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DELAY AND IMPROVEMENT OF TOMATO SEED GERMINATION BY OSMOPRIMING UNDER AMBIENT AND ACCELERATE STORAGE CONDITIONS

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Abstract: It is known that tomato seeds are highly valuable products to be exported. Some of the seeds have low quality after the post-harvesting process and while being kept in storage. However, these low-quality seeds can be improved through seed priming. This experiment aimed to delay and improve the germination of tomato seeds under two different conditions: Ambient and accelerated conditions. The experiment followed a Completely Randomised Design (CRD) with four replicates. This method is to prepare the tomato seeds aged 0 to 14 months soaked in seven various chemical solutions before and after storage for five hours, followed by drying. The seven solutions included Reverse Osmosis (RO) water, 2% KNO_3, 500 ppm GA_3, 2% vitamin C, 2% H_2O_2, 50 ppm Ethephon, and PEG 6000 (-1.5 MPa). The results indicated that seed priming with 2% vitamin C before storage resulted in the highest germination percentage compared to other treatments. At the same time, post-storage priming with 2% H_2O_2 led to the highest germination percentage when evaluated under both laboratory and greenhouse conditions. Additionally, seed priming with 2% vitamin C and 2% H_2O_2 increased catalase enzyme activity, improving seed quality and preventing deterioration.

Keywords: Seed priming, germination percentage, solutions, delay, improvement.

Introduction

Seeds are an extremely crucial factor in agricultural production since seeds are the starting point of the process. If farmers decide to use high-quality seeds, their chances of success in the agricultural production business will increase. Notably, according to export standards, high-quality seeds are included with a high seed germination rate and high seed vigour (Sikhao, 2016). However, the current production of tomato seeds still faces critically significant problems. Some seeds have a reduced germination rate and poor seed vigour due to small size, limited stored food, and improper post-harvest processing and storage, leading to losses for farmers and seed exporting companies (Siri *et al.*, 2013).

Seed production in Thailand has over 100 seed business firms that produce seeds for export. The prices of produced seeds range from 2,000 to 200,000 Baht per kg under the two conditions, including seed purity at the level of 98% to 99% and a seed germination rate of 90% or more (National Biotechnology Policy Committee, 2016). Therefore, seed production provides high returns to produce farmers and seed exporting companies. Nevertheless, the current production of tomato seeds still has many problems. These issues hinder the seed trading business such as a low germination rate, poor seed vigour, and improper post-harvest processing and storage. Due to seed deterioration, some seeds are discarded since their quality does not meet the standard requirements in terms of germination and vigour (Thai Seed Association, 2022). As a result, the discarded seeds of at least 10% or more lead to a loss of income for the producers or farmers.

Poor germination or discarded seeds may result from various factors during

the developmental process. This includes fluctuating environmental conditions, including soil fertility, water, sunlight, temperature, and relative humidity during planting, flowering, harvesting, and storage. These fluctuations can cause seeds to deteriorate faster than normal. Due to these environmental fluctuations, metabolism within the seeds stimulates larger amounts of free radicals than normal (Dewal & Pareek, 2004) such as those produced by reduction and oxidation reactions during photosynthesis and respiration (Siriphanich, 2007). The changes or impairments of the biomolecule functions result in the efficiency of various type of enzymes that act as anti-oxidation enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which act as the main mechanism for controlling the amounts of free radicals within the cell (Wang & Huang, 2004). The changes cause the cells to have large amounts of free radicals that are out of balance (Wacharakupt *et al.*, 2006). Therefore, it leads the seeds to undergo deterioration. As a result, the seeds would reduce the ability to germinate in narrow germination and decrease storability (Duangpatra, 1986).

However, as mentioned above regarding seed deterioration, Heydecker *et al*. (1975) discovered that when deteriorated seeds were prepared for germination using the seed priming method (Sveinsdo *et al.*, 2009), their germination rate increased, and they germinated more quickly and uniformly. This has been observed in many plants such as wheat, broccoli, melons, carrots, tomatoes, and peppers (Nascimento & de Aragao, 2004). Moreover, the seed preparation process for germination involves adding moisture to the seeds or soaking the seeds in a solution with an appropriate concentration and temperature for an extended period to cause the seeds to change into various metabolisms that occur internally and prepare for germination. The changes include cell membranes, various deteriorated subcellular membranes having enough time to rearrange (reorganisation of membranes), self-repairing, and lessening and completely eliminating toxins (Gray *et al*., 1990; Bray, 1995). It was also reported that preparation for seed germination experiment decreased the levels of Malondialdehyde (MDA). This index measures the lipid peroxidation reaction, which is harmful to the cells (Bailly *et al.*, 1998). In contrast, it has increased antioxidant enzymes, superoxide dismutase, catalase, glutathione reductase, and antioxidant substances, vitamin C and vitamin E (Bailly *et al*., 2000).

