American Journal of Plant Sciences, 2026, 17(3), 284-301
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Evaluation of Pre-Germination Treatments as Potential Promoters of Germination and Development in Maize (*Zea mays* L.)

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How to cite this paper: Hernández-Escalona, R., Carrillo Castañeda, G.M., Cruz-Izquierdo, S. and Antúnez-Ocampo, O.M. (2026) Evaluation of Pre-germination Treatments as Potential Promoters of Germination and Development in Maize (*Zea mays* L.). *American Journal of Plant Sciences*, **17**, 284-301.
https://doi.org/10.4236/ajps.2026.173019

Received: December 1, 2025
Accepted: March 21, 2026
Published: March 24, 2026

Abstract

Pre-germination treatments are a promising tool for improving germination and early seedling development in economically important crops such as maize. The objective of this study was to evaluate different pre-germination treatments and identify the most effective ones for germination and early seedling development of the Zacatecas 58 maize variety. Variables such as daily germination (DG), total germination (GT), and time to 50% germination (T_{50}) (indicators of germination speed and synchronization) were measured. For seedling development, stem and root length and dry matter weight were measured, and the data were analyzed using analysis of variance for completely randomized designs. The most effective treatments for germination among the chemical substances were acetylsalicylic acid (ASA) at 0.06% (81% germination) and calcium chloride ($CaCl_2$) at 3% (84% germination), both superior to the control (75%) in germination speed and synchronization. The plant extracts that exhibited a hormetic effect were garlic at 15%, chili pepper at 15%, and onion at 25% and 50%. Regarding plant development, onion extracts significantly stimulated stem and root length. Biopriming with strains of *Pseudomonas fungipugnans* and *P. hunanensis* obtained from corn roots improved root length and dry weight, while *Bacillus* strains from tomato roots had no apparent effect on the corn seedlings. It is concluded that concentration and application time are critical factors and that plant extracts, biopriming, and chemical compounds represent viable and economical alternatives for improving seedling germination and vigor. A certain species-specific relationship between the root microbiota and its host was also demonstrated.

Keywords

Biopriming, Chemical Compounds, Plant Extracts, Germination, Development

1. Introduction

Maize is one of the most important crops worldwide, ranking first in production with over 1200 million tons and representing a staple food for more than 1000 million people [1]. In Mexico, this cereal holds relevance not only for its nutritional and economic value, but also for its cultural significance as a center of origin and diversification of the species, with approximately 60 races [2]. Due to the high demand for grain, practices associated with cultivation require intensive use of synthetic agrochemical products such as fertilizers and insecticides, which results in less fertile soils [3], environmental contamination, interruption of the natural nutrient cycle, and destruction of biological communities in soils [4]. The intensive agrochemical use on approximately 6.6 million hectares planted in the country [5] contributes to a hostile soil environment. This, combined with prolonged seed storage, creates significant barriers for maize seed germination and seedling emergence.

Faced with such a complicated scenario, pre-germinative treatments are a promising alternative in preparing seeds for an initial stage full of adversities. Different priming techniques such as hydropriming (controlled hydration), through chemical compounds in solution and biopriming (application of growth-promoting bacteria such as *Pseudomonas* and *Bacillus*), as well as plant extracts with bioactive compounds like organosulfur compounds (abundant in members of the genus *Allium*) and flavonoids (present in the genus *Capsicum*) types have demonstrated effectiveness in improving parameters such as germination speed, emergence uniformity, seedling vigor, and resistance to adverse environmental conditions [6]-[8].

Despite the potential demonstrated by these technologies in situations such as droughts and high salinity in tomato, pepper, and other crops [9], there is a need to optimize specific protocols for different species and varieties, considering factors such as concentrations, application times, and environmental conditions.

Based on the above, this work aims to evaluate different pre-germinative treatments, and to identify the most promising ones for their effects on germination and initial development of maize seedlings, of the Zacatecas 58 variety.

2. Materials and Methods

The experiments were conducted between January 2024 and June 2025 at the facilities of Colegio de Postgraduados, Montecillo Campus, Texcoco, Estado de

México. Seeds of maize variety Zacatecas 58, harvested in 2020 on the same institution's premises, were used.

