

## INVIGORATIVE POTENTIAL ASSESSMENT OF PRIMING AGENTS THROUGH GERMINATION AND VARIOUS SEEDLING ATTRIBUTES OF ARTIFICIALLY AGED SEEDS IN CARROT (*DAUCUS CAROTA* L.)

UZMA ARIF<sup>1</sup>, SADAR UDDIN SIDDIQUI<sup>2</sup>, MUHAMMAD FAREED KHAN<sup>1</sup>  
AND SHAKEEL AHMAD JATOI<sup>2\*</sup>

<sup>1</sup>Department of Plant Breeding and Molecular Genetics, University of Poonch, Rawalakot, Pakistan

<sup>2</sup>Plant Genetic Resources Institute, NARC, Islamabad-45500, Pakistan

\*Corresponding author's email: [sajatoi@gmail.com](mailto:sajatoi@gmail.com)

### Abstract

The decrease in seed viability, a commonly observed phenomenon even in optimal storage conditions, poses a significant challenge, particularly concerning genebank seed collections. Addressing the issue of low viability in genebanks requires dedicated efforts, especially in conducting studies on seed invigoration. This study explored the invigorating effects of KNO<sub>3</sub>, NaCl, KH<sub>2</sub>PO<sub>4</sub>, PEG, salicylic acid, ethanol, GA<sub>3</sub>, and ethrel, both individually and in specific combinations, on artificially aged carrot seeds. When evaluating various priming agents individually, it was noted that GA<sub>3</sub> consistently outperformed others, revealing a remarkable 91.4% increase in germination compared to the control. Following closely behind, KNO<sub>3</sub> demonstrated a 65% improvement in germination. With slight variations, the remaining parameters, namely shoot length (cm), root length (cm), fresh and dry seedling biomass (g) also exhibited a similar trend. On the other hand, KNO<sub>3</sub> when applied in combination with NaCl, salicylic acid, KH<sub>2</sub>PO<sub>4</sub>, PEG and ethanol, it was noted that its combination with NaCl and KH<sub>2</sub>PO<sub>4</sub>, revealed improved germination revealing 6.7% and 20.4% over control, respectively. While the collective application of invigorants resulted in enhanced germination compared to the control, the improvement was either modest or comparable to their individual application. The remaining combinations did not demonstrate superior performance compared to the control group. Among the tested chemical compounds, salicylic acid exhibited neither individual nor combined invigorating potential in carrot seeds. Instead, it proved to be detrimental, as no germination was observed with this compound. When used alone or in combination, KNO<sub>3</sub> emerged as the most versatile invigorating agent, predominantly displaying positive or synergistic effects, with a few exceptions.

**Key words:** Invigoration, Seed priming, Invigorative potential, Germination, Artificial ageing.

### Introduction

The carrot (*Daucus carota*) stands as a crucial root vegetable crop, and in Pakistan, it is cultivated across 28,708 hectares, yielding a total production of 728,137 tonnes in the year 2021 (Anon., 2022). The average carrot yield in the country lags significantly behind that achieved in other global nations. Carrots rank among the top ten economically significant vegetable crops worldwide. In 2021, world production of carrot was 59842322 tonnes, with China producing 18175607 tons of the world total. Other major producers are Uzbekistan 3155745, the United States 1432740, Russia 1303288, Germany 961970 and UK 888851 tonnes (Anon., 2022).

Carrot is a directly seeded vegetable crop, and the plant population relies heavily on seed quality. Numerous factors impact seed yield and quality, such as optimal plant spacing, floral set, planting material, nutrition, the health of the mother plant, root size, and root age (Noor *et al.*, 2020). Aged and old seeds are among prominent factors that is responsible for the poor crop stand of various food crops particularly vegetables. The loss of vigor due to ageing is normally observed as delayed emergence, quite slow growth, over responsive to stress susceptibility. In storage, different external factors affect the seed longevity among which temperature, relative humidity and seed moisture content are sufficiently investigated (Khan *et al.*, 2021). The yield of carrot is much lower in third world countries. One of the main causes is the lack of a good stand establishment and reduced early growth under adverse environment. The

germination and emergence of carrot seeds is often slow and non-uniform under normal as well as stress conditions. Uniformity and percentage of seedling emergence of direct-planted crops have a major impact on final yield and quality (Khan *et al.*, 2016).

Enhancing seed performance can be achieved through pre-sowing seed treatments administered prior to planting in the field. Pre-soaking treatments for seed invigoration have been proven to enhance seed performance, leading to consistent germination, the development of normal and robust seedlings, and a faster, higher rate of germination and emergence across various crops. Additionally, these treatments aid seedlings in thriving under biotic or abiotic stress conditions (Oliveira & Gomes-Filho, 2016). Various seed priming techniques like hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermopriming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices) and biopriming (hydration using biological compounds) are available. However, each treatment has certain advantages and disadvantages as well depending upon plant species, stage of plant development, concentration/dose of priming agent and incubation (Ashraf & Foolad, 2005).

The invigorations studies help develop new strategies to design new biotech-based treatments to modulate and improve seed germination and invigoration under *In vivo* conditions. It also helps to provide an integrated and multi-disciplinary approach to speed up basic and translational

research in seed technology. A comprehensive study was undertaken to determine the invigorative potential of diverse chemicals alone as well as in certain selective combinations under four groups using varying concentrations on artificially aged carrot seed.

### Material and Methods

Morpho-physiological studies on seed invigoration were carried out at the Seed Preservation Laboratory of the Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC) in Islamabad, Pakistan in 2018.

**Seed material:** The aged seeds of only one genotype viz. Indian desert look, provided by Horticultural Research Institute, NARC were used in this experiment under completely randomized design. Seeds were subjected to different priming treatment observations were recorded for germination rate (%), shoot and root length (cm) and fresh & dry seedling biomass (g). Carrot seed was artificially aged by subjecting it to high temperature (40°C) for 48 hours in sealed container under high relative humidity condition.

**Invigorants/priming agents:** Different priming agents which include brine salt, inorganic salt, alkali metal, alcohol, hormone viz. NaCl, KNO<sub>3</sub>, Salicylic acid, KH<sub>2</sub>PO<sub>4</sub>, PEG, Ethanol, GA<sub>3</sub> and Etheral alone as well as in different combinations have been used in four separate experiments conducted at different dates. Each experiment was considered as one group (Table 1) and each experiment had its own control treatment. For better understanding of diverse invigorants across groups, relative performance of each parameter in every group was calculated that helped to neutralize the effect of 04

different control-treatments.

**Germination (%):** Seed germination test was performed by using between papers (BP) methods of germination in three replications for each treatment. After 24 hours of soaking, 50 seeds per replication from each treatment without washing were placed on 22 x 23 cm sized paper towel (Victory brand, Shinbashi Paper Company, Shizuoka, Japan). The Germination test was conducted as per ISTA Rules (Anon., 2016). The seeds were placed on surface of double sheet of paper towel which were moistened with distilled water, then covered with another sheet of paper towel. The rolled sandwiches were placed in plastic beakers and covered with polythene bags placed in the incubator maintained at 25±2 for 14 days. The seeds with 0.5cm radicle and plumule were considered as normal germinated. The final count was made on the 14th day. The abnormal seedlings and the dead seeds were removed from substrate. The germination percentage was calculated based on the number of normal seedlings. After 14<sup>th</sup> day germinating seedlings were used to assess the total seedling length and their fresh and dry weight analysis.

**Surface sterilization of seed material:** Commercial Clorox bleach containing 0.5% Sodium hypochlorite was used for surface sterilization of seed. After sterilization seeds were washed twice with distilled water, and then subjected to priming treatments given in Table 1. For each treatment the seeds were soaked in priming media in glass petri dishes at room temperature for 24 hours. The control seeds were soaked in distilled water.