This accomplishment involves many factors such as plant type, variety, age, seed quality, temperature, time, and chemical substances used to soak the seeds, to name a few. Therefore, the study explores (1) The use of various chemical substances in seed priming on biochemical changes, (2) The quality of the tomato seeds deteriorated at different levels, and (3) Post-preparation process for germination indicates the mechanism of change that has occurred and leads to appropriate ways to delay and improve seed quality. In addition, this finding also develops the seed trading business and is usefully applicable for seed business producers as well.

Materials and Methods

Plant Materials

Tomato seeds (*Lycopersicon esculentum* Mill.) cv. Sida was harvested in January 2018, with a shelf life of 0 month and an initial germination rate of 99%. Seed production was conducted in the Department of Agriculture Technology, Faculty of Technology, Maha Sarakham University.

Tomato Seed under Ambient and Accelerate Storage Conditions

For storage under ambient conditions, dried tomato seeds were stored at room temperature (30°C to 32°C) in a controlled environment using 2-mil thick plastic bags. Preparation of seeds for accelerated aging storage: 1 g batches of dried tomato seeds were placed in cloth bags. These bags were then positioned on a wire mesh in an aging jar filled with water, ensuring a 2 cm

water level both below and above the mesh. This setup maintained approximately 100% relative humidity. The sealed jar was incubated in a Takedarika GL-60 aging chamber at 43°C for 96 hours (ISTA, 2013).

Seed Priming

Seed priming treatments on the germination of tomato seeds stored under normal and accelerated aging conditions for 0 to 14 months. Seeds were soaked in seven solutions, including RO water, 2% KNO_3, 500 ppm, 2% vitamin C, 2% H_2O_2, 50 ppm Ethephon, and PEG 6000 (-1.5 MPa) at a water potential of -1.5 MPa. All treatments were conducted at 25°C for 5 hours, followed by drying to reduce the seed moisture content to near the initial level (7% to 8%) (Peeya *et al*., 2014). The changes in seed quality were then assessed both in laboratory conditions and in a greenhouse.

Seed Quality Testing

Tomato seeds stored under ambient conditions were randomly selected and subjected to germination testing using the Top-of-Paper (TP) method. A total of 400 seeds (4 replicates of 100 seeds) were evaluated. The seeds were placed in a germination chamber with alternating temperatures of 28°C to 30°C. Germination was assessed based on two criteria:

(1) Radicle emergence: Seeds were evaluated from day 3 to day 5 after sowing. Radicle emergence was defined as the protrusion of the primary root through the seed coat by at least 5 mm.

(2) Seed germination: Normal seedling emergence was counted daily from day 5 to day 14, according to ISTA (2013).

Consequently, tomato seeds were subjected to a germination test in peat moss-filled trays. A total of 400 seeds (4 replicates of 100 seeds) were evaluated according to ISTA (2013). Germination was assessed based on two criteria:

(1) Hypocotyl emergence: Emergence was evaluated from day 3 to day 5 after sowing. Hypocotyl emergence was defined as the protrusion of the hypocotyl above the growing medium by at least 2 mm.

(2) Seed germination: Normal seedling emergence was counted daily from day 5 to day 14.

$$\text{Germination (\%)} = \frac{\text{Total normal seedling}}{\text{Total seed}} \times 100$$

Seed radicle emergence under laboratory and greenhouse conditions. A total of 400 tomato seeds (4 replicates of 100 seeds) that had undergone germination preparation were randomly selected. Seed radicle emergence was assessed using the TP method. The seeds were then placed in a germination chamber with alternating temperatures and the number of radicles that emerged was counted on days 3, 4, and 5 after sowing. Correspondingly, the percentage of radicle emergence was calculated (Siri & Diumkhunthod, 2013).