2.1. Pre-Germinative Treatments

2.1.1. Hydropriming

Hydropriming is based on controlled seed hydration in such a way that metabolic activation of germination is advanced, but without reaching radical emergence [10]. In the experiment, seeds were hydrated for 25 h based on the preliminary water absorption curve which showed radical emergence at 27 h of continuous hydration. This duration was selected to be 2 h less than the time to germination, followed by standard hydropriming protocols [11]. After hydration dried with a fan for 2 h and 30 min and then stored. Weight was checked periodically until it matched the initial weight (23.45 g weight of 100 seeds). This drying restored the seeds to the original moisture content a key principle of priming.

2.1.2. Plant Extracts

1) Germination

From fresh samples, aquous extracts of five plant species were prepared: garlic (*Allium sativum* L.), purple onion (*Allium cepa* L.), chili pepper (*Capsicum chinense* Jacq.), ginger (*Zingiber officinale* Roscoe), and aloe (*Aloe vera* L.) at concentrations of 15%, 25%, 50%, and 75% (v/v) in final volumes of 32 mL per treatment. For their preparation, water and mortars were pre-cooled to 16˚C and −18˚C respectively to minimize the thermal degradation of bioactive compounds during maceration. Each plant material (0.52 g) was weighed and macerated for 3 to 4 min (garlic, pepper, and onion) and 5 to 6 min (ginger and aloe) adding water gradually. Finally, each extract was adjusted to the required concentration according to Table 1.

Table 1. Volumes of plant extracts and water required to achieve the experimental concentrations based on a total volume of 32 mL.

Required Concentration (%)	Extract Volume (mL)	Water Volume (mL)
15	4.8	27.2
25	8	24
50	16	16
75	24	8

2) Seedling Development Assay

From the germination tests, two of the most promising extracts were selected: onion extracts at 25% (C25) and 50% (C50). These were evaluated for seedling development variables. Three treatments were established: seeds soaked in C25, seeds soaked in C50, and a control soaked in distilled water (I). An additional untreated control (T) was also included. For each treatment, 20 seeds were

soaked for 20 h in 10 mL of the corresponding extract and the control in distilled water (I).

2.1.3. Chemical Compounds

Five chemical compounds were selected based on their prevelance in the seed priming literature to evaluate effects on germination: acetylsalicylic acid ($C_9H_8O_4$ or ASA) at 0.06% and 0.12% concentration, potassium nitrate (KNO_3) 1% and 3%, copper sulfate ($CuSO_4$) 1% and 2%, calcium chloride ($CaCl_2$) 2% and 3%, and sodium chloride (NaCl) at 0.5% and 1% concentration. Initially, the concentrations coincided with those reported in the literature, and based on the results, it was decided to increase or decrease the concentration for the second stage of the experiment. The treatment consisted of immersing the seeds in the chemical compounds in solution prior to germination. Solutions of 40 mL were prepared from each substance; the required amounts of substance were weighed (Table 2), placed in distilled water and stirred until completely dissolved. For each concentration seeds were placed in petri dishes, solutions were added and maintained for 20 h. Finally, they were thoroughly washed with distilled water and placed to germinate.

Table 2. Quantities of substance needed to prepare the experimental solutions based on a total volume of 40 mL.

Chemical Substance	Solution Concentration (%)	Amount of Substance (g)
ASA	0.06	0.03
	0.12	0.05
KNO_3	1	0.40
	3	1.20
$CuSO_4$	1	0.40
	2	0.80
$CaCl_2$	2	0.80
	3	1.20
NaCl	0.5	0.20
	1	0.40

2.1.4. Biopriming of Seedling Development Assay

Biopriming was employed for evaluation in initial development; this treatment is based on exposing seeds to microorganisms with beneficial properties [12]. For this experiment, strains from the collection of the Cell Biology laboratory of Colegio de Postgraduados, Montecillo Campus were used. *Pseudomonas* and other species associated with tomato and maize roots were selected for this work (Table 3).

The selection criterion for *Pseudomonas* strains was based on their ability to develop and maintain fluorescence with increasing salinity of the culture medium. Strains of other species were selected only based on salinity tolerance. Each strain preserved in test tubes was spread on King's B medium using an inoculation loop

in Petri dishes with salinity levels at 1%, 2%, and 3%. Fluorescence was determined using an ultraviolet light lamp.

Table 3. Bacterial strains used in biopriming treatments.