**Table 1. Different chemical compounds used for seeds priming treatments on artificially aged carrot seeds.**

Treatment Group-1	Invigorant and concentrations	Treatment Group-3	Invigorant and concentrations
T1	Control (distilled water)	T1	Control (distilled water)
T2	NaCl 10%	T2	PEG 5%
T3	NaCl 20%	T3	PEG 10%
T4	NaCl 30%	T4	PEG 15%
T5	Salicylic acid 0.1%	T5	Ethanol 0.5%
T6	KNO <sub>3</sub> 0.1%	T6	Ethanol 1%
T7	NaCl 10% + 0.1 KNO <sub>3</sub>	T7	Ethanol 2%
T8	NaCl 20% + 0.1 KNO <sub>3</sub>	T8	PEG 5% + 0.1 KNO <sub>3</sub>
T9	NaCl 30% + 0.1 KNO <sub>3</sub>	T9	PEG 10% + 0.1 KNO <sub>3</sub>
T10	NaCl 10% + 0.1 Salicylic acid	T10	PEG 15 % + 0.1 KNO <sub>3</sub>
T11	NaCl 20% + 0.1 Salicylic acid	T11	Ethanol0.5% + 0.1 KNO <sub>3</sub>
T12	NaCl 30% + 0.1 Salicylic acid	T12	Ethanol1% + 0.1 KNO <sub>3</sub>
		T13	Ethanol 0.5 % + 0.1 KNO <sub>3</sub>
Group-2		Group-4	
T1	Control (distilled water)	T1	Control (distilled water)
T2	1%	T2	GA <sub>3</sub> 5 ppm
T3	KH <sub>2</sub> PO <sub>4</sub> 0.1%	T3	GA <sub>3</sub> 50 ppm
T4	KH <sub>2</sub> PO <sub>4</sub> 0.6%	T4	GA <sub>3</sub> 100 ppm
T5	KH <sub>2</sub> PO <sub>4</sub> 1% + 0.1 KNO <sub>3</sub>	T5	GA <sub>3</sub> 150ppm
T6	KH <sub>2</sub> PO <sub>4</sub> 0.1% + 0.1 KNO <sub>3</sub>	T6	GA <sub>3</sub> 200ppm
T7	KH <sub>2</sub> PO <sub>4</sub> 0.6% + 0.1 KNO <sub>3</sub>	T7	Ethetal 5ppm
T8	KH <sub>2</sub> PO <sub>4</sub> 1% + 0.1 Salicylic acid	T8	Ethetal 50ppm

T9	KH <sub>2</sub> PO <sub>4</sub> 0.1% + 0.1 Salicylic acid	T9	Etheral100ppm
T10	KH <sub>2</sub> PO <sub>4</sub> 0.6% + 0.1 Salicylic acid	T10	Etheral 150ppm
		T11	Etheral 200ppm

Table 2. Effect of multitude of invigorating agents on germination and other seedling traits in carrot.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh seedling biomass (g)	Dry seedling biomass (g)
<b>Group-1</b>					
T1 Control	53.33 bd	4.50 ac	7.80 a	0.318 ab	0.0117 be
T2 NaCl 10%	48.00 cf	3.60 bd	5.50 d	0.267 bc	0.0070 eg
T3 NaCl 20%	69.33 ab	4.57 ab	7.73 ab	0.381 ab	0.0170 ab
T4 NaCl 30%	66.67 bc	3.90 ad	5.87 cd	0.326 ab	0.0143 bd
T5 Salicylic acid	0.000 g	0.00 f	0.00 e	0.000 e	0.0000 h
T6 KNO <sub>3</sub>	88.00 a	5.03 a	7.20 ac	0.435 a	0.0157 ac
T7 NaCl 10% + 0.1% KNO <sub>5</sub>	44.00 df	3.50 be	5.27 d	0.296 ac	0.0093 dg
T8 NaCl 20% + 0.1% KNO <sub>3</sub>	57.33 bd	4.10 ac	6.23 bd	0.289 ac	0.0110 cf
T9 NaCl 30% + 0.1% KNO <sub>5</sub>	69.33 ab	3.77 bd	5.87 cd	0.352 ab	0.0207 a
T10 NaCl 10% + 0.1% Salicylic acid	50.67 be	2.80 de	4.73 d	0.276 bc	0.0053 fh
T11 NaCl 20% 0.1 % Salicylic acid	32.00 ef	2.33 e	4.97 d	0.083 de	0.0037 gh
T12 NaCl 30% 0.1% Salicylic acid	29.33 f	3.33 ce	5.17 d	0.164 cd	0.0053 fh
Mean square (Treatment)	1587.39*	5.23422*	12.3417*	0.04690*	0.0001119*
Mean square (Error)	128.44	0.50389	0.8539	0.00783	0.0001155
<b>Group-2</b>					
T1 Control	58.67 a	4.00 bc	8.97 ac	0.2810 b	0.0120 ab
T2 KH <sub>2</sub> PO <sub>4</sub> 1%	70.67 a	4.67 ab	9.80 ab	0.3417 ab	0.0130 ab
T3 KH <sub>2</sub> PO <sub>4</sub> 0.1%	70.67 a	3.40 c	9.40 ab	0.3093 ab	0.0097 bc
T4 KH <sub>2</sub> PO <sub>4</sub> 0.06%	68.00 a	4.20 ab	9.27 ab	0.3277 ab	0.0143 ab
T5 KH <sub>2</sub> PO <sub>4</sub> 1% + 0.1% KNO <sub>3</sub>	65.33 a	4.67 ab	9.80 ab	0.3343 ab	0.0123 ab
T6 KH <sub>2</sub> PO <sub>4</sub> 0.1% + 0.1% KNO <sub>3</sub>	72.00 a	4.00 bc	8.43 bd	0.3593 ab	0.0157 ab
T7 KH <sub>2</sub> PO <sub>4</sub> 0.06% + 0.1% KNO <sub>3</sub>	74.67 a	4.30 ab	10.47 a	0.3870 a	0.0173 a
T8 KH <sub>2</sub> PO <sub>4</sub> 1%+0.1% Salicylic acid	10.67 b	4.87 a	6.77 de	0.0630 c	0.0027 d
T9 KH <sub>2</sub> PO <sub>4</sub> 0.1% + 0.1% Salicylic acid	14.00 b	3.30 c	7.07 ce	0.0673 c	0.0027 d
T10 KH <sub>2</sub> PO <sub>4</sub> 0.06% + 0.1% Salicylic acid	12.33 b	02.3 d	6.07 e	0.0670 c	0.0040 cd
Mean square (Treatment)	2403.79*	1.87111*	6.58033*	0.05281*	0.00008848*
Mean square (Error)	96.53	0.20533	1.30033	0.00351	0.00001533
<b>Group-3</b>					
T1 Control	33.3 ac	4.27 <sup>NS</sup>	5.93 <sup>NS</sup>	0.1923 ac	0.0327 ab
T2 PEG 5%	37.3 ab	3.87	5.33	0.2273 ab	0.0123 ab
T3 PEG 10%	48.0 a	4.30	5.97	0.2523 a	0.0107 ab
T4 PEG 15%	29.3 bc	4.63	6.77	0.1820 ac	0.0063 b
T5 Ethanol 0.5%	10.7 d	3.93	4.73	0.0487 e	0.0047 b
T6 Ethanol 1%	21.3 cd	3.53	5.33	0.1250 ce	0.0523 a
T7 Ethanol 2%	24.0 bd	3.67	4.50	0.1157 ce	0.0090 b
T8 PEG 5% + 0.1 KNO <sub>3</sub>	26.7 bc	4.40	6.73	0.1287 ce	0.0050 b
T9 PEG 10% + 0.1 KNO <sub>3</sub>	24.0 bd	4.60	5.60	0.1460 bd	0.0080 b
T10 PEG 15% + 0.1 KNO <sub>3</sub>	18.7 cd	4.63	6.53	0.1097 ce	0.0060 b
T11 Ethanol 0.5% + 0.1 KNO <sub>3</sub>	28.0 bc	3.97	5.93	0.0713 de	0.0073 b
T12 Ethanol 1% + 0.1 KNO <sub>3</sub>	21.3 cd	3.83	6.00	0.0553 e	0.0037 b
T13 Ethanol 2% + 0.1 KNO <sub>3</sub>	28.0 bc	4.00	5.40	0.1630 ac	0.0080 b
Mean square (Treatment)	253.470*	0.41688	1.49021	0.01185*	0.0005878
Mean square (Error)	84.513	0.70872	2.21205	0.00284	0.0006306
<b>Group-4</b>					
T1 Control	18.67 de	5.40 b	6.23 ab	0.1133 cd	0.0037 c
T2 GA <sub>3</sub> 5ppm	33.33 ad	6.63 ab	4.50 b	0.1333 bd	0.0067 ac
T3 GA <sub>3</sub> 50ppm	45.33 a	5.97 b	6.67 a	0.2583 a	0.0123 a
T4 GA <sub>3</sub> 100ppm	44.00 ab	5.13 b	6.10 ab	0.2457 ab	0.0097 ab
T5 GA <sub>3</sub> 150ppm	24.00 ce	10.80 a	5.90 ab	0.1503 ad	0.0060 bc
T6 GA <sub>3</sub> 200ppm	32.00 ad	4.73 b	5.20 ab	0.2050 ac	0.0077 ac
T7 Etheral 5ppm	28.00 be	4.77 b	5.77 ab	0.1203 cd	0.0047 bc
T8 Etheral 50ppm	14.67 e	5.07 b	5.43 ab	0.0803 d	0.0033 c
T9 Etheral 100ppm	37.33 ac	4.17 b	5.47 ab	0.1580 ad	0.0067 ac
T10 Etheral 150ppm	24.00 ce	4.33 b	6.00 ab	0.1030 cd	0.0050 bc

