 h prior to germination; 16 mL of solution were prepared for each substance. To prepare the solutions, the quantities of each substance described in Table 2 were weighed, water was added, and the mixture was stirred until completely dissolved. For each concentration, the seeds were placed in Petri dishes, the solutions were added, and the seeds were incubated for 24 h. Finally, they were thoroughly washed with distilled water and placed in the germination chamber.

Biopriming

Biopriming was selected to evaluate seedling development responses. This technique is based on exposing seeds to microorganisms with beneficial properties (Rendón and Gómez, 2018). In this experiment, strains from the collection of the Cellular Biology Laboratory at Colegio de Postgraduados, Montecillo Campus, were used. *Pseudomonas* spp. and other species associated with tomato and maize roots were evaluated. Selection of *Pseudomonas* strains was based on their ability to grow and maintain fluorescence under increasing salinity in the culture medium. Strains from other species were selected solely according to salinity tolerance. Each strain preserved in test tubes was streaked using an inoculation loop onto Petri dishes containing King's B medium with salinity levels of 1, 2, and 3% (Table 3). Fluorescence was assessed using an ultraviolet (UV) lamp. The best-performing strains were then inoculated onto Petri dishes with King's B medium and incubated at 26-28 °C for 24 h. The following day, bacterial suspensions were prepared in test tubes containing sterile water. Each suspension was adjusted to a standardized turbidity of 0.90 at an optical density of 660 nm using a spectrophotometer. Twenty seeds per treatment and the control were placed in sterile Gerber jars. For seed coating, 10 mL of bacterial suspension was added to each jar and allowed to stand for 15 minutes. The seeds were then removed and sown immediately.

Germination assays

For each treatment and control, 100 seeds were used and distributed into four Petri dishes (25 seeds per dish). Then, 4 mL of water, plant extract, or solution depending on the treatment was added to each dish, always over three sheets of Sanitas paper. The dishes

Table 3. Bacterial strains evaluated in tomato development tests.

Species	Code	Origin	Type
Unidentified	RJ6	Tomato	Rhizospheric
<i>Bacillus albus</i>	RJ12	Tomato	Rhizospheric
<i>Bacillus paranthracis</i>	RJ13	Tomato	Endophytic
<i>Bacillus pacificus</i>	RJ16	Tomato	Endophytic
<i>Bacillus amyloliquefaciens</i>	RJ25	Tomato	Rhizospheric
<i>Pseudomonas hunanensis</i>	1M	Maize	Endophytic
<i>Pseudomonas fungipugnans</i>	3L	Maize	Rhizospheric
<i>Pseudomonas fungipugnans</i>	4L	Maize	Rhizospheric
<i>Enterobacter bugandensis</i>	5L	Maize	Rhizospheric

were incubated at 26-28 °C, and the number of germinated seeds was recorded daily. A seed was considered germinated when a visible radicle protrusion (≥ 2 mm) was observed outside the seed coat. The Petri dish was considered the experimental unit ($n=4$).

Germination data analysis

From the recorded data, the Cumulative Germination (CG) curve was plotted; expressed as the cumulative sum of the mean daily germination percentages of the four Petri dishes used in each treatment. For each treatment, the TG was calculated as the mean final germination percentage of the four Petri dishes. The TG results are presented with their respective standard deviations (SD, equation 1) and confidence intervals (CI, equation 2) for a 95% confidence level, calculated using the four replicate values.

$$SD = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N}} \quad (1)$$

$$CI = \bar{x} \pm z(\sigma / \sqrt{N}) \quad (2)$$

where: N denotes the sample size; x_i represents the individual observations within each treatment; \bar{x} is the sample mean; and z is the critical value associated with the selected confidence level.

In addition, T_{50} the time required for 50% of the viable seeds to germinate was estimated using the CG data and the slope of a linear function model, according to the following formulas:

$$m = \frac{y_2 - y_1}{x_2 - x_1}$$

$$y = mx + n$$

where: m is the slope of a line; x_n and y_n these are coordinates representing intercept points along a straight line (in this case, x corresponds to time and y represents the germination value at that time), and n denotes the y-intercept of the line.

The germination interval was also determined as the elapsed time between the onset and completion of germination (D_0 - D_f). Graph construction and data processing were performed using Microsoft Excel (Microsoft Office suite).

Development assays

The seeds were sown in sterile substrate in polystyrene germination trays, which remained in the greenhouse for 30 to 35 days; the approximate time before transplanting (Gómez-Salazar *et al.*, 2023). The sample size was 10 plants per treatment, where each plant represented an experimental unit ($n=10$). The measured variables were stem length, root length, and dry matter weight of the seedlings. To determine dry matter weight, the

seedlings were first placed in an oven for 24 h at 43 °C, and then their weight was checked every hour until it stabilized.

Statistical analyses

The treatments evaluated for developmental variables were distributed in a completely randomized design with 10 replicates per treatment. The data for each vigor variable were subjected to a one-way Analysis of Variance (ANOVA). When the ANOVA indicated significant differences ($p < 0.05$), the treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $p = 0.05$. Prior to the ANOVA, assumptions (Normality and Homogeneity of Variances) were verified. All analyses were performed using R version 4.4.3.

RESULTS AND DISCUSSION

Pregermination Treatments

Hydropriming

Seed germination benefited from this treatment, as it increased total germination (TG) to 90%, with improved standard deviations and confidence intervals. Synchrony and germination rate also improved, as evidenced by a high maximum daily germination (MDG; 64%) and a lower T_{50} (39.7 h), even though the overall germination period extended by one additional day under the treatment (Table 4). The germination process began and proceeded more actively following a typical temporal pattern, with no appreciable modifications, as reflected by the cumulative germination (CG) curve (Figure 1).