Species	Code	Crop	Origin
Pseudomonas fungipugnans	3L	Maize	Rhizospheric
Pseudomonas fungipugnans	4L	Maize	Rhizospheric
Enterobacter bugandensis	5L	Maize	Rhizospheric
Pseudomonas uvaldensis	13L	Maize	Rhizospheric
Pseudomonas hunanensis	1M	Maize	Endophytic
Not identified	4M	Maize	Endophytic
Bacillus cereus	RJ12	Tomato	Rhizospheric
Bacillus paranthracis	RJ13	Tomato	Endophytic
Bacillus pacificus	RJ16	Tomato	Endophytic
Bacillus amyloliquefaciens	RJ25	Tomato	Rhizospheric

The best strains were seeded in Petri dishes with King's B medium and placed to grow in the incubator between 26˚C and 28˚C for 24 h. The next day, bacterial suspensions were prepared in test tubes with sterile water. Each suspension was adjusted to a standarized turbidity of 0.90 at an optical density of 660 nm in a spectrophotometer. Twenty seeds for each bacterial treatment were deposited in sterile Gerber flasks. For seed coating, 10 mL of standardized bacterial suspension were added to each flask and left to stand for 15 minutes. Seeds were then removed and sown immediately.

2.2. Germination Tests

In each treatment and the controls, 100 seeds were used, distributed in 4 Petri dishes in quantities of 25; and water (8 mL), plant extract (8 mL) or solution (10 mL) was added depending on the treatment, always on three sheets of Sanitas paper. They were placed in the incubator between 26˚C and 28˚C, and the number of germinated seeds was recorded daily for each dish. A seed was considered germinated upon visible radicle protrusion (≥2 mm) outside the seed coat. The petri dish was considered the experimental unit ($n = 4$).

2.3. Germination Data Analysis

From the data records, Cumulative Germination (CG) curves were created, expressed as the cumulative sum of the mean daily germination percentages of the four Petri dishes used in each treatment. For each treatment, the CG 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.

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

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

where N: number of replicates ($n = 4$); x_i : treatment data; $\bar{x}$: sample mean; z: critical value associated with the confidence level. T_{50} was also determined, which is the time in which 50% of viable seed germinates, calculated from CG data and the slope of a linear function model based on Equation (3) and Equation (4):

$$m = \left(y_2 - y_1\right)/\left(x_2 - x_1\right) \quad (3)$$

$$y = mx + n \quad (4)$$

where m is the slope of a line, x and y are coordinates representing intercept points on a line (in this case x is time and y represents germination corresponding to that time) and n: is the intercept of a line on the y axis. The germination interval was also established, which is the time elapsed between the beginning and end of germination (D_o-D_f). Microsoft Office Excel application was used for creating graphics and data processing.

2.4. Vigor Tests

Seeds were sown in polystyrene germination trays filled with sterile substrate and remained in the greenhouse for 30 to 35 days; the time at which seedlings enter the second stage of root development, depending only on themselves to survive [13]. The sample size was 10 plants per treatment with each seedling constituting an experimental unit (n = 10). The variables measured were stem length, root length, and dry matter weight of seedlings. To determine dry matter weight, they were first left in the oven for 48 h at 43˚C, then the weight was checked every hour until it remained constant.

2.5. Statistical Analyses

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

3. Results and Discussion

3.1. Pre-Germinative Treatments

3.1.1. Hydropriming

Germination of maize seeds was negatively affected by this treatment. TG had a 53% decrease between control and hydroprimed seeds. The other variables that

were measured also had better parameters in the control seeds than in the treated ones. (Table 4). Only T_{50} is better in treated seeds, but it corresponds to a very low TG. The temporal germination pattern with evident distances has CG curves with similar trajectories (Figure 1).

Table 4. Germination parameters of maize seeds subjected to hydropriming and control.

	TG (%)	DG (%)	T_{50} (h)	D_o-D_f (d)	SD	CI
Control	75	54	39.5	3	±9.75	9.31
Hydropriming	22	16	29.5	2	±9.29	9.10

TG: total germination; DG: 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.

The observed negative effect of hydropriming stands in contrast to previous reports [11] [14]. 24 h of hydration for maize seeds has been suggested by several authors [15]; however, it has been found that hydration times exceeding 4 h are harmful to maize seeds [16], it has been described that uncontrolled water absorption by seeds can occur, as it depends largely on the tissue's affinity for water, which could cause imbibition damage that would affect germination [17]. This variability in results reassures that the temporal limit of soaking is critical [16], so determining the optimal imbibition duration is crucial before conditioning to achieve satisfactory effects [18]. This demonstrates the high sensitivity to the duration of hydropriming treatments.