T11 Etheral 200ppm	21.33 ce	4.80 b	5.10 ab	0.0777 d	0.0037 c
Mean square (Treatment)	302.933*	10.3576	1.10364	1.10364*	0.0000231
Mean square (Error)	91.636	6.1988	1.59515	1.59515	0.0000123

**Table 3. The relative change in seedling parameters in response to the application of different invigorants in carrots as compared to the control.**

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh seedling biomass (g)	Dry seedling biomass (g)
<b>Group-1</b>					
T1 Control	0.0	0.0	0.0	0.0	0.0
T2 NaCl 10%	-9.94	-20.37	-29.37	-16.14	-41.67
T3 NaCl 20%	30.08	1.50	-0.76	19.71	41.39
T4 NaCl 30%	25.08	-13.60	-25.02	2.41	16.67
T5 Salicylic acid	-100.00	-100.00	-100.00	-100.00	-100.00
T6 KNO <sub>3</sub>	65.10	11.95	-7.66	36.90	30.56
T7 NaCl 10% + 0.1% KNO <sub>3</sub>	-17.45	-22.57	-32.43	-6.92	-21.67
T8 NaCl 20% + 0.1% KNO <sub>3</sub>	7.50	-9.15	-20.05	-9.01	-8.33
T9 NaCl 30% + 0.1% KNO <sub>3</sub>	30.02	-16.42	-24.33	10.80	-2.78
T10 NaCl 10% + 0.1% Salicylic acid	-4.88	-38.19	-39.32	-13.31	-56.94
T11 NaCl 20% 0.1% Salicylic acid	-39.96	-48.15	-36.61	-74.00	-69.44
T12 NaCl 30% 0.1% Salicylic acid	-45.03	-26.54	-34.12	-48.53	-55.56
<b>Group-2</b>					
T2 KH <sub>2</sub> PO <sub>4</sub> 1%	20.4	16.8	9.3	21.6	8.3
T3 KH <sub>2</sub> PO <sub>4</sub> 0.1%	20.4	-15.0	4.8	10.1	-19.2
T4 KH <sub>2</sub> PO <sub>4</sub> 0.06%	15.8	5.0	3.3	16.6	19.2
T5 KH <sub>2</sub> PO <sub>4</sub> 1% + 0.1% KNO <sub>3</sub>	11.3	16.8	9.3	19.0	2.5
T6 KH <sub>2</sub> PO <sub>4</sub> 0.1% + 0.1% KNO <sub>3</sub>	22.7	0.0	-6.0	27.9	30.8
T7 KH <sub>2</sub> PO <sub>4</sub> 0.06% + 0.1% KNO <sub>3</sub>	27.2	7.5	16.7	37.7	44.2
T8 KH <sub>2</sub> PO <sub>4</sub> 1% + 0.1% Salicylic acid	-81.8	21.8	-24.6	-77.6	-77.5
T9 KH <sub>2</sub> PO <sub>4</sub> 0.1% + 0.1% Salicylic acid	-76.1	-17.5	-21.2	-76.0	-77.5
T10 KH <sub>2</sub> PO <sub>4</sub> 0.06% + 0.1% Salicylic acid	-79.6	-42.5	-32.4	-76.2	-66.7
<b>Group-3</b>					
T2 PEG 5%	12.0	-9.5	-10.4	18.2	-63.0
T3 PEG 10%	44.0	0.1	0.1	31.1	-68.3
T4 PEG 15%	-12.0	8.4	14.2	-5.4	-80.2
T5 Ethanol 0.5%	-68.0	-7.4	-20.6	-74.7	-86.0
T6 Ethanol 1%	-36.0	-17.3	-11.1	-35.0	60.7
T7 Ethanol 2%	-28.0	-13.7	-24.5	-39.8	-71.8
T8 PEG 5% + 0.1 KNO <sub>3</sub>	-20.0	3.0	13.2	-33.1	-85.5
T9 PEG 10% + 0.1 KNO <sub>3</sub>	-28.0	8.2	-6.1	-24.1	-74.9
T10 PEG 15% + 0.1 KNO <sub>3</sub>	-44.0	8.4	9.9	-43.0	-82.0
T11 Ethanol 0.5% + 0.1 KNO <sub>3</sub>	-16.0	-6.5	-0.5	-63.0	-77.1
T12 Ethanol 1% + 0.1 KNO <sub>3</sub>	-36.0	-10.2	0.7	0.0	-88.6
T13 Ethanol 2% + 0.1 KNO <sub>3</sub>	-16.0	-6.7	-9.6	-15.3	-76.6
<b>Group-4</b>					
T2 GA <sub>3</sub> 5ppm	78.3	22.8	-27.4	18.0	66.8
T3 GA <sub>3</sub> 50ppm	142.4	10.5	7.5	128.6	207.5
T4 GA <sub>3</sub> 100ppm	135.3	-4.9	-1.6	117.4	141.8
T5 GA <sub>3</sub> 150ppm	28.3	100.0	-4.8	33.0	50.0
T6 GA <sub>3</sub> 200ppm	71.1	-12.3	-16.1	81.4	91.8
T7 Etheral 5ppm	49.7	-11.7	-7.0	6.5	16.8
T8 Etheral 50ppm	-21.6	-6.2	-12.4	-28.9	-16.8
T9 Etheral 100ppm	99.6	-22.8	-11.8	39.8	66.8
T10 Etheral 150ppm	28.3	-19.8	-3.2	-8.8	25.0
T11 Etheral 200ppm	14.1	-11.1	-17.7	-31.2	-8.3

**Seedling growth rate:** For each treatment 30 seeds per replication were used to determine the rate of seedling growth and each treatment was replicated three times. Normal seedling with complete shoots and roots were used for seedling growth rate. The data was recorded on

the 15th day of sowing. For each normally grown seedling, shoot and root length (from point of initiation to its peak) was recorded and then averaged. The weight of fresh and dry seedling biomass of each replication was recorded. The complete seedlings along with seed remains

were dried at 100°C in an oven for 24 hours and expressed as g/seedling.

### Statistical analysis

Data was subjected to analysis for variance using completely randomized design (CRD) for each group of invigorants separately using computer program STATISTIX 8.1 (Anonymus, 2003). Statistical significance and mean separation were done using LSD.

### Results and Discussion

**Impact of NaCl, salicylic acid and KNO<sub>3</sub> on seed invigoration (Group-1):** In this study, NaCl (10%, 20%, 30%), salicylic acid (0.1%), KNO<sub>3</sub> (0.1%), alone and in combination were used to investigate their invigorative effect on aged carrot seeds. The carrot seeds revealed varying responses for various seedlings attributes against different chemicals having invigorative properties. Significant differences for most of the seedling attributes like germination (%), shoot & root length, fresh & dry seedling biomass in carrot were revealed using different invigorants (NaCl, Salicylic acid and KNO<sub>3</sub>) under group-1 (Table 2).