Table 4. Germination parameters of tomato seeds subjected to hydropriming and the control treatment.

	TG (%)	MDG (%)	T_{50} (h)	D_0-D_f (d)	SD	CI
Control	86	40	47.8	6	7.65	± 7.49
Hydropriming	90	64	39.7	7	5.16	± 5.05

TG: total germination; MDG: maximum daily germination; T_{50} : time to 50% germination of viable seeds; D_0-D_f : germination time interval; SD: standard deviation of TG; CI: confidence interval of TG.

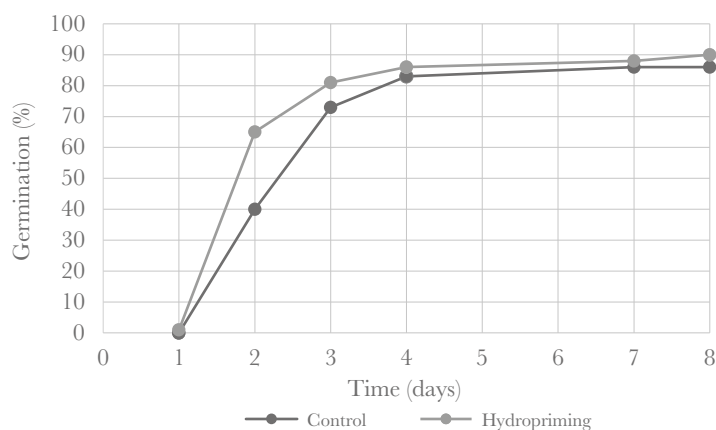


Figure 1. Germination behavior of tomato seeds subjected to hydropriming and control. Cumulative germination percentage determined at the indicated times.

Hydropriming promotes advancement to a late point within Phase II of the triphasic germination pattern, during which critical metabolic processes occur, including increases in the ATP/ADP ratio, mitochondrial repair, protein synthesis, and enzyme activation (Lemmens *et al.*, 2019). Consequently, upon rehydration, seeds exhibit clear advantages: more efficient metabolism, embryo enlargement, and endosperm weakening, thereby reducing the time and resources required for radicle protrusion (Chen and Arora, 2012). In aged seeds, moisture loss can lead to membrane damage and diminished enzymatic activity; controlled hydration can partially restore germination potential (Rendón and Gómez, 2018).

Natural Extracts

Germination

The results identified chili extracts at 15 and 25%, garlic extract at 25%, and onion extract at 50% as the most effective treatments for improving germination. The primary effects of the extracts were observed on total germination (TG) and process synchrony, as all treatments exceeded the control values for these parameters. However, only the 25% garlic and 25% chili extracts increased germination rate, as indicated by improved T_{50} values relative to the control. Notably, the 25% extracts yielded consistent outcomes, exhibiting favorable performance across multiple parameters (Table 5). The cumulative germination (CG) curve captured the key phases of the typical temporal germination pattern of tomato seeds (Figure 2).

Table 5. Germination parameters of tomato seeds subjected to different plant extracts and the control treatment.

	Control	Extracts (%)									
		15%					25%				
		Garlic	Onion	Chili	Ginger	Aloe	Garlic	Onion	Chili	Ginger	Aloe
TG (%)	86	84	90	95	86	90	91	94	93	92	91
MDG (%)	37	45	41	54	48	42	43	37	41	48	42
T_{50} (h)	54.5	62.1	58.5	59.3	62.5	61.6	49.8	54.6	52.1	58.0	50.5
D_o-D_f (d)	7	7	7	6	7	6	7	8	6	8	7
SD	5.16	7.57	9.52	3.82	6.92	9.52	3.82	5.16	2.00	8.64	5.03
CI	±5.05	±7.41	±9.32	±3.74	±6.78	±9.32	±3.74	±5.05	±1.96	±8.46	±4.92
		50%					75%				
TG (%)	86	89	92	81	83	93	84	89	90	85	85
MDG (%)	37	52	54	42	52	51	56	45	42	49	38
T_{50} (h)	54.5	44.5	58.2	60.2	62.5	62.3	59.1	47.7	50.1	58.7	51.0
D_o-D_f (d)	7	6	6	7	6	6	6	7	8	8	7
SD	5.16	8.24	3.26	11.01	11.94	8.86	3.26	6.00	4.00	5.03	5.03
CI	±5.05	±8.07	±3.19	±10.78	±11.70	±8.68	±3.19	±5.87	±3.91	±4.29	±4.29

TG: total germination; MDG: maximum daily germination; T_{50} : time to 50% germination of viable seeds; D_o-D_f : germination time interval; SD: standard deviation of TG; CI: confidence interval of TG.