Catalase Activity

Catalase activity of tomato seeds ambient stored for 14 months was measured to compare the enzyme activity among three seed treatments: (1) Untreated seeds, (2) seeds primed before storage, and (3) seeds primed after storage. Four replicates of 1 g of 14-month-old tomato seeds were weighed and placed in a chilled mortar containing liquid nitrogen. 10 ml of 0.1 M phosphate buffer (pH 7.8) containing 0.2 g Polyvinylpyrrolidone (PVP), 10 mM β-mercaptoethanol, 10 mM KCl, 1 mM MgCl2, and 1 mM EDTA was added and homogenised. Subsequently, the homogenate was centrifuged at 10,000 rpm for 30 minutes at 4°C and filtered through Whatman No. 1 filter paper. The clear supernatant (crude enzyme) was stored at 4°C for subsequent determination of antioxidant enzyme activity. Catalase activity was measured according to the method of Kaewnaree *et al*. (2007).

Results and Discussion

The Quality of Tomato Seeds Stored under Ambient Conditions

Seed quality declined over time when stored under ambient conditions. While seeds stored for 0 month had the highest germination and seedling emergence rates (99%) (Table 1), seeds stored for 2 to 6 months still exhibited high germination (94% to 96%), and seedling emergence (88% to 93%) rates. However, at 12 months, germination and seedling emergence rates decreased significantly. Greenhouse results revealed similar emergence rates for seeds stored for 0, 4, and 8 months. Nonetheless, normal seedling development decreased significantly for seeds stored for 8 months. At 12 months, emergence and normal seedling development declined rapidly. These findings indicate that seed viability began to decline after 8 months of storage and deteriorated significantly at 12 months.

The Quality of Tomato Seeds Stored under Accelerated Aging Conditions

Seed germination was assessed after subjecting seeds to accelerated aging under controlled (laboratory) and uncontrolled (greenhouse) conditions. Results revealed that germination of seeds subjected to accelerated aging decreased progressively with increasing aging duration. In the laboratory, seeds that were not aged (0 months) exhibited 100% germination and a maximum of 97% normal seedling emergence, which differed significantly from all other aged seed lots (Table 2). However, when considering the percentage of seedlings developing from roots, seeds aged for 4 to 8 months demonstrated high germination rates of 94% to 96% and normal seedling emergence rates of 88% to 93%, resulting in a 93.6% to 96.9% conversion rate from germination to normal seedling emergence. After 12 months of aging, seed germination decreased to 88%, and normal seedling emergence dropped to 75%, resulting

Table 1: Radicle emergence, hypocotyl emergence, and normal seedlings of tomato seeds tested under laboratory and greenhouse storage conditions

Storage Period (months)	Laboratory Conditions			Greenhouse Conditions		
	Radical Emergence[1/] (%)	Normal Seedling (%)	Abnormal Seedling (%)	Hypocotyl Emergence[2/] (%)	Normal Seedling (%)	Abnormal Seedling (%)
0	99a[3/]	99a (99.0)[4/]	0.0	92a	92a (97.9)[5/]	2.1
2	96b	93a (96.9)	3.1	91a	91a (100.0)	0.0
4	95b	90ab (94.7)	5.3	86ab	84b (95.5)	4.5
6	94b	88b (93.6)	6.4	84ab	82b (98.8)	1.2
8	90b	85 (90.0)	10.0	83b	80bc (95.1)	4.9
10	87c	87b (87.2)	12.8	76c	73c (94.4)	5.6
12	80d	75c (93.3)	6.7	75c	70c (76.5)	23.5
14	75e	74d (85.2)	14.8	68d	64d (81.1)	18.2
F-test	**	**	-	**	**	-
C.V. (%)	6.91	7.09	-	8.11	9.68	-

** significantly different at $p \leq 0.01$.

[1/] Radical emergence: The root has emerged from the seed coat and is 5 millimetres long.

[2/] Hypocotyl emergence: Seedlings have emerged 5 millimetres from the growing medium.

[3/] Means in the same column with different letters are statistically different at $p \leq 0.01$ by DMRT.

[4/] Percentage of normal seedlings/root germination.