**Germination:** The relative germination over control displayed 65.1% improved germination in carrot seeds, at 0.1% KNO<sub>3</sub> followed by 30.1% improved germination at 20% NaCl alone as well as in combination with 0.1% KNO<sub>3</sub> (Table 3). The lowest improvement in carrot seed germination (7.6%) was noted on T8 (NaCl 20% + 0.1% KNO<sub>3</sub>). The rest of the treatments revealed negative response with poor germination than the control ranging from -4.9 at T10 (NaCl 10% + 0.1% Salicylic Acid) to -84.9% at T12 (NaCl 30% + 0.1% Salicylic Acid). Salicylic acid was the only chemical compound that neither alone nor in-combination has shown any invigorative potential in carrot seeds.

**Shoot length (cm):** The relative shoot length observed at T6 (0.1% KNO<sub>3</sub>) alone was 11.8% longer than control and it was followed by 1.6% recorded at T3 (NaCl 20%) in carrot seedlings (Table 3). The enhanced trend noted in shoot length under various treatments closely resembled the pattern observed during germination. All remaining treatments did not show any improvement in shoot length over control.

**Root length (cm):** The longest root (7.8 cm) was observed at T1 (control) and it was followed by 7.7cm root at T3 (NaCl 20%) and 7.2 cm at T6 (0.1% KNO<sub>3</sub>). Among the other chemical invigorants used, though revealed differential responses for relative root length, however, all the combinations produced short roots as compared to control (Table 3). The root length observed on different combinations ranged between 4.7cm at T10 (NaCl 20% + 0.1Salicylic acid) to 7.8cm at T1 (Control). The relative decrease in root length observed at different combination over control treatment ranged between -0.9% at T3 (NaCl 20%) to -39.4%. at T10 (NaCl 10% + 0.1% Salicylic Acid). The rooting pattern observed at various treatments seems to be negatively affected by various invigorates as none of the

treatment combinations yielded better rooting response over control.

**Fresh seedling biomass (g):** By comparing the mean values for FSB, it was observed that T6 (0.1% KNO<sub>3</sub>) yielded 0.435g FSB that was followed by 0.3807 g recorded at T3 (20% NaCl). Perusal of the data also highlighted some trends e.g., with the increasing concentration from 10% to 20% of NaCl alone, resulted in increased FSB up to 20% that further decline at 30% NaCl. Similarly, with the increase in NaCl concentration from 10% to 30% in combination with KNO<sub>3</sub>, there was a gradual decrease in FSB from 0.296 g to 0.352 g. It was observed that 36% improvement in FSB was observed at T6 (0.1KNO<sub>3</sub>) followed by 19.8% at T3 (20% NaCl) alone (Table 3). Similarly, 10.7% and 2.5% improvement in FSB over control was also noted on T9 (NaCl 30% + 0.1 KNO<sub>3</sub>) and T4 (30% NaCl), respectively.

**Dry seedling biomass (g):** The highest DSB (0.0207 g) was recorded at T9 (NaCl 30% + 0.1% KNO<sub>3</sub>) that was followed by 0.0170 g at T3 (NaCl 20%). Four treatments gave varying invigorative response by revealing high DSB over control and the relative increase ranged from 72.5% to 19.2% at T9 (NaCl 30%+0.1% KNO<sub>3</sub>) and T4 (NaCl 30%), respectively. DSB production trend almost complemented the pattern of FSB production.

Best invigoration (65.1%) was attained by KNO<sub>3</sub> (0.1%) followed 30% at by NaCl and 20.1% at NaCl (30%) indicating that lower concentration of NaCl was ineffective rather negative as data revealed that further higher concentration will have negative impact for NaCl. Salicylic acid alone at the test rate killed the seed potential 50% to '0' and in combination maintained its negative impact; increasing with NaCl concentration whereas KNO<sub>3</sub> was able to improve the invigoration in combination with NaCl at higher concentrations only. The shoot length and root length growth and development behaved in almost similar manner as germination for all the treatment except for KNO<sub>3</sub> when used alone, where the root growth was negatively affected though remained at par with control. It was interesting to note the unusually high concentration 20% of NaCl could enhance invigoration of carrot seedling while further high-level start to decline.

The group of chemicals being used as invigorant in this study revealed useful trends. Three NaCl levels were employed, and it is evident that all seedling parameters displayed lower values at 10% NaCl. These values subsequently increased to levels surpassing even the control at 20% NaCl, only to decline once more at 30% NaCl. This trend remained dominant for all the seedling attributes studied. On the other hand, salicylic acid (0.1%) proved deleterious as no germination was recorded with this chemical. Contrary to salicylic acid, KNO<sub>3</sub> (0.1%) proved to be most effective chemical having invigorative potential. KNO<sub>3</sub> revealed improved performance as compared to control by 28% germination, 90% shoot length, 62% root length, 36% fresh seedling biomass and 31% relative increase in dry seedling biomass. However, the same concentration of KNO<sub>3</sub> as well as salicylic acid

when combined with 03 levels of NaCl, mix behaviour was observed for all the parameters. The common finding in such combinations was noted that none of the treatment combinations yielded better response than the corresponding values at control treatment.

Enhanced carrot seed quality can better be explained by the fact the invigorative treatments normally initiate, repair, and detoxify the internal membrane system of seed and physiologically progress by carrying out certain initial step of germination without radical emergence (Tajbakhsh *et al.*, 2016). The activity of super oxidant dismutase (SOD) and peroxidase normally leads to increased respiration rate that ultimately improved germination and affecting other seedling attributes (Jie *et al.*, 2020). It was observed that NaCl (20%) seemed to be optimum for promoting the action of these compounds in carrot as germination and other attributes were positively influenced at this concentration. However, at the low (10%) as well as high (30%) NaCl concentration depicted a declined response in most of the parameters. At the increased NaCl concentration (30) the process of seed imbibition might be decreased due to the osmotic potential decreased in the growth medium influencing the water potential to decrease (Martinez *et al.*, 2021). This study showed that seed priming with 20% NaCl could be a better choice for carrot seeds. In a similar study on coriander, increased germination as well as growth parameters in Tunisian coriander seeds primed with NaCl compared with unprimed seeds was reported (Elouaer & Hannachi, 2013).

Seed priming with 0.1% KNO<sub>3</sub> also provided very encouraging results (65% enhanced germination) as compared to untreated seeds. The relative increase over control was equivalent to that of 20% NaCl. In another study where onion seed priming with 2% KNO<sub>3</sub> resulted improved behaviour observed through seedling-based parameters (Dong *et al.*, 2014). Piri *et al.*, (2009) also suggested that the priming material KNO<sub>3</sub> gave higher seedling, fresh weight, dry weight, and root volume over control. Nego *et al.*, (2018) also recommended that KNO<sub>3</sub> to be a suitable priming agent to enhance cucumber seed germination.

It was observed that carrot seed priming with 0.1% salicylic acid alone displayed an inhibitory effect on carrot seed germination that resulted in death of all the seeds. Similarly, when it was used in combination with other priming agents in carrot, it also negatively influenced their invigorative potential. Similar inhibitory effect of Salicylic acid on germination of many plants species has also been recorded in different studies (Negi & Prasad, 2001; Chandra *et al.*, 2007; El-Mergawi, 2019). The inhibitory effect of salicylic acid on germination could be attributed to toxic stress (Canakci & Munzuroglu, 2007), however, further studies to explore the inhibitory mechanism of this chemical compound need to be carried out, particularly at very low concentration.

**Seed Invigoration by using KH<sub>2</sub>PO<sub>4</sub> with combination of salicylic acid and KNO<sub>3</sub> (Group-2):** The set of chemicals under group-2 viz. KH<sub>2</sub>PO<sub>4</sub> (1%, 0.1%, 0.06%) alone and in combination with 0.1% KNO<sub>3</sub> and 0.1%

salicylic acid revealed varying responses for various seedling attributes in carrot. The potential invigorants under group-2 displayed significant differences for all the seedling traits including germination (%), shoot & root length, fresh & dry seedling biomass (Table 2).

**Germination (%):** The mean germination ranged from 74.67% to 9.33% and six treatment combinations yielded a better germination response than untreated seedlings. The relative increase in germination over control ranged from 27.2% at T7 (0.06% KH<sub>2</sub>PO<sub>4</sub> + 0.1% KNO<sub>3</sub>) to 11.3% at T5 (1% KH<sub>2</sub>PO<sub>4</sub> + 0.1% KNO<sub>3</sub>) revealing invigorative potential (Table 3).