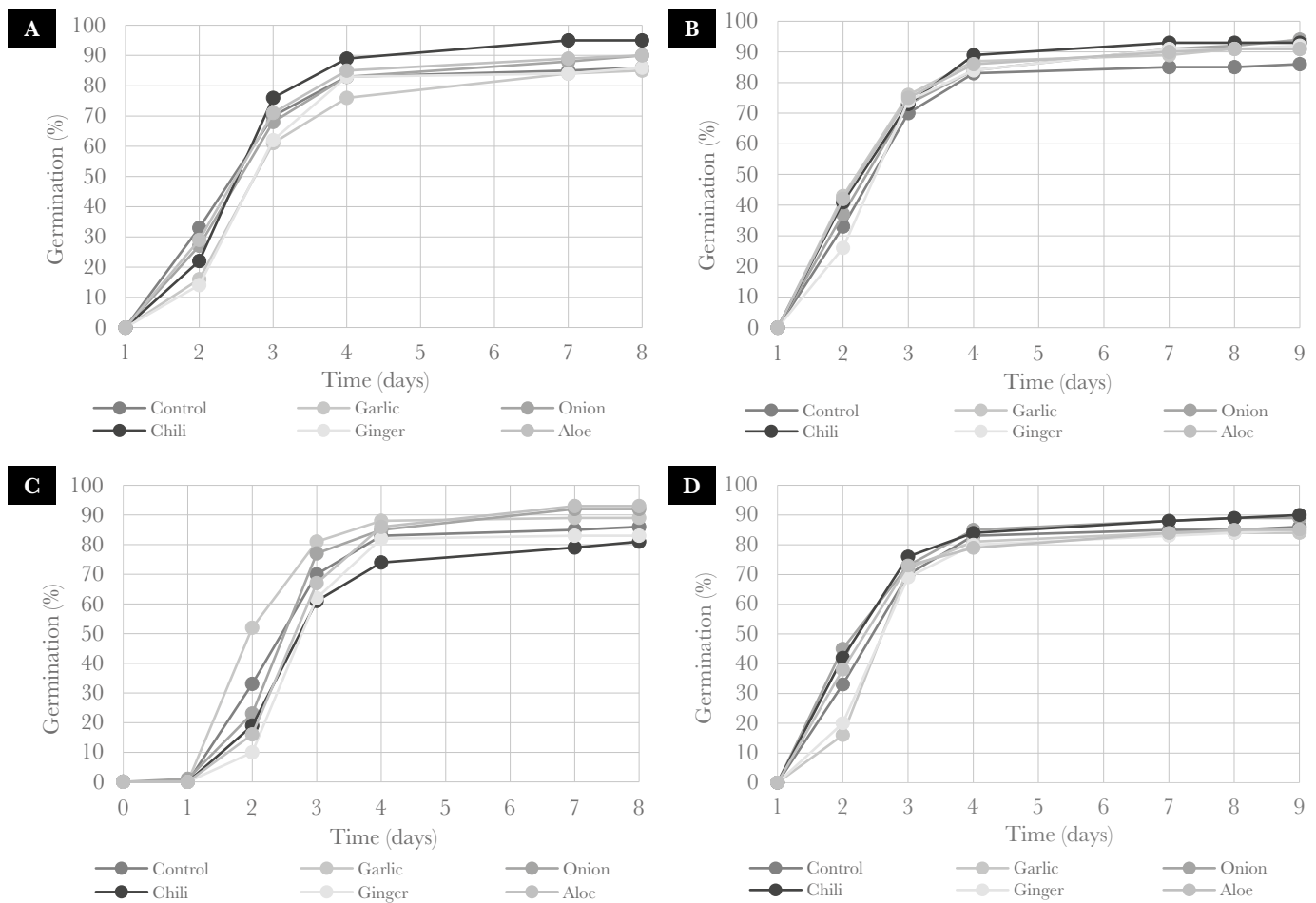


Figure 2. Germination behavior of tomato seeds exposed to different plant extracts and the control treatment. Cumulative germination percentages at the indicated time points for extracts at 15% (A), 25% (B), 50% (C), and 75% (D) concentrations.

Plant extracts exhibited a hormetic effect, a phenomenon in which a stress factor recognized for its toxicity at high doses elicits stimulatory responses at lower concentrations (Agathokleous *et al.*, 2023). Onion extract was among those that displayed this response, a logical outcome given its richness in diverse bioactive compounds, including organosulfur compounds, phenolic acids, flavonoids, minerals, and amino acids (Sagar *et al.*, 2022). Additionally, phytosterols present in onion act as precursors of brassinosteroids, which may promote germination by reducing seed sensitivity to abscisic acid (Pareek *et al.*, 2017; Finch-Savage, 2013).

Regarding the effect of chili extract on plants, there are differing opinions; It has been shown that it does not affect the germination of *Ipomoea purpurea* L. (Del Rosario García-Mateos *et al.*, 2013); while studies in *Capsicum annum* demonstrated negative effects of the extract on seed germination (Barchenger and Bosland, 2016). In this work, the chili extract enhanced germination performance. Capsaicin, one of its principal bioactive compounds, is a nitrogen compound that may serve as a precursor for amino acid synthesis during the activation of germinative metabolism (Finch-Savage, 2013).

Additionally, chili contains quercetin, a distinctive subclass of flavonoid known to participate in the regulation of diverse physiological processes, including germination (Singh *et al.*, 2021).

Regarding garlic, organosulfur compounds are particularly abundant, especially allicin, which exhibits high permeability across biological membranes. This compound functions as a signaling molecule capable of modulating endogenous hormonal balance by increasing auxin and gibberellic acid levels, while allelochemicals act as natural biostimulants that trigger reactive oxygen species signaling pathways (Hayat *et al.*, 2020).

Development

Chili extract at 25% (CH25) and onion extract at 50% (O50) were evaluated for development variables. Statistical analysis revealed significant differences between treatments and the control across all three evaluated variables. For stem and root length, no significant differences were detected between treatments, although CH25 (8.16 cm) and O50 (8.30 cm), respectively, exhibited slightly superior values. In contrast, differences in dry matter weight were more pronounced; treatments were separated into three distinct groups, and imbibition (I) outperformed the remaining treatments, exceeding the control by more than 40% (Table 6).

Imbibition performed adequately, and the combination of imbibition with the bioactive compounds present in the extracts further enhanced stem and root length; however, no significant differences were detected among the extract treatments. The marked differences observed in dry matter weight provide evidence of structural changes in the root system, likely associated with increased secondary root formation. Lemmens *et al.*, (2019) concluded that seed prehydration is effective not only in enhancing germination; they also showed that, after sowing, primed seeds absorb water and reactivate metabolism more rapidly, thereby improving establishment and vigor.