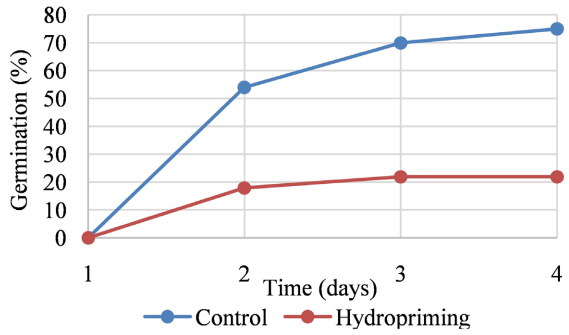

Figure 1. Germination behavior of maize seeds subjected to hydropriming and control. Cumulative germination percentage at the indicated times.

This indicates that while the treatment altered the final germination percentage, it did not fundamentally change the temporal pattern of germination. In another study different pre-germinative treatments in maize varieties and the key moments of the process, such as germination onset and the interval of greatest activity, remain practically temporally invariable [19] [20].

In this study, identification of efficient treatments was based on achieving not only a high TG but also improved speed (lower T_{50}) and synchronization as some authors have suggested [21]. For this, it was necessary that, in addition to achiev-

ing higher values of TG than the controls, they also met other qualities such as positive indicators in T_{50}, DG, and germination interval (D_o-D_f), which reflect speed, synchrony, and brevity, respectively.

3.1.2. Plant Extracts

1) Germination

The extracts that significantly favored germination compared to the control were pepper and garlic at 15%, and onion at 25% and 50% concentration. The effect of the onion extract was dose-dependent, and at 50%, it caused the best T_{50} (38.4 h), DG (68%), and a germination interval (D_o-D_f) of only three days, demonstrating improvements in speed, synchronization, and was based on achieving not only a high TG but also improved speed (lower T_{50}) and shorter germination period. The other treatments stand out for significantly increasing TG with better standard deviations and confidence intervals (Table 5). The temporal germination pattern does not have considerable deviations, as reflected by the CG curves; germination begins on the second day and is accelerated until the fourth day, after which there is no considerable activity (Figure 2).

Table 5. Germination parameters of maize seeds subjected to different plant extracts and control.

		Extracts (Concentration %)									
		15 (%)					25 (%)				
Variable	**Control**	**Garlic**	**Onion**	**Chili**	**Ginger**	**Aloe**	**Garlic**	**Onion**	**Chili**	**Ginger**	**Aloe**
TG (%)	76	83	74	82	80	80	80	83	73	69	78
DG (%)	57	51	47	51	57	57	56	56	47	45	51
T_{50} (h)	40.0	43.5	43.5	42.8	56.4	40.8	41.1	42.2	42.2	42.4	42.3
D_o-D_f (d)	6	7	6	6	6	6	6	3	6	3	6
SD	±9.52	±3.82	±12.00	±5.16	±3.26	±6.53	±4.61	±6.00	±8.86	±13.21	±12.00
CI	9.33	3.75	11.75	5.06	3.20	6.40	4.52	5.87	8.69	12.95	11.75
		50					**75**				
TG (%)	76	80	82	70	69	73	73	79	75	75	72
DG (%)	57	46	68	55	57	57	51	59	48	48	49
T_{50} (h)	40.0	44.8	38.4	39.2	38.5	39.3	42.7	39.6	42.7	40.2	41.6
D_o-D_f (d)	6	6	3	6	6	6	7	7	6	6	6
SD	±9.52	±14.96	±7.65	±16.81	±12.38	±6.83	±9.45	±10.51	±6.83	±3.82	±10.32
CI	9.33	14.66	7.50	16.47	12.13	6.69	9.26	10.3	6.69	3.75	10.12

TG: total germination; DG: 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.

The extracts show a potential hormetic effect, which is a biphasic adaptive response with stimulation at low doses and inhibition at high doses, often linked to

adaptive responses to stressful conditions [22]. Onion extract was one of these cases. Onion is rich in biocompounds of different chemical nature that can favor germination [23]. Allicin and gibberellic acid are known germination promoters and phytosterols are precursors of brassinosteroids that decrease germinative inhibition caused by abscisic acid [24]-[26].