It was noted that different concentration of KH<sub>2</sub>PO<sub>4</sub> alone and in combination with KNO<sub>3</sub> showed invigorative potential for carrot seeds by inducing germination better than control. When used alone, the highest concentration of KH<sub>2</sub>PO<sub>4</sub> (1%) yielded similar germination (70.67%) to that of recorded at 0.1% of KH<sub>2</sub>PO<sub>4</sub>. The invigorative response was enhanced a bit more when 0.1% KNO<sub>3</sub> was used in combination with KH<sub>2</sub>PO<sub>4</sub>. In this case an increasing trend in germination was observed from 65.33% at T5 (1% KH<sub>2</sub>PO<sub>4</sub> + 0.1% KNO<sub>3</sub>) to 74.67% at T7 (0.06% KH<sub>2</sub>PO<sub>4</sub> + 0.1% KNO<sub>3</sub>). It further confirms that lower concentration of KH<sub>2</sub>PO<sub>4</sub> (0.1% and 0.06%) performs synergistic and better than 1% when used in combination with 0.1% KNO<sub>3</sub>. Salicylic acid negatively affected the germination to zero and behaved as antagonistic to another potential invigorant. Three treatments, where salicylic acid has been used in combination with KH<sub>2</sub>PO<sub>4</sub> revealed poor germination response as compared to control treatment. Salicylic acid induced inhibitory effect in carrot and declining germination drastically and the relative decrease ranged from -79.6% to -84.1% at T9 (0.1% KH<sub>2</sub>PO<sub>4</sub>+0.1% salicylic acid) and T10 (0.06% KH<sub>2</sub>PO<sub>4</sub> + 0.1% salicylic acid), respectively (Table 3). The role of salicylic acid appeared to be deleterious and having no invigorative potential.

**Shoot length (cm):** The longest shoot (4.87 cm) was recorded on T8 (1% KH<sub>2</sub>PO<sub>4</sub> +0.1% Salicylic acid) whereas the shortest shoot (2.67 cm) emerged at T10 (0.06% KH<sub>2</sub>PO<sub>4</sub> + 0.1% Salicylic acid). Three treatments viz. T3 (0.1% KH<sub>2</sub>PO<sub>4</sub>), T9 (0.1% KH<sub>2</sub>PO<sub>4</sub> + 0.1% Salicylic acid) and T10 (0.06% KH<sub>2</sub>PO<sub>4</sub> + 0.1% Salicylic acid), induced poor shoots as compared to control and for rest of the treatments shoot induction was improved than arising from untreated seeds. The relative enhancement in shoot induction ranged from 5% to 21.8% at T4 (0.06% KH<sub>2</sub>PO<sub>4</sub>) and T8 (1% KH<sub>2</sub>PO<sub>4</sub> + 0.1% Salicylic acid), respectively (Table 3). Although salicylic acid remained highly antagonistic in germination whereas it behaved positively in case of shoot length.

It was observed that 1% KH<sub>2</sub>PO<sub>4</sub> alone (T2) as well as in combination with 0.1% KNO<sub>3</sub> (T5) remained indifferent as both revealed similar mean shoot length. Similarly, 0.1% KH<sub>2</sub>PO<sub>4</sub> in combination with 0.1% KNO<sub>3</sub> performed better than the 0.1% KH<sub>2</sub>PO<sub>4</sub> alone. Same was the case with T4 (0.06% KH<sub>2</sub>PO<sub>4</sub>) and T7 (0.06% KH<sub>2</sub>PO<sub>4</sub> + 0.1% KNO<sub>3</sub>). The response in terms of longest shoot length produced at T8 (1% KH<sub>2</sub>PO<sub>4</sub> + 0.1%

Salicylic acid) seemed unusual, as in most of the cases salicylic acid played an inhibitory role in carrot. Further investigation is required to elucidate whether this peculiar response is incidental or indicative of an underlying phenomenon. Nevertheless, in correlation with the germination response, it becomes evident that only approximately 10.67% of germinating seedlings, capable of withstanding salicylic acid stress, exhibited enhanced shoot induction.

**Root length (cm):** The invigorants under group-2 revealed the longest root (10.5 cm) at T7 (0.6%  $\text{KH}_2\text{PO}_4$ + 0.1%  $\text{KNO}_3$ ) that was followed by 9.8 cm at T2 (1%  $\text{KH}_2\text{PO}_4$ ) and T5 (1%  $\text{KH}_2\text{PO}_4$  + 0.1%  $\text{KNO}_3$ ). Among treatments, excluding control, 5 yielded better root induction over control and the relative increase ranged from 16.7% at T7 ( $\text{KH}_2\text{PO}_4$  0.06% + 0.1%  $\text{KNO}_3$ ) to 3.3% at T4 (0.06%  $\text{KH}_2\text{PO}_4$ ; Table 3).

A linear decrease in root length from 9.8cm to 9.3cm was observed with the increase in  $\text{KH}_2\text{PO}_4$  concentration. Similar trend was repeated here again that was observed in case of shoot length, that 1%  $\text{KH}_2\text{PO}_4$  alone (T2) as well as in combination with 0.1  $\text{KNO}_3$  (T5) remained indifferent as both revealed similar mean root length. All the treatment combinations containing salicylic acid resulted in a reduced root length as compared to control, revealing it negative impact on root induction in carrot. However, in the case of shoot length, response on this combination was not same.

**Fresh seedling biomass-FSB (g):** The highest FSB (0.387 g) was recorded at T7 ( $\text{KH}_2\text{PO}_4$  0.06% + 0.1%  $\text{KNO}_3$ ) that was followed by 0.359 g at T6 ( $\text{KH}_2\text{PO}_4$  0.1% + 0.1%  $\text{KNO}_3$ ). Six treatments produced FSB better than control and relative increase ranged between 10.1% to 37.7% at T2 ( $\text{KH}_2\text{PO}_4$  1%) and T7 ( $\text{KH}_2\text{PO}_4$  0.06% + 0.1%  $\text{KNO}_3$ ), respectively (Table 3). It is crucial to highlight that all combinations, whether using  $\text{KH}_2\text{PO}_4$  alone or in conjunction with  $\text{KNO}_3$ , resulted in enhanced FSB compared to the control. However, in all combinations of  $\text{KH}_2\text{PO}_4$  and salicylic acid, FSB was lower than that of the control. Salicylic acid once again exhibited a detrimental effect on FSB.

A linear increase in FSB was noted with the decreasing concentration of  $\text{KH}_2\text{PO}_4$  from 1% to 0.1% when used in combination with 0.1%  $\text{KNO}_3$ . The response was even better when  $\text{KH}_2\text{PO}_4$  was used alone in T3 ( $\text{KH}_2\text{PO}_4$  0.1%) and T4 (0.06%  $\text{KH}_2\text{PO}_4$ ), with the exception of T2 ( $\text{KH}_2\text{PO}_4$  1%) where it was slightly better. This showed that T7 ( $\text{KH}_2\text{PO}_4$  0.06% + 0.1%  $\text{KNO}_3$ ) could be the suitable choice as most of the parameters revealed an improved response over control on this treatment combination.

**Dry seedling biomass-DSB (g):** The highest DSB (0.0173 g) was displayed by T7 ( $\text{KH}_2\text{PO}_4$  0.06% + 0.1%  $\text{KNO}_3$ ) and it was followed by 0.0157 g at T6 ( $\text{KH}_2\text{PO}_4$  0.1%+0.1%  $\text{KNO}_3$ ). Among the treatments, T4 (0.06%  $\text{KH}_2\text{PO}_4$ ) to T8 ( $\text{KH}_2\text{PO}_4$  1%+0.1% Salicylic acid) displayed better DSB production as compared to control. The relative increase in DSB over control ranged between 44.2% to 2.5% at T7 ( $\text{KH}_2\text{PO}_4$  0.06% + 0.1%  $\text{KNO}_3$ ) and T5 (1%  $\text{KH}_2\text{PO}_4$ +0.1%  $\text{KNO}_3$ ), respectively (Table 3). On

the other hand, four treatments revealed the relative decrease over control in DSB that ranged between -66.7 at T8 ( $\text{KH}_2\text{PO}_4$  1%+0.1% Salicylic acid) & T9 (0.1%  $\text{KH}_2\text{PO}_4$  + 0.1% Salicylic acid) to -19.2% at T3 ( $\text{KH}_2\text{PO}_4$  0.1%). A linear increase in DSB was noted with the decreasing concentration of  $\text{KH}_2\text{PO}_4$  from 1% to 0.1% when used in combination with 0.1%  $\text{KNO}_3$ . All the treatment combinations of  $\text{KH}_2\text{PO}_4$  and salicylic acid revealed poor DSB that was even lower than DSB produced at control. Salicylic acid again displayed a negative impact on DSB as well.