Regarding the extracts, chili contains carotenoids that act as precursors of strigolactones, which have the capacity to stimulate root development (Cortes *et al.*, 2019), as well as quercetin, which plays a key role in activating plant physiological traits such as growth and photosynthesis (Singh *et al.*, 2021). In onion, flavonoids and phenolic compounds are among the most abundant constituents and are noteworthy due to their active involvement in regulating plant growth and mediating defense responses (Rodríguez *et al.*, 2024).

Table 6. Results of Tukey's test for comparing means to evaluate the treatments with plant extracts applied to tomato seeds for development.

Stem length(cm)			Root length (cm)			Dry matter weight (mg)		
Treatment	Mean	Group	Treatment	Mean	Group	Treatment	Mean	Group
CH25	8.16	a	O50	8.30	a	I	108.30	a
O50	7.81	a	CH25	8.05	a	O50	97.95	ab
I	7.55	a	I	7.99	a	CH25	91.95	b
T	6.14	b	T	6.31	b	T	74.10	c

Chemical compounds

The CuSO₄ solutions at 1 and 2% were identified as the most effective treatments. These solutions primarily improved total germination (TG), whereas the remaining germination variables were not substantially modified relative to the control (Table 7). The cumulative germination (CG) curves followed similar trajectories consistent with the typical temporal germination pattern of tomato (Figure 3).

Table 7. Germination parameters of tomato seeds subjected to different chemical compound solutions and the control treatment.

Variables	Solutions (Concentration %)					
	0	0.5	0.06	2	1	1
TG (%)	Control	NaCl	AAS	CaCl ₂	KNO ₃	CuSO ₄
TG (%)	86	85	88	89	87	91
MDG (%)	40	47	36	40	46	41
T ₅₀ (h)	50.8	58.5	54.0	53.1	46.7	53.6
D ₀ -D _f (d)	7	6	6	7	6	6
SD	5.16	8.24	3.26	5.03	2.00	3.82
CI	±5.05	±8.07	±3.19	±4.92	±1.96	±3.74
	0	1	0.12	3	3	2
TG (%)	86	84	80	86	89	90
MDG (%)	40	36	33	30	43	39
T ₅₀ (h)	50.8	55.3	53.1	60.0	57.2	51.8
D ₀ -D _f (d)	7	7	7	7	7	6
SD	5.16	3.26	14.37	10.58	5.16	2.70
CI	±5.05	±3.19	±14.08	±10.36	±5.05	±2.64

TG: total germination; MDG: maximum daily germination; T₅₀: time to 50% germination of viable seeds; D₀-D_f: germination time interval; SD: standard deviation of TG; CI: confidence interval of TG.

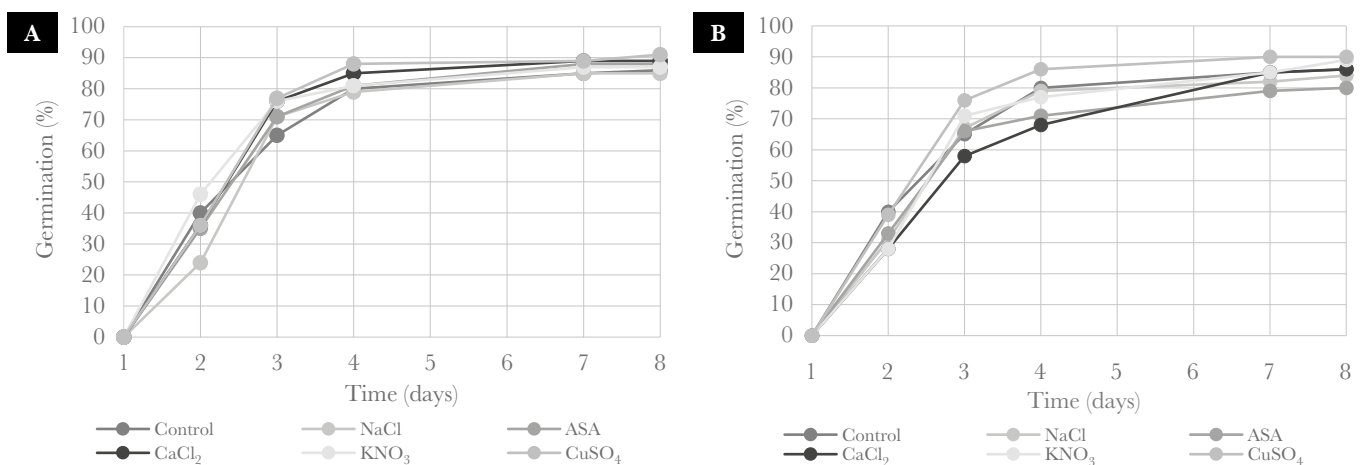


Figure 3. Germination behavior of tomato seeds exposed to different chemical compound solutions and the control treatment. Cumulative germination percentages (A and B) across the indicated time points. (A) NaCl 0.5%, acetylsalicylic acid (C₉H₈O₄; ASA) 0.06%, CaCl₂ 2%, KNO₃ 1%, and CuSO₄ 1% solutions. (B) NaCl 1%, ASA 0.12%, CaCl₂ 3%, KNO₃ 3%, and CuSO₄ 2% solutions.

Copper (Cu) is an essential element for plants; nonetheless, the available evidence regarding its use is contradictory. Iqbal *et al.*, (2018) reported toxic effects of Cu-derived compounds, including growth inhibition and reduced germination. Conversely, the findings of De la Cruz *et al.* (2023) in pepper indicate that CuSO₄ supplementation can stimulate regenerative processes in cotyledons, enhancing embryo production and promoting germination.