Reference [27] also verified the stimulating effects of garlic extract at low concentrations on TG, supporting the results of this work. Garlic contains organosulfur compounds such as allicin, a highly permeable compound to biological membranes. It has been proven that allicin acts in tomato seeds as a signaling molecule that increases the levels of germination activators such as auxin and gibberellic acid [28].

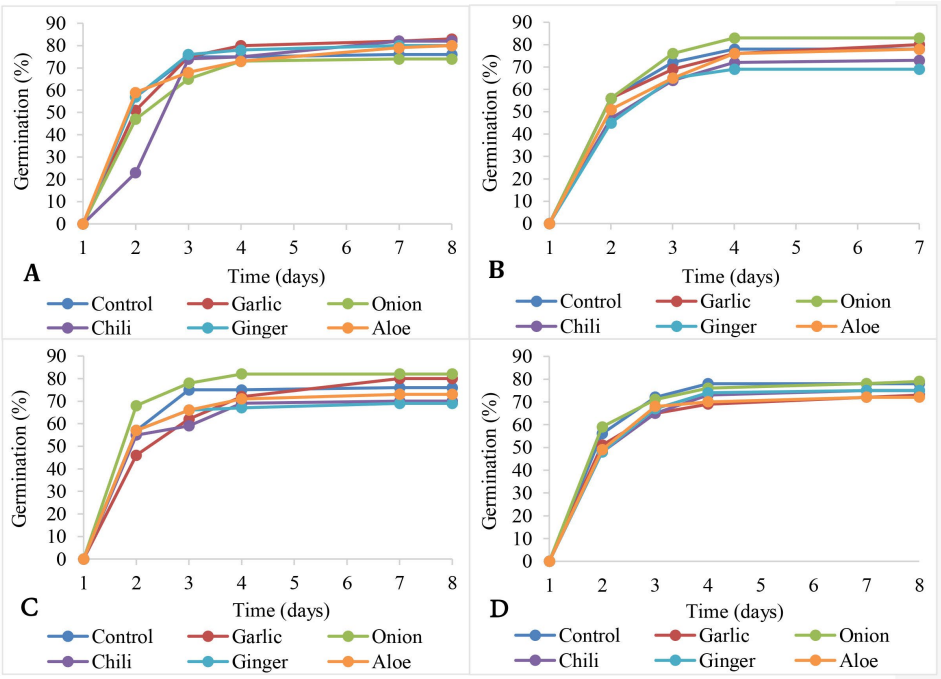

Figure 2. Germination patterns of maize seeds subjected to germination in different plant extracts and control. Cumulative germination percentages at the indicated times with extracts at 15% (**A**), 25% (**B**), 50% (**C**), and 75% (**D**) concentration.

No studies were found on the effects of chili pepper extracts on maize seeds. The effects on other species are contradictory; in the species *Lactuca sativa* L. and *Ipomoea purpurea* L., no effects on germination were found; while in *Amaranthus hybridus* L., the response was adverse [29]. Capsaicin is a predominant metabolite in chili pepper [30], due to its chemical nature as a nitrogenous organic compound, perhaps it could serve as a precursor for amino acid synthesis in the activation of germination [24]. Furthermore, the genus *Capsicum* also contains quercetin, an essential compound in triggering multiple plant physiological processes such as germination [31].

2) Development

Based on previous results, onion extracts at 25% and 50% concentration were

evaluated in vigor tests. Analysis of variance detected statistical differences between treatments and control for each of the measured parameters; although between treatments they did not differ considerably. The root length analysis did not meet the normality assumption, so a Box-Cox data transformation with λ = 0.55 was necessary. After transformation, the assumptions were met (Normality: p = 0.11 and Homogeneity of variance: p = 0.54) and statistical differences between treatments were maintained. Despite data transformation, means are reflected based on original data in Table 6. Seed imbibition (I) had a particularly outstanding effect on dry matter weight, almost three times higher than the weight of the untreated control sample (T). Although no differences were detected between treatments, C25 displayed the best results in stem (13.23 cm) and root (22.00 cm) lengths, and the second best in dry matter weight (Table 6).

Table 6. Tukey's test results for mean comparison of plant extract treatments applied to maize seeds for development.