Many studies have revealed germination enhancement by priming that improved membrane integrity as well as the increase in protein and nucleic acid synthesis. The enhanced germination due to potassium dihydrogen phosphate might be due to ions observations during priming. Moreover, potassium salt had been reported to raise the ambient oxygen level by making less oxygen available for the citric acid cycle (Bewley *et al.*, 2013). The increasing concentration of potassium and nitrogen plays an important role for the translocations of photo assimilates and this has also been reflected in carrot when we used different concentrations of  $\text{KH}_2\text{PO}_4$  combined with 0.1%  $\text{KNO}_3$ . Seed germination requires various macronutrients, including Potassium nitrogen and phosphorous (Fageria, 2016) and potassium is essential to the performance of multiple enzyme function required for plant growth (Marschner, 2012).

Several other studies have also documented the significance of  $\text{KH}_2\text{PO}_4$  in relative growth rate and all seed germination characteristics in different crops like wheat (Yari *et al.*, 2010) and okra (Sahib *et al.*, 2014). Seed priming with salicylic acid affected germination and other attributes in carrot and the inhibitory effect of salicylic acid on germination of many plants species has also previously recorded (Negi & Prasad, 2001; Chandra *et al.*, 2007; El-Mergawi, 2019). The inhibitory effect of salicylic acid on germination can be attributed to toxic stress (Canakci-& Munzuroglu, 2007).

**Invigorative effect of PEG, ethanol and  $\text{KNO}_3$  on forcedly aged carrot seeds (Group-3):** The set of chemicals in group-3 viz. PEG (5%, 10%, 15%), Ethanol (0.5%, 1%, 2%) and  $\text{KNO}_3$  (0.1%) have been used as invigorant to explore their invigorative effect on aged carrot seeds. The diverse invigorative responses for various seedling attributes in carrot have been observed.

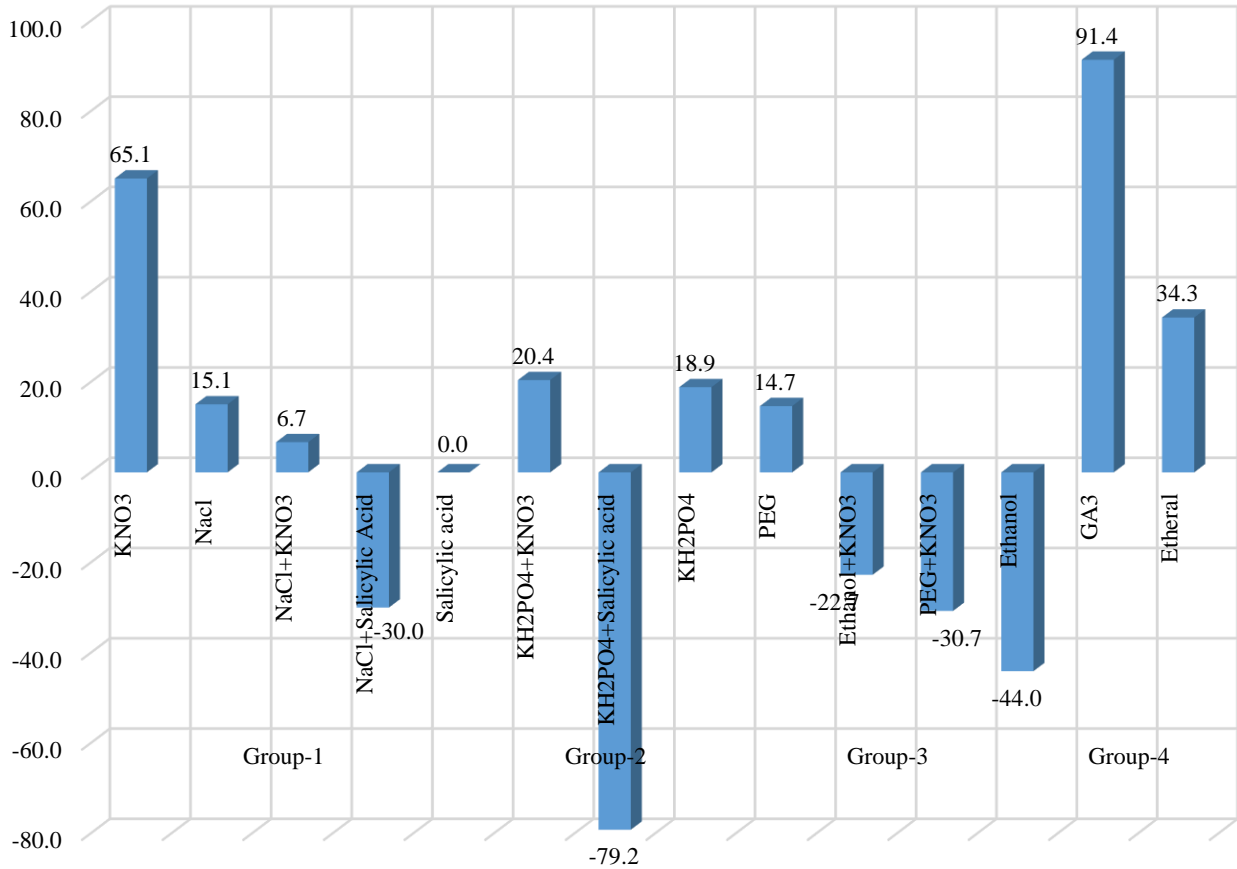
**Germination (%):** Germination has been affected significantly when different combination of invigorants was applied to forcedly aged carrot seeds (Table 2). The highest germination (48%) was observed at PEG10% that was followed by 37% recorded at PEG5% revealing improved germination as compared to control. The relative increase in germination over control was 44% and 12% at T3 (PEG 10%) and T2 (PEG 5%), respectively (Table 3). For the rest of the treatments, various invigorants when used alone or in combinations, revealed decrease in germination over control. Various increasing /decreasing trends in germination have been observed in this study. For example, germination (37%) recorded at

PEG5% was increased to 48% when the PEG dose was raised from 5% to 10%. However, it again dropped to 29.3% when PEG level was further increased to 15%. In this group of invigorants, PEG at 10% seems to be suitable revealing better invigorative response.