This apparent discrepancy may be explained by differential sensitivity among species or by excessively high application concentrations. Carrillo *et al.* (2005) support this hypothesis by showing considerable variation in Cu accumulation among different roselle (*Hibiscus sabdariffa* L.) varieties, suggesting that differential responses may constitute genotype-specific protective mechanisms. The observation that CuSO₄ is not universally lethal and that, at certain concentrations, it can stimulate germination suggests the existence of an optimal range in which Cu functions as an essential micronutrient before reaching toxic thresholds.

As demonstrated in this study, germination onset and critical points such as the day of maximum MDG and the interval of highest germinative activity follow a nearly invariable temporal germination pattern, consistent with the results reported by Michelin *et al.* (2016). Identifying the most effective treatment depends on multiple factors, and TG alone is often not a sufficiently comprehensive indicator. Parameters such as MDG, T₅₀, and the germination interval (D₀-D_f) must also be considered. If the objective is to determine the most efficient treatment, it should also confer greater speed and synchrony to the germination process; only then can establishment success and seedling survival be effectively improved (García and González, 2021).

Biopriming with tomato-associated bacteria

Analysis of variance for treatments involving *Bacillus* strains isolated from tomato roots revealed statistically significant differences among treatments for the evaluated development variables, albeit with specific considerations for root length and dry matter weight. For root length, the assumption of homogeneity of variances was not met ($p=0.01$); therefore, an arcsine transformation was applied to data normalized as proportions. For dry matter weight, a Box-Cox transformation was required ($\lambda=-0.3$). In both cases, the respective transformations corrected the assumption violations, and the analyses of variance continued to indicate significant treatment effects. The means presented in Table 8 are reported using the original (untransformed) data. Among the evaluated strains, RJ16 consistently performed best across the three variables, showing no major differences relative to RJ25 and RJ13, yet maintaining superior values in all cases (Table 8).

These bacteria belong to the group commonly referred to as Plant Growth-Promoting Bacteria (PGPB) (Castaño *et al.*, 2021b). The mechanisms of action of PGPB are typically classified as direct and indirect. Direct mechanisms include the biosynthesis of phytohormones and vitamins, as well as stimulation of germination. Indirectly, PGPB can synthesize antibiotics and fungicidal compounds, produce siderophores, and act as biological control agents against phytopathogens (Álvarez-García *et al.*, 2020). The genus *Bacillus* is dominant among endophytic bacteria (Cochard *et al.*, 2022), which are known

Table 8. Results of Tukey's test for comparing means to evaluate the inoculation of tomato seeds on development with tomato root-associated bacteria.

Stem length (cm)			Root length (cm)			Dry matter weight (g)		
Treatment	Mean	Group	Treatment	Mean	Group	Treatment	Mean	Group
RJ16	6.95	a	RJ16	5.95	a	RJ16	76.62	a
RJ25	6.91	ab	RJ13	5.30	ab	RJ25	70.06	ab
RJ6	6.90	ab	RJ25	5.13	ab	RJ13	65.93	bc
RJ13	6.85	ab	RJ12	4.82	bc	RJ12	65.37	bc
RJ12	6.80	ab	T	4.53	bc	RJ6	64.06	bc
T	5.55	b	RJ6	3.81	c	T	62.37	c

to establish intimate associations with plants, may be vertically transmitted, and can act through the secretion of minute concentrations of metabolites (Álvarez-García *et al.*, 2020). This could help explain the pronounced effects observed for *Bacillus pacificus* (RJ16) and *Bacillus paranthracis* (RJ13).

Regarding *B. pacificus* (RJ16), information on its effects in tomato is limited. Kouam *et al.* (2023b) reported that it increased stem length and the dry weight of roots and shoots. Ma *et al.* (2024) demonstrated that it enhances chlorophyll content and promotes root development in *Arabidopsis thaliana* and *Medicago sativa*, with the latter effect also corroborated in the present study. In these species, it also exhibited the capacity to mitigate drought damage. *Bacillus amylobliquefaciens* (RJ25), another strain with strong performance, has been extensively studied in tomato as a biocontrol agent (Chou *et al.*, 2022; Imran *et al.*, 2021). As a PGPB, Imran *et al.* (2021) confirmed that it increases germination and improves root and shoot length, with results comparable to those reported by Ali *et al.* (2025b). *Bacillus paranthracis* (RJ13) is a recently described species with limited information available; however, it belongs to a group closely related to *Bacillus cereus* (Campos-Avelar *et al.*, 2025), a species known to promote both aerial and root development in tomato (Andrade-Sifuentes *et al.*, 2022).

Biopriming with Maize-associated bacteria

Analysis of variance for treatments with *Pseudomonas* and *Enterobacter* strains isolated from maize roots, evaluated on tomato seedling development variables, revealed statistically significant differences in stem length and dry matter weight, whereas no differences were detected for root length. For stem length, the reference treatment was 1M, which was slightly higher than 3L and 4L, although differences were modest. Dry matter weight exhibited a different pattern: 5L and 3L were the best treatments, with weight increases of up to 75% relative to the control. Treatment 3L showed the most stable performance, ranking second for both evaluated variables, yet it did not differ significantly from the top-performing treatments (Table 9).

Pseudomonas fungipugnans (3L), *Pseudomonas humanensis* (1M), and *Enterobacter bugandensis* (5L) were the most effective treatments for promoting vegetative development, with *P. fungipugnans* (3L) showing particularly strong performance. The effects of *Pseudomonas* strains derived from maize roots on tomato seedlings were previously demonstrated by

Table 9. Results of Tukey's mean comparison test for evaluating the effect of inoculating tomato seeds on seedling development with bacteria associated with maize roots.