Stem Length (cm)			Root Length (cm)			Dry Matter Weight (mg)		
Treatment	Mean ± SD	Group	Treatment	Mean ± SD	Group	Treatment	Mean ± SD	Group
C25	13.23 ± 1.43	a	**C25**	22.00 ± 1.32	a	**I**	625.35 ± 28.81	a
C50	12.87 ± 1.39	a	**I**	21.94 ± 1.98	a	**C25**	579.75 ± 28.57	a
I	12.44 ± 2.54	a	**C50**	20.00 ± 1.99	a	**C50**	530.70 ± 47.78	ab
T	6.30 ± 2.01	b	**T**	14.50 ± 1.66	b	**T**	242.14 ± 40.75	b

Amino acids in onion, such as tryptophan, methionine, and phenylalanine are precursors of phytohormones such as auxins, ethylenes, and salicylic acid respectively; all of them are relevant in plant development [26] [32]. Another interesting result in development tests was the notable difference in dry matter weight with the imbibition control, a result that was not so as pronounced in the other study variables where treatments did not differ between them practically from each other. This result implies modifications in distribution and root development through pre-hydration. Reference [33] with wheat seeds showed that seed imbibition produces faster water absorption and more efficient recovery of metabolic activity; activates biochemical mechanisms of cell repair, increases RNA content and DNA replication; and, particularly in the coleoptile and roots, promotes cell division.

3.1.3. Chemical Compounds

ASA (0.06%) and $CaCl_2$ (3%) were the best solutions among those evaluated in this experiment, increasing TG compared to the control with better standard deviations and confidence intervals. ASA considerably improved synchronization from a high DG (62%) and it also reduced T_{50} (39.6 h). $CaCl_2$, beyond achieving the highest TG, did not significantly improve other control sample indicators such as T_{50} or DG (Table 7). The temporal germination pattern does not have important deviations from the typical maize CG curve. Germination is accelerated

from when it begins on the second day until the fourth day, after which there are no important increases (Figure 3).

Table 7. Germination parameters of maize seeds subjected to different chemical compound solutions and control.

Variable	Solutions (Concentration %)					
	0	0.5	0.06	2	1	1
	Control	NaCl	ASA	$CaCl_2$	KNO_3	$CuSO_4$
TG (%)	76	47	81	65	67	79
DG (%)	54	39	62	51	37	43
T_{50} (h)	41.0	38.5	39.6	39.3	45.7	46.0
D_o-D_f (d)	6	3	6	7	3	6
SD	±9.55	±16.12	±6.00	±11.48	±8.86	±8.24
CI	9.37	15.80	5.87	11.25	8.69	8.08
	0	1	0.12	3	3	2
TG (%)	76	64	71	84	66	81
DG (%)	55	47	47	53	42	26
T_{50} (h)	40.0	40.3	42.1	43.0	42.8	62.3
D_o-D_f (d)	6	7	6	6	6	7
SD	±9.55	±10.00	±8.86	±4.61	±15.09	±11.77
CI	9.37	9.79	8.69	4.52	14.79	11.53

TG: total germination; DG: 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.

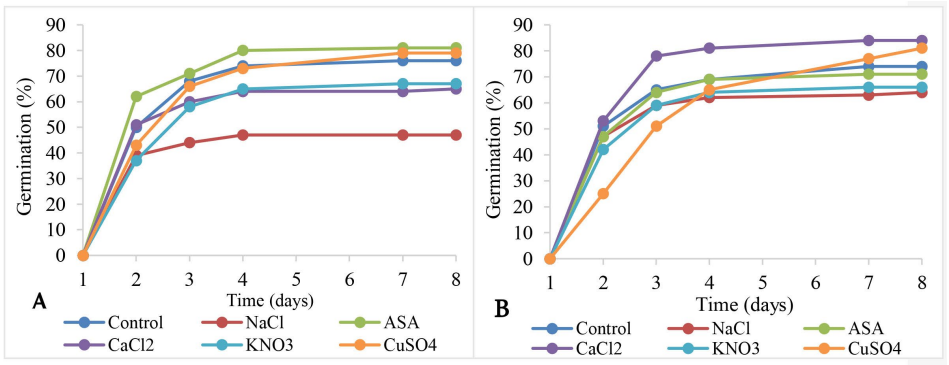

Figure 3. Germination patterns of maize seeds placed to germinate in different solutions of chemical compounds and control. Cumulative germination percentages (**A** and **B**) throughout the indicated times. (**A**) Solution of 0.5% NaCl, 0.06% ASA, 2% $CaCl_2$, 1% KNO_3 and 1% $CuSO_4$. (**B**) Solutions of 1% NaCl, 0.12% ASA, 3% $CaCl_2$, 3% KNO_3 and 2% $CuSO_4$.