[5/] Percentage of normal seedlings/seedlings that emerged from peat moss.

in an 85.2% conversion rate from germination to normal seedling emergence. At the same time, greenhouse trials revealed that non-aged seeds (0 month) and seeds aged 4 to 8 months did not exhibit statistically significant differences in seedling emergence, with 88% to 94% emergence. However, when monitoring normal seedling development, seeds aged for 8 months demonstrated a statistically significant decrease in normal seedling development compared to non-aged seeds and those aged for 4 months. Furthermore, after 12 months of aging, seedling emergence declined rapidly to 72%, and normal seedling development decreased to 39%. This results in a 54.2% conversion rate from emergence to normal seedling development (Table 2). Accordingly, these findings suggest that the initial decline in germination occurred at 8 months of aging, with a critical decline at 12 months.

The Results of the Effect of Seed Priming Before and After Storage on the Delay and Improvement of Germination in Tomato Seeds

Seed quality after seed priming and storage at different ages was evaluated under laboratory conditions. The effects of pre-storage priming with various chemicals on the germination of tomato seeds stored for different durations were investigated (0, 8, 10, 12, and 14 months). Results indicated significant differences in germination rates among treatments. In particular, seeds primed with 2% vitamin C (Table 3) consistently exhibited the highest germination rates. Statistical analysis confirmed significant differences among all treatments. The optimal priming conditions, including solution concentration, temperature, and duration, increase seed moisture content, and stimulate metabolic activities prior to germination. However, according to Sathiyamoorthy and Nakamura (1995), the process is halted before

Table 2: Radicle emergence, hypocotyl emergence, and normal seedlings of tomato seeds tested under laboratory and greenhouse conditions after accelerated aging

Aging Period (months)	Laboratory Conditions			Greenhouse Conditions		
	Radical Emergence[1/] (%)	Normal Seedling (%)	Abnormal Seedling (%)	Hypocotyl Emergence[2/] (%)	Normal Seedling (%)	Abnormal Seedling (%)
0	100a[3/]	99a (97.0)[4/]	1.0	91a	91a (96.9)[5/]	3.1
2	95b	93a (96.5)	3.5	91a	91a (100.0)	0.0
4	94b	90ab (94.2)	5.8	86ab	84b (95.5)	4.5
6	94b	88b (91.6)	7.4	84ab	82b (98.8)	1.2
8	90b	84b (89.0)	11.0	83b	80bc (95.1)	4.9
10	85c	87b (87.2)	12.8	76c	73c (94.4)	5.6
12	80d	75c (93.3)	6.7	75c	70c (76.5)	23.5
14	72e	71d (85.2)	14.8	68d	64d (81.1)	18.2
F-test	**	**	-	**	**	-
C.V. (%)	7.11	9.18	-	10.11	7.68	-

** significantly different at $p \leq 0.01$.
[1/] Radical emergence: The root has emerged from the seed coat and is 5 millimetres long.
[2/] Hypocotyl emergence: Seedlings have emerged 5 millimetres from the growing medium.
[3/] Means in the same column with different letters are statistically different at $p \leq 0.01$ by DMRT.
[4/] Percentage of normal seedlings/root germination.
[5/] Percentage of normal seedlings/seedlings that emerged from peat moss.

Table 3: Germination percentage of tomato seeds priming with various chemicals and stored under ambient conditions

Treatment	Storage Period (months)										F-test	C.V. (%)
	0		8		10		12		14			
T1	94bA	-	86bB	(8.8%)	84bB	(10.0%)	75bC	(20.9%)	73bC	(23.1%)	**	3.19
T2	98aA	-	90aA	(8.2%)	90aA	(8.2%)	87aB	(10.2%)	82aB	(16.3%)	**	2.59
T3	98aA	-	94aA	(4.1%)	94aA	(4.1%)	93aA	(5.1%)	90aB	(8.2%)	**	7.89
T4	98aA	-	93aA	(5.1%)	93aA	(5.1%)	86aB	(12.2%)	86aB	(12.2%)	**	6.25
T5	99aA	-	96aA	(3.0%)	96aA	(3.0%)	95aA	(4.0%)	94aB	(5.1%)	**	2.16
T6	96aA	-	91B	(5.2%)	91B	(5.2%)	90B	(6.3%)	89aB	(7.3%)	**	9.57
T7	94aA	-	91aA	(3.2%)	91aA	(3.2%)	90aA	(4.3%)	86aB	(8.5%)	**	5.65
T8	94aA	-	87bA	(7.4%)	86bA	(8.5%)	79bC	(16.0%)	75bC	(20.2%)	**	3.19
F-test	**		**		**		**		**			
C.V. (%)	6.60		7.56		4.56		7.42		5.31			

T1 = Non treated, T2 = R.O. water, T3 = 2% KNO_3, T4 = GA_3 500 ppm, T5 = 2% Vitamin C, T6 = 2% H_2O_2, T7 = ethephon 50 ppm, T8 = PEG 6000 (-1.5 MPa).