Stem length (cm)			Dry matter weight (g)		
Treatment	Mean	Group	Treatment	Mean	Group
1M	8.81	a	5L	148.50	a
3L	8.81	a	3L	144.30	a
4L	8.61	a	4L	128.25	ab
5L	8.47	ab	1M	117.95	ab
T	6.98	b	T	84.85	b

Rojas-Solís *et al.* (2016), who reported increases in hypocotyl and root length, as well as seedling fresh weight. *Pseudomonas* spp. are described as major components of the endophytic community in maize roots, with functions linked to plant growth promotion through the production of compounds such as NH_3 , siderophores, auxins, and extracellular enzymes (Singh and Goodwin, 2022). Similar effects have also been documented following inoculation of tomato seeds with species from this genus (Pandey and Gupta, 2020). The pronounced increase in dry matter weight, despite the absence of significant differences in root length, provides evidence of variation in root system development. This suggests modifications in lateral rooting and an increase in secondary roots traits that are critical for nutrient acquisition from the soil (Chen *et al.*, 2022).

P. fungipugnans is a recently described species for which a potential antifungal effect has been hypothesized (Schnyder *et al.*, 2025); however, the present work demonstrates that it may also exert additional effects associated with the promotion of vegetative development. With respect to *P. hunanensis*, its performance as a PGPB in tomato infested with *Fusarium oxysporum* under saline stress conditions has been previously validated (Verma *et al.*, 2024). Likewise, *E. bugandensis* has been reported to promote growth in maize and wheat under water and salinity stress (Tian *et al.*, 2025; Verma *et al.*, 2024).

CONCLUSIONS

Hydropriming increased and accelerated germination. Chili, garlic, and onion extracts exhibited concentration-dependent biostimulant effects, evidencing a hormetic phenomenon in which low concentrations improved both germination and vegetative development. CuSO_4 was the best-performing chemical-solution treatment, although its effects were not outstanding. Biopriming results confirm the relevance of host-microorganism specificity in beneficial interactions, as well as cross-effects of maize-associated bacteria acting on tomato. Moreover, the outcomes obtained with recently described species expand current knowledge regarding their biological effects.

This study provides scientific evidence to support the development of pregermination treatment protocols for tomato. Integrating physical (hydropriming), allelopathic (plant extracts), and biotechnological (biopriming) approaches offers a robust theoretical basis for more efficient production systems. Improved understanding of hormetic mechanisms and response specificity facilitates scale-up toward commercial applications, contributing to the development of sustainable technologies for modern agriculture.

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REFERENCES

- Agathokleous, E., Calabrese, E. J., & Barceló, D. (2023). Environmental hormesis: New developments. *The Science Of The Total Environment*, 906, 167450. <https://doi.org/10.1016/j.scitotenv.2023.167450>
- Ali, D. F. I., El-Nahrawy, S., El-Zawawy, H. A. H., & Omara, A. E. (2025b). Effective Applications of *Bacillus subtilis* and *B. amyloliquefaciens* as Biocontrol Agents of Damping-Off Disease and Biostimulation of Tomato Plants. *Stresses*, 5(1), 9. <https://doi.org/10.3390/stresses5010009>
- Álvarez-García, J., Santoyo, G., & Del Carmen Rocha-Granados, M. (2020). *Pseudomonas fluorescens*: Mecanismos y aplicaciones en la agricultura sustentable. *Revista Latinoamericana de Recursos Naturales*, 16(1), 01-10. <https://doi.org/10.33154/rlrn.2020.01.01>
- Andrade-Sifuentes, A., Fortis-Hernández, M., Preciado-Rangel, P., Sáenz-Mata, J., Coria-Arellano, Y., & López, C. G. (2022). Promoción del crecimiento de tomate saladette con *Bacillus cereus* y estiércol solarizado en invernadero. *Revista Mexicana de Ciencias Agrícolas*, 13(7), 1259-1270. <https://doi.org/10.29312/remexca.v13i7.3120>
- Barchenger, D. W., & Bosland, P.L.W. (2016). Exogenous applications of capsaicin inhibits seed germination of *Capsicum annuum*. *Scientia Horticulturae*, 203, 29-31. <https://doi.org/10.1016/j.scienta.2016.03.009>
- Campos-Avelar, I., Montoya-Martínez, A. C., Escalante-Beltrán, A., Parra-Cota, F. I., & De los Santos Villalobos, S. (2025). Do Organic Amendments Foster Only Beneficial Bacteria in Agroecosystems?: The Case of *Bacillus paranthracis* TSO55. *Plants*, 14(7), 1019. <https://doi.org/10.3390/plants14071019>
- Carrillo, C.G., Juárez, M.J., Peralta, V.J.R., Gómez, E. and Gardea, T.J.L. (2005). Modulation of uptake and translocation of iron and copper from root to shoot in common bean by siderophore-producing microorganisms. *Journal of Plant Nutrition*, 28(10), pp. 1853-1865. <https://doi.org/10.1080/01904160500251340>
- Castaño, A. M. P., Durango, D. P. M., Polanco-Echeverry, D., & Cardona-Arias, J. A. (2021b). Rizobacterias promotoras de crecimiento vegetal (PGPR): una revisión sistemática 1990-2019. *Revista de Investigación Agraria y Ambiental*, 12(2), 161-178. <https://doi.org/10.22490/21456453.4040>
- Chen, K., & Arora, R. (2012). Priming memory invokes seed stress-tolerance. *Environmental And Experimental Botany*, 94, 33-45. <https://doi.org/10.1016/j.envexpbot.2012.03.005>
- Chen, C., Wang, M., Zhu, J., Tang, Y., Zhang, H., Zhao, Q., Jing, M., Chen, Y., Xu, X., Jiang, J., & Shen, Z. (2022). Long-term effect of epigenetic modification in plant-microbe interactions: modification of DNA methylation induced by plant growth-promoting bacteria mediates promotion process. *Microbiome*, 10(1). <https://doi.org/10.1186/s40168-022-01236-9>
- Chou, H., Huang, Y., Lin, Y., & Deng, W. (2022). Selection, Formulation, and Field Evaluation of *Bacillus amyloliquefaciens* PMB01 for Its Application to Manage Tomato Bacterial Wilt Disease. *Agriculture*, 12(10), 1714. <https://doi.org/10.3390/agriculture12101714>
- Cochard, B., Giroud, B., Crovadore, J., Chablais, R., Arminjon, L., & Lefort, F. (2022). Endophytic PGPR from Tomato Roots: Isolation, *In Vitro* Characterization and *In Vivo* Evaluation of Treated Tomatoes (*Solanum lycopersicum* L.). *Microorganisms*, 10(4), 765. <https://doi.org/10.3390/microorganisms10040765>
- Cortes, J. S. A., Godoy, J. A., Cortés, J. D. A., & Mora, R. M. S. (2019). Principales reguladores hormonales y sus interacciones en el crecimiento vegetal. *Nova*, 17(32), 109-129. <https://doi.org/10.22490/24629448.3639>
- Da Silva Carvalho, R., Da Silva, M. A., Borges, M. T. M. R., & Forti, V. A. (2021). Plant extracts in agriculture and their applications in the treatment of seeds. *Ciência Rural*, 52(5). <https://doi.org/10.1590/0103-8478cr20210245>
- De la Cruz, M. F. S., Gardezi, A. K., Castañeda, G. C., Escobar, H. M. O., Villa, O. R. M., Panta, J. E. R., Buenfil, J. A. L., Saavedra, M. U. L., Aguilar, G. H., & Gamboa, C. A. A. (2023). efecto del sulfato de cobre pentahidratado ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) y rizobacterias en la germinación de jamaica (*Hibiscus sabdariffa* L.). *Tropical And Subtropical Agroecosystems*, 26(1). <https://doi.org/10.56369/tsaes.4286>
- Del Rosario García-Mateos, M., Sánchez-Navarro, C., Solís, J. M., & Pérez-Grajales, M. (2013). Actividad fitotóxica de extractos de chile manzano (*Capsicum pubescens* R & P). DOAJ (DOAJ: Directory Of Open Access Journals). <https://doaj.org/article/6744c7f3c5404b3da871de7e4a1d4316>