ASA has been widely studied as a promoter of plant development in maize and other species under saline stress conditions, with a fundamental role in photosynthesis regulation and osmotic adjustment [34] [35]. Its positive effect on germina-

tion synchronization observed here is likely a direct consequence of the time and concentration at which it is employed, in addition to certain sensitivity of the species [36].

Regarding $CaCl_2$, it has been used in previous studies to prepare maize seeds for drought and soil salinity [37] [38], and it has improved the vigor and growth of cereals [39]. The low water potential of solutions conditions the onset of pre-germinative metabolic processes but prevents germination. When treated seeds are sown, they have rapid and uniform germination even under saline stress conditions, perhaps due to the influence of Ca^{2+} ions on membranes and improved antioxidant proteins such as superoxide dismutase enzyme [40].

3.1.4. Biopriming with Maize Bacteria

Analysis of variance of vigor parameters with maize strains detected statistical differences for root length and dry matter weight, while no differences were detected for stem length. The analysis of variance of treatments for root length required a Box-Cox data transformation, with $\lambda = 0.80$, since the assumption of Homogeneity of variances ($p = 0.04$) was not met. Despite transformation, statistical differences were maintained, now with assumptions met (Normality: $p = 0.11$ and Homogeneity of variance: $p = 0.07$).

The most stable results were presented with strain 3L, being the second-best treatment in both parameters and having no significant differences from better treatments. Strains 1M and 4L were effective at improving stem length (10.50 cm) and dry matter weight (326.00 mg), respectively (**Table 8**).

Table 8. Tukey's Test results for mean comparison to evaluate maize seed inoculation with maize root-associated bacteria on development.

Stem Length (cm)*		Root Length (cm)			Dry Matter Weight (mg)		
Treatment	**Mean ± SD**	**Treatment**	**Mean ± SD**	**Group**	**Treatment**	**Mean ± SD**	**Group**
5L	10.60 ± 1.08	**1M**	10.5 ± 0.68	a	**4L**	326.00 ± 20.08	a
4L	10.10 ± 1.02	**3L**	9.70 ± 0.97	ab	**3L**	304.00 ± 20.49	ab
4m	10.10 ± 0.54	**4L**	9.30 ± 0.83	ab	**1M**	300.00 ± 29.15	ab
3L	10.00 ± 1.27	**4M**	8.90 ± 0.22	ab	**5L**	298.00 ± 14.83	ab
1M	10.00 ± 1.17	**5L**	8.70 ± 1.20	ab	**4M**	290.00 ± 31.62	ab
13L	9.00 ± 1.41	**13L**	8.50 ± 0.40	bc	**13L**	275.00 ± 20.81	bc
T	8.62 ± 0.47	**T**	6.80 ± 0.67	c	**T**	230.50 ± 14.14	c

The values in the table are shown based on the means of the original data; *In the case of Stem Length, no statistically significant differences were detected.

Strains of *P. fungipugnans* (3L and 4L) and *P. hunanensis* (1M) had the most outstanding effects. According to [41], Plant Growth Promoting Bacteria (PGPB) of the genus *Pseudomonas* are fundamental in the maize rhizosphere microbiome and maintain high abundance regardless of the genotype. Regarding their effects, inoculation of floury maize seeds produced seedlings with better capacity to fix

nitrogen, solubilize phosphorus, and produce compounds that stimulate plant development, which corresponded to longer roots and taller plants [42]. In this work, statistically significant results were not detected in stem length; however, root length and especially dry matter weight evidence modifications of root architecture and an increase of secondary roots [43] which is a key component of seedling vigour.