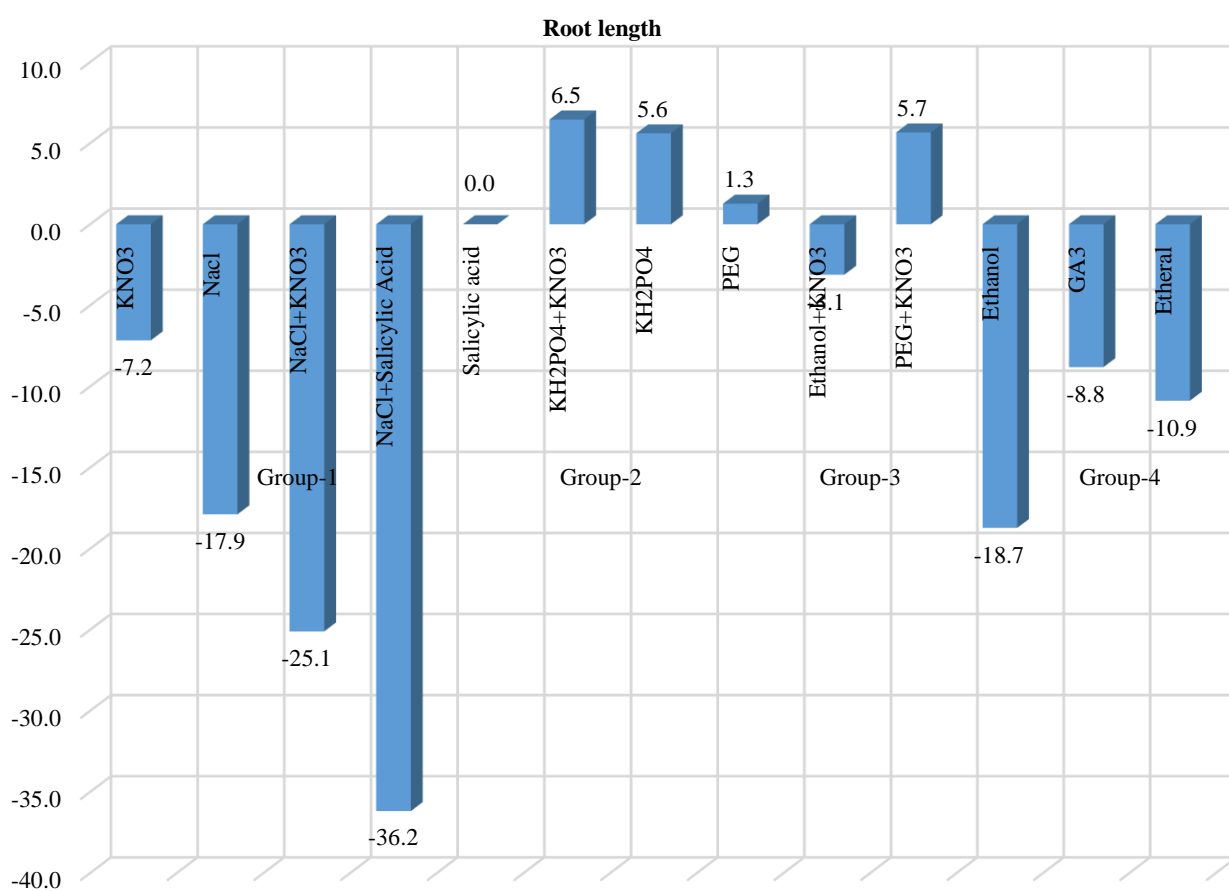
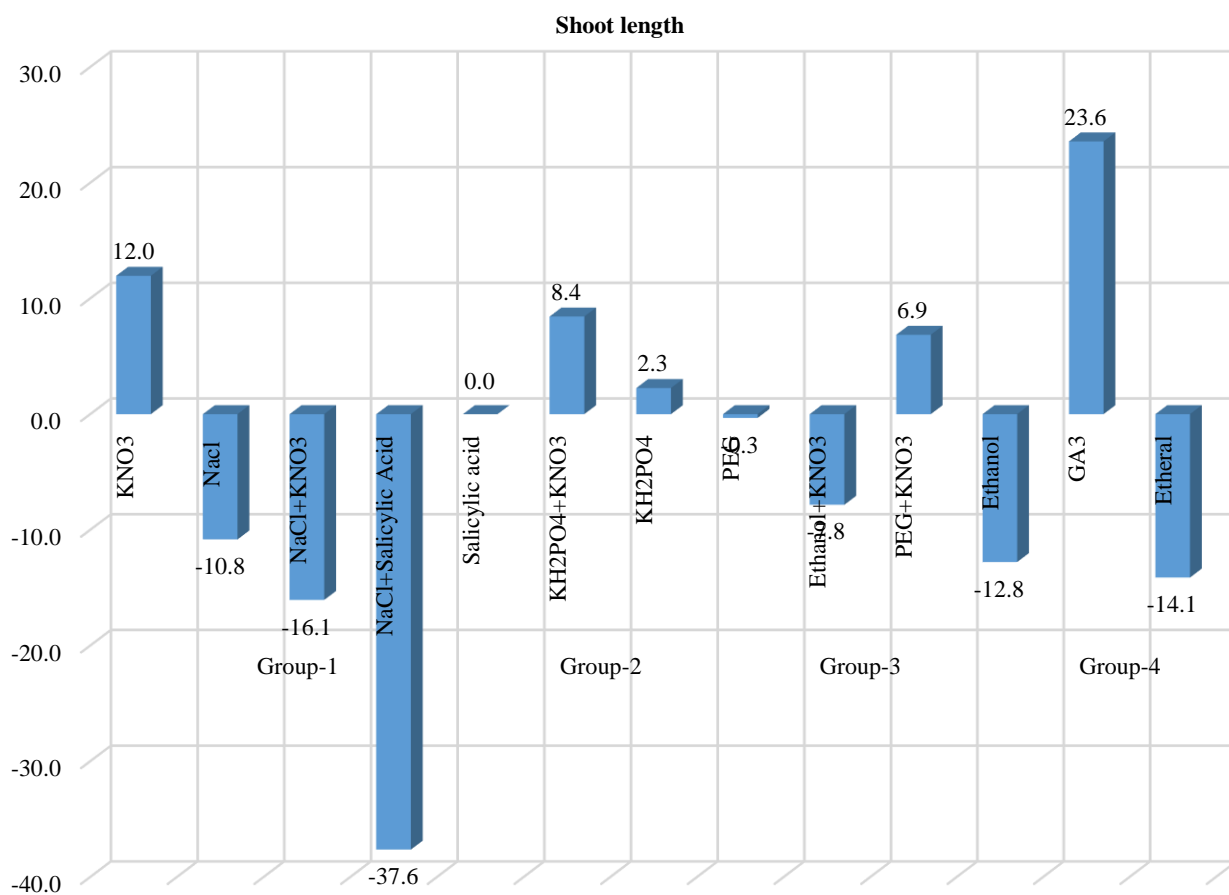
**Shoot length (cm):** Analysis of variance revealed non-significant differences in shoot length when different invigorants under group-3 were applied (Table 2). Shoot length recorded against various treatment combinations ranged from 4.63cm at T4 (PEG 15%) to 3.53 cm at T6 (Ethanol 1%). It was observed that PEG either alone (10%

& 15%) or in combination with 0.1% KNO<sub>3</sub> induced shoot longer than shoots produced at control. The relative increase ranged from 2.97% (T8: 5% PEG + 0.1 KNO<sub>3</sub>) to 8.41% observed in T4 (PEG 15%) and T10 (PE15% G + 0.1 KNO<sub>3</sub>) (Table 3). However, at 15% PEG (T4) as well as in combination with 0.1% KNO<sub>3</sub> (T10) shoot length (4.63 cm) recorded at both treatments was same. Shoot induced at rest of the treatments was lower than the control. The relative decrease observed for shoot length ranged between -9.5% (T2: PEG 5%) to -17.25% (T6: Ethanol 1%).