- Dhal, P., Sahu, G., Dhal, A., Mohanty, S., & Dash, S. K. (2022). Priming of vegetable seeds: a review. *The Pharma Innovation Journal*, *11*(2), 519-525. <https://www.thepharmajournal.com/archives/?year=2022&vol=11&issue=2&ArticleId=10560>
- FAOSTAT. (2023). Food and Agriculture Organization of the United Nations Statistics Division. <http://www.fao.org/faostat/> : Fecha de consulta: 04 de julio de 2025
- Finch-Savage, B. (2013). Seeds: Physiology of development, germination and dormancy (3rd edition) - J.D. Bewley, K.J. Bradford, H.W.M. Hihorst H. Nonogaki. 392 pp. Springer, New York – Heidelberg – Dordrecht – London 2013978-1-4614-4692-7. *Seed Science Research*, *23*(4), 289. <https://doi.org/10.1017/s0960258513000287>
- García, A. F., & González, J. J. M. (2021). Evaluación cuantitativa de la germinación de *Quercus variabilis* Blume en tres tamaños de semilla. *Revista Mexicana de Ciencias Forestales*, *12*(68), 202-211. <https://doi.org/10.29298/rmcf.v12i68.1104>
- Gómez-Salazar, A., López-Salvador, G., Jerez-Mompie, E., González-Cañizares, P., & Guerrero-Domínguez, L. (2023). Influencia de dos bioestimulantes en el crecimiento y desarrollo de plantas de jitomate (*Solanum lycopersicum* L.). <https://ediciones.inca.edu.cu/index.php/ediciones/article/view/1658>
- Hayat, S., Ahmad, H., Nasir, M., Khan, M. N., Ali, M., Hayat, K., Khan, M. A., Khan, F., Ma, Y., & Cheng, Z. (2020). Some Physiological and Biochemical Mechanisms during Seed-to-Seedling Transition in Tomato as Influenced by Garlic Allelochemicals. *Antioxidants*, *9*(3), 235. <https://doi.org/10.3390/antiox9030235>
- Imran, M., Abo-Elyousr, K. A. M., Mousa, M. A. A., & Saad, M. M. (2021). A study on the synergetic effect of *Bacillus amyloliquefaciens* and dipotassium phosphate on *Alternaria solani* causing early blight disease of tomato. *European Journal Of Plant Pathology*, *162*(1), 63-77. <https://doi.org/10.1007/s10658-021-02384-8>
- Iqbal, M. Z., Habiba, U., Nayab, S., & Shafiq, M. (2018). Effects Of Copper On Seed Germination And Seedling Growth Performance Of Lens Culinaris Medik. *Journal Of Plant Development*, *25*, 85-90. <https://doi.org/10.33628/jpd.2018.25.1.85>
- Kouam, I. D., Mabah, J., Ntsoli, P. G., Tchamani, L., Yaouba, A., Katte, B., & Bitom, D. (2023b). Growth promotion potential of *Bacillus* spp. isolates on two tomato (*Solanum lycopersicum* L.) varieties in the West region of Cameroon. *Open Agriculture*, *8*(1). <https://doi.org/10.1515/opag-2022-0154>
- Lamichane, J. R., Debaeke, P., Steinberg, C., You, M. P., Barbetti, M. J., & Aubertot, J. (2018). Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant And Soil*, *432*(1-2), 1-28. <https://doi.org/10.1007/s11104-018-3780-9>
- Lemmens, E., Deleu, L. J., De Brier, N., De Man, W. L., De Proft, M., Prinsen, E., & Delcour, J. A. (2019). The Impact of Hydro-Priming and Osmo-Priming on Seedling Characteristics, Plant Hormone Concentrations, Activity of Selected Hydrolytic Enzymes, and Cell Wall and Phytate Hydrolysis in Sprouted Wheat (*Triticum aestivum* L.). *ACS Omega*, *4*(26), 22089-22100. <https://doi.org/10.1021/acsomega.9b03210>
- Ma, X., Zhang, B., Xiang, X., Li, W., Li, J., Li, Y., Tran, L. P., & Yin, H. (2024). Characterization of *Bacillus pacificus* G124 and Its Promoting Role in Plant Growth and Drought Tolerance. *Plants*, *13*(20), 2864. <https://doi.org/10.3390/plants13202864>
- Michelin, K. T. P., Jean, A., Donatien, G., Keilah, L. P., Stephen, L. T., & Jules-Roger, K. (2016). *In vitro* allelopathic effects of extracts and fractions of five plants on tomato seed germination and vigor index. *Cogent Biology*, *2*(1), 1220661. <https://doi.org/10.1080/23312025.2016.1220661>
- Pandey, S., & Gupta, S. (2020). Evaluation of *Pseudomonas* sp. for its multifarious plant growth promoting potential and its ability to alleviate biotic and abiotic stress in tomato (*Solanum lycopersicum*) plants. *Scientific Reports*, *10*(1). <https://doi.org/10.1038/s41598-020-77850-0>
- Pareek, S., Sagar, N. A., Sharma, S., & Kumar, V. (2017). Onion (*Allium cepa* L.). *Fruit and Vegetable Phytochemicals*, 1145-1162. doi:10.1002/9781119158042.ch58
- Rendón, J. A. S., & Gómez, E. F. F. (2018). Ecotecnologías para la restauración ecológica: los tratamientos de semillas y las micorrizas. <http://repositorio.geotech.cu/jspui/handle/1234/3607>
- Rodríguez, D. J., Ramírez-Pérez, C., Ramírez-Rodríguez, H., Villarreal-Quintanilla, J. Á., Hernández-Pérez, A., Díaz-Jimenez, M. L. V., & Peña-Ramos, F. M. (2024). Bioestimulantes de plantas del semidesierto en el crecimiento radicular y aéreo de plántulas de tomate. *Ecosistemas y Recursos Agropecuarios*, *11*(3). <https://doi.org/10.19136/era.a11n3.4145>
- Rojas-Solís, D., Hernández-Pacheco, C. E., & Santoyo, G. (2016). Evaluation of *Bacillus* and *Pseudomonas* to colonize the rhizosphere and their effect on growth promotion in tomato (*Physalis ixocarpa* Brot. ex Horm.). *Revista Chapingo Serie Horticultura*, *XXII*(1), 45-57. <https://doi.org/10.5154/r.rchsh.2015.06.009>