Regarding the particular action of the species in question; *P. hunanensis* (1M) is a species discovered only in 2014 [44]. Its main attributes described are the ability to degrade hydrocarbons in contaminated soils, as well as to reduce the autotoxic effect of phenolic acids also by degradation. In these types of situations, it has demonstrated effectiveness as a growth promoter in melon, strawberry, and poplar [45]. In tomato, antifungal effect against *Fusarium oxysporum* and germination and development stimulator under salinity conditions has been proven [46]. Its positive effects under non stress conditions, expands the beneficial roles of this species. On the other hand, *P. fungipugnans* (3L) is a species on which the promoting effects validated in this work have not been documented; a possible antifungal effect has been hypothesized; but much remains to be discovered about this species since it has been recently characterized [47].

3.1.5. Biopriming with Tomato Bacteria

Evaluation of *Bacillus* strains obtained from tomato roots (treatments RJ12, RJ13, RJ16, and RJ25) in maize seedling development showed no statistically significant differences in any of the analyzed variables. For root length, because the normality assumption was not met in the original data ($p = 0.03$), a logarithmic transformation was applied that corrected data distribution ($p = 0.13$). However, the analysis still showed no significant differences between treatments and control (**Table 9**).

Table 9. Development test results expressed as means of the evaluation of maize seeds inoculated with bacteria from tomato roots.

Stem Length (cm)		Root Length (cm)		Dry Matter Weight (mg)	
Treatment	**Mean ± SD**	**Treatment**	**Mean ± SD**	**Treatment**	**Mean ± SD**
T	12.90 ± 1.73	**T**	10.07 ± 1.95	**T**	349.66 ± 74.83
RJ12	12.91 ± 1.78	**RJ12**	11.65 ± 3.07	**RJ12**	372.37 ± 95.17
RJ13	13.85 ± 3.13	**RJ13**	12.15 ± 1.54	**RJ13**	371.57 ± 68.58
RJ16	12.55 ± 1.94	**RJ16**	12.80 ± 1.52	**RJ16**	395.25 ± 62.94
RJ25	12.08 ± 0.71	**RJ25**	11.92 ± 1.46	**RJ25**	407.40 ± 61.23

All strains from tomato roots were of the genus *Bacillus* and none of them displayed relevant results. Several studies verified the mixed effects of maize seed inoculation with species of this genus. The complex interactions between *Bacillus* species and agroecosystem components could justify their inability to obtain the desired effects. On the other hand, the multifunctional nature of these bacteria and their application methods determine their future impact, in close correlation

with the agroecosystem [48]. It has been proven that bacterial strains associated with a certain species can interact negatively with others [49]. Therefore, the lack of effect from the tomato-associated *Bacillus* strains on maize observed here likely reflects this host-specificity. Additionally, there are also possible long-term consequences or effects at the cellular level, but this type of variables was not included in this work.

4. Conclusions

Treatments with chemical compounds proved to be the most effective for germination, with acetylsalicylic acid (ASA at 0.06%) and calcium chloride ($CaCl_2$ at 3%) being the most relevant among those tested. Plant extracts demonstrated a hormetic effect, with low and moderate concentrations being stimulating, and onion extract at 50% concentration providing the optimal balance for germination metrics. Conversely, hydropriming treatment was counterproductive, evidencing the criticality of precise application time. In plant development, onion extracts (25% and 50%) doubled stem length and improved root development, while imbibition in water achieved important biomass accumulation. Biopriming showed host-bacteria specificity: strains of *Pseudomonas fungipugnans* and *Pseudomonas hunanensis* obtained from maize roots improved root length and dry weight, while *Bacillus* strains from tomato roots produced no significant effects.

The results confirm that concentration and application timing are critical factors for the success of pre-germination treatments. Plant extracts and chemical compounds represent viable and economical alternatives for improving seed quality, and the hormetic response should be considered in the design of future treatments. The specific relationship between bacteria and hosts reveals evolutionary mechanisms of plant-microorganism coexistence that can be harnessed to produce more effective biofertilizers and biostimulants derived from the bacterial species best associated with the target plant. These findings contribute to the development of sustainable technologies for maize seed conditioning, offering alternatives to improve agricultural productivity from the early stages of cultivation. However, since this study was conducted under optimal and controlled conditions, the most promising treatments, specifically onion extract, acetylsalicylic acid, and native *Pseudomonas* strains, must be validated under field conditions and against relevant abiotic stresses to assess their practical agronomic value and their potential to improve resilience.

Acknowledgements

This work was supported by funds from Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) through a scholarship, as well as the facilities and equipment of COLPOS (Colegio de Postgraduados-Montecillo Campus).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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