** significantly different at $p \leq 0.01$.

ABCD Different letters assigned to the same treatment indicate a statistically significant difference at $p \leq 0.01$.

abcd Different lowercase letters assigned within the same storage period indicate statistically significant differences at $p \leq 0.01$.

germination by reducing the seed moisture content to the initial level. This phenomenon, known as seed priming, aligns with the findings of Bewley and Black (1982) and Bradford (1986), who suggested that prior physiological and biochemical changes enable rapid germination with minimal water uptake. Furthermore, seed priming involves controlling water potential at a low or negative level, allowing for Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA), and protein repair and reorganisation and eliminating free radicals and peroxides (Table 3). Priming, a technique involving soaking seeds in a solution with controlled osmotic potential, enhances seed hydration and stimulates metabolic processes prior to germination. Notably, the process is arrested by reducing the seed moisture content to the initial level, allowing for subsequent rapid germination. This is in accordance with the findings of Bewley and Black (1982) and Bradford (1986), who suggested that prior physiological and biochemical changes enable rapid germination with minimal water uptake. The principle of seed priming involves controlling water potential at a low or negative level, thereby slowing down water uptake. This allows for sufficient time for DNA, RNA, and protein repair and reorganisation, as well as membrane repair (Petruzzeli, 1986; Rao *et al*., 1987). Additionally, seed priming helps eliminate free radicals and peroxides.

Consequently, seed quality after seed priming and storage at different ages was evaluated under greenhouse conditions.

The effects of different priming treatments on the root germination of tomato seeds stored for varying durations (0, 8, 10, 12, and 14 months) were investigated. Accordingly, significant differences in root germination rates were observed among treatments. Seeds primed with 2% H_2O_2 consistently exhibited the highest root germination. However, for seeds stored for 8 and 10 months, 2% KNO_3 priming yielded the highest root germination rates while 2% H_2O_2 priming was optimal for seeds stored for 12 and 14 months (Table 4). Note that germination tests were conducted under greenhouse conditions.

Table 4: Germination percentage of tomato seeds priming with various chemicals and stored under accelerated conditions

Treatment	Storage Period (months)										F-test	C.V. (%)
	0		8		10		12		14			
T1	94bA	-	80bB	(14.9%)	74bC	(21.3%)	74bC	(21.3%)	73bC	(21.3%)	**	8.10
T2	98aA	-	82aB	(16.3%)	80aB	(18.4%)	78aC	(20.4%)	73bD	(25.5%)	**	12.41
T3	98aA	-	95aA	(3.1%)	93aB	(5.1%)	86aC	(12.2%)	76bD	(22.4%)	**	9.09
T4	98aA	-	87abB	(11.2%)	87abB	(11.2%)	86aB	(12.2%)	86aB	(12.2%)	**	11.05
T5	99aA	-	83aB	(16.2%)	83aB	(16.2%)	81aC	(18.2%)	80aC	(19.2%)	**	8.36
T6	96aA	-	90aB	(6.3%)	90aB	(6.3%)	90aB	(6.3%)	90aB	(6.3%)	**	9.57
T7	94aA		90aB	(4.3%)	90aB	(4.3%)	86aC	(8.5%)	86aC	(8.5%)	**	5.25
T8	94aA		87bB	(7.4%)	86bB	(8.5%)	76bC	(19.1%)	75bC	(20.2%)	**	4.69
F-test	**		**		**		**		**			
C.V. (%)	7.60		6.16		14.06		8.62		9.01			

T1 = Non treated, T2 = R.O. water, T3 = 2% KNO_3, T4 = GA_3 500 ppm, T5 = 2% Vitamin C, T6 = 2% H_2O_2, T7 = ethephon 50 ppm, T8 = PEG 6000 (-1.5 MPa).

** significantly different at $p \leq 0.01$.

ABCD Different letters assigned to the same treatment indicate a statistically significant difference at $p \leq 0.01$.

abcd Different lowercase letters assigned within the same storage period indicate statistically significant differences at $p \leq 0.01$.