- Sagar, N. A., Pareek, S., Benkeblia, N., & Xiao, J. (2022). Onion (*Allium cepa* L.) bioactives: Chemistry, pharmacotherapeutic functions, and industrial applications. *Food Frontiers*, 3(3), 380-412. <https://doi.org/10.1002/fft2.135>
- SIAP (2023) Panorama Agroalimentario 2023. Servicio de Información Agroalimentaria y Pesquera. https://drive.google.com/file/d/1FWHntHMgju_uOse_MsOF9jZQDAm_FOD9/view .Fecha de consulta: 04 de julio de 2025
- Singh, R., & Goodwin, S. B. (2022). Exploring the Corn Microbiome: A Detailed Review on Current Knowledge, Techniques, and Future Directions. *PhytoFrontiers™*, 2(3), 158-175. <https://doi.org/10.1094/phytofr-04-21-0026-rvw>
- Singh, P., Arif, Y., Bajguz, A., & Hayat, S. (2021). The role of quercetin in plants. *Plant Physiology And Biochemistry*, 166, 10-19. <https://doi.org/10.1016/j.plaphy.2021.05.023>
- Schnyder, A., Kalawong, R., Eberl, L., & Agnoli, K. (2025b). *Pseudomonas fungipugnans* sp. nov., a potentially antifungal bacterium isolated from moss. *International Journal Of Systematic And Evolutionary Microbiology*, 75(1). <https://doi.org/10.1099/ijsem.0.006624>
- Tian, X., Liu, Q., Song, J., Zhang, X., Yang, G., Li, M., Qu, H., Tastanbek, & Tan, Y. (2025). Effect of *Enterobacter bugandensis* R-18 on Maize Growth Promotion Under Salt Stress. *Microorganisms*, 13(8), 1796. <https://doi.org/10.3390/microorganisms13081796>
- Verma, P., Bhattacharya, A., Bharti, C., & Arora, N. K. (2024). Antifungal metabolites produced by *Pseudomonas hunanensis* SPT26 effective in biocontrol of fusarium wilt of *Lycopersicon esculentum* under saline conditions. *World Journal Of Microbiology And Biotechnology*, 40(10). <https://doi.org/10.1007/s11274-024-04092-x>