Catalase Activity

Catalase activity in tomato seeds increased significantly with increasing storage duration (Table 5). That is, fresh seeds (0 month) exhibited the lowest catalase activity at 2.47 units/g seed. However, a rapid increase was observed at 8 months, reaching 4.01 units/g seed. Thereafter, the increase gradually continued, with the maximum activity of 8.95 units/g seed recorded at 14 months of storage. Meanwhile, seed priming with 2% vitamin

Table 5: Catalase activity in tomato seeds stored for different durations

Storage Period (months)	Catalase (unit/g seed)
0	2.47g[1/]
2	2.91f
4	3.28f
6	4.11e
8	4.73d
10	6.37c
12	8.33b
14	8.95a
F-test	**
C.V. (%)	7.18

** significantly different at $p \leq 0.01$.

[1/] Means within a column followed by the same letter are insignificant at $p \leq 0.01$ by DMRT.

C before storage decreased catalase activity compared to other treatments (Figure 1) while post-storage priming with 2% H_2O_2 resulted in the lowest catalase activity (Figure 2). In addition, it has been reported that H_2O_2 priming increased catalase activity. Seed preparation with H_2O_2 could efficiently and sustainably reduce tomato salinity stress (Saidi *et al*., 2024). Moreover, priming improves and restores the gene expression of catalase and eliminates the impacts of aging on enzyme activity (Kibinza *et al*., 2006). In essence, catalase continues to help protect cells from Reactive Oxygen Species (ROS) (Romero-Puertas *et al*., 2002). In addition, catalase accumulates in the cytosol simultaneously with hydrogen peroxide localisation during seed priming (Bray, 1995).

Conclusions

The following conclusions can be drawn after examining the effects of various chemical seed treatments on the deterioration and germination of tomato seeds during storage under normal and accelerated aging conditions.

1. **Seed priming with vitamin C:** Seed priming with a 2% vitamin C solution resulted in the highest germination rates under both laboratory and greenhouse conditions. Tomato seeds soaked in the 2% vitamin C solution at 25°C for 5 hours, followed by drying to approximately 7% to 8% moisture content, effectively delaying seed deterioration during storage.

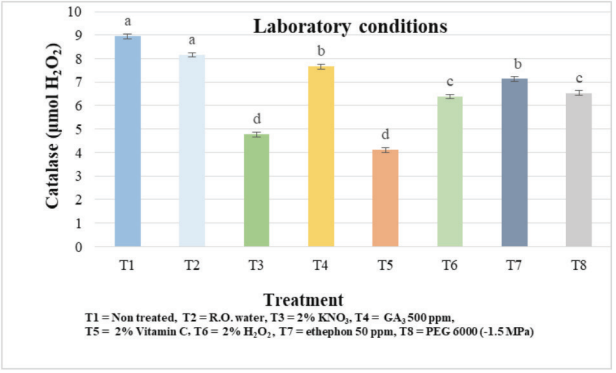

Figure 1: Comparison of catalase activity in tomato seeds priming with different chemicals before storage for 14 months under laboratory conditions

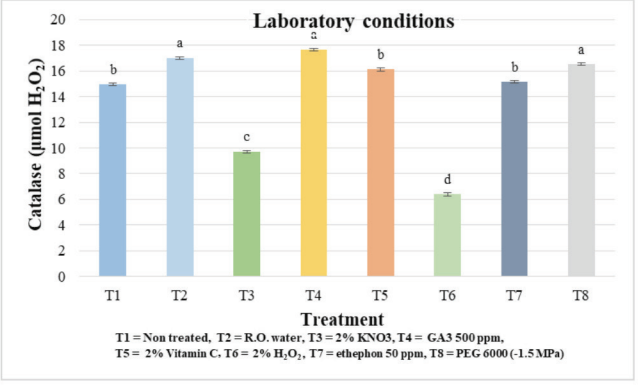

Figure 2: Comparison of catalase activity in tomato seeds priming with different chemicals after storage for 14 months under greenhouse conditions

2. **Improvement with H_2O_2 treatment:** Seed germination of 8-month-old deteriorated seeds was significantly improved by soaking them in a 2% H_2O_2 solution at 25°C for 5 hours. Consequently, the seeds were dried to their initial moisture content (7% to 8%). This treatment enhanced seed germination compared to untreated seeds. The study also revealed that the critical storage duration for maximum seed viability and vigour deterioration was 12 months.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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