**Germination**







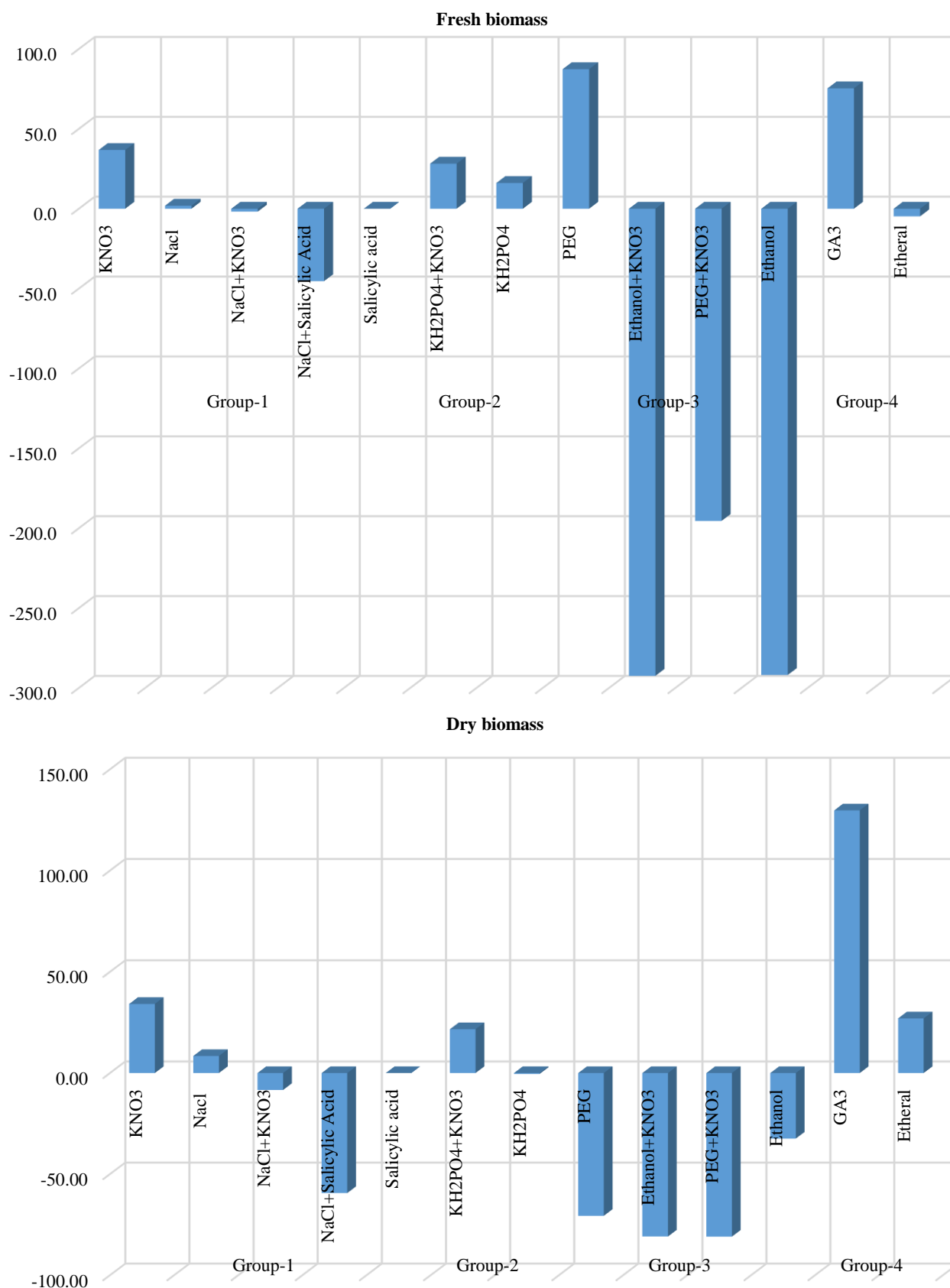


Fig. 1. Mean relative response (%) over control of multitude of invigorants on different seedling parameters in carrot.

**Root length (cm):** Non-significant differences for root length were recorded when different invigorants viz. PEG, Ethanol, KNO<sub>3</sub> were applied (Table 2). Root length recorded at T2 (PEG 5%) and control were same, however,

four different treatments (T4: PEG 15%, T8: 5% PEG + 0.1 KNO<sub>3</sub>, T10: PE15% G + 0.1 KNO<sub>3</sub>) and T12 (1% Ethanol + 0.1 KNO<sub>3</sub>) revealed longer roots as compared to control. The relative increase in roots observed at these treatments

was 0.7% (T12: 1% Ethanol + 0.1 KNO<sub>3</sub>) to 14.19% at T4 (PEG 15%) (Table 3). The root length revealed at rest of the treatments was lower than the root induced at control. The relative decrease observed ranged between -0.45% (T11: 0.5% Ethanol + 0.1 KNO<sub>3</sub>) to 14.19% (T4: PEG 15%).

**Fresh seedling biomass-FSB (g):** FSB revealed significant differences against treatment combinations of the diverse seed invigorants (Table 2). FSB observed at control remained at par with T4 (PEG 15%) and T13 (2% Ethanol + 0.1 KNO<sub>3</sub>), whereas T6 (Ethanol 1%), T7 (Ethanol 2%), T8 (5% PEG + 0.1 KNO<sub>3</sub>) and T10 (15% PEG + 0.1 KNO<sub>3</sub>) displayed FSB at par among each other. Similarly, T5 (Ethanol 0.5%) and T12 (1% Ethanol + 0.1 KNO<sub>3</sub>) also shared the same FSB. Among different invigorants applied, only PEG (5% and 10%) revealed improved FSB over control treatment. The relative increase was 18.2% and 31.1% at T2 (PEG 5%) and T3 (PEG 10%), respectively (Table 3). FSB recorded at other treatment combinations was lower than the control and the relative decrease observed ranged from -5.4% at T4 (PEG 15%) to -71.4% at T12 (1% Ethanol + 0.1 KNO<sub>3</sub>).

**Dry seedling biomass-DSB (g):** Dry seedling biomass (DSB) recorded against various invigorants depicted significant variation (Table 2). There was only one treatment (T6: Ethanol 1%) revealing higher DSB over the control. The relative increase recorded was 60.7% (Table 3). For the rest of the invigorants the DSB observed was less than the control.

Seed priming with PEG appeared to be an effective method to improve seed germination, seedling emergence and stress tolerance of several crop plants under unfavourable conditions (Chen & Arora, 2011; Zhang *et al.*, 2015). Improvement of germination by priming with PEG might be directly related to the modification of seed water relations (Nagarajan & Rane, 2020). PEG improves cell membrane stability. Seed primed with PEG improved the enzymatic antioxidant activities in plant seeding which subsequently improved plant growth during seedling stand (Zang *et al.*, 2015). Furthermore, the study suggested that vacuums created inside the seed as a result of priming made water flow easier, thus contributing to tissue hydration. These findings are in accordance with Sadeghi *et al.* (2011) who observed increased germination percentages, germination index and seed vigour in soybean. Similar results were also obtained by Kaur *et al.* (2017) in okra, osmo-priming with 5% PEG led to better yield and biochemical quality parameter by tolerating adverse environmental effects. Sweet pepper seeds osmo primed with PEG 6000 (-1.5 MPa) recorded the highest germination percentage, number of roots and root length.

On the other hand, the slow germination of seeds primed with different concentrations of ethanol might be due to low antioxidant activity. Our results are in accordance with (Afzal *et al.*, 2013) who also reported that higher level of ethanol reduces germination, root, shoot and biomass in tomato.

#### Response of carrot seed against GA<sub>3</sub> and ethereal

**(group-4):** The set of invigorants in group-4 viz. GA<sub>3</sub> (5 ppm-200 ppm) and Ethereal (5 ppm - 200 ppm) have been used to explore their priming effect on aged carrot seeds. Analysis of variance revealed significant differences for all the parameters investigated in carrot against various concentrations of GA<sub>3</sub> and Ethereal (Table 2).

**Germination:** In case of GA<sub>3</sub>, germination ranged between 24% (150 ppm ethereal) to 45.3% (50 ppm GA<sub>3</sub>) with a mean germination 35.73%. The germination observed over both the highest (200 ppm) and lowest concentration (5 ppm) of GA<sub>3</sub> remained at par with each other. At all the levels of GA<sub>3</sub>, improved germination over control was recorded. The relative increase in germination ranged between 28.6% at GA<sub>3</sub> (150 ppm) to 142.9% at 50ppm of GA<sub>3</sub> (Table 3). At different concentrations of ethereal, germination ranged between 14.7% to 37.3% with a mean value of 25.07% (Table 2). Except at 50 ppm, all concentrations revealed higher germination rate over control and the relative increase ranged from 14.3% (200 ppm) to 100.0% at 100 ppm, with a mean relative increase of 48.21 % (Table 3).

The germination response at GA<sub>3</sub> (150 ppm) as well as on Ethereal (150 and 200 ppm) also remained at par. No relationship among the increasing or decreasing concentrations of either GA<sub>3</sub> or ethereal with the germination was observed.

**Shoot length (cm):** The longest shoot (10.8 cm) was observed at GA<sub>3</sub> (150 ppm) that was followed by 6.61 cm at GA<sub>3</sub> (5 ppm) whereas the lowest shoot length (4.16 cm) was observed at ethereal (100 ppm). Shoot length observed at all concentration of Ethereal as well as at GA<sub>3</sub> (50, 100 and 200 ppm) remained at par among each other. The shoot length better than control was observed at 5, 50 and 150 ppm of GA<sub>3</sub> and the relative increase in shoot length was 22.7%, 11% and 100.4%, respectively (Table 3). Within this group of invigorants, the highest priming effect was observed at 150 ppm GA<sub>3</sub> (10.8 cm) inducing 10.8 cm long shoot.

**Root length (cm):** The longest root (6.67 cm) was observed at 50 ppm of GA<sub>3</sub> and it was followed by 6.1 cm at GA<sub>3</sub> (100 ppm). There was only one treatment (50 ppm of GA<sub>3</sub>) where root length (6.7 cm) was better than control (6.2 cm) and the relative increase in root length was 7.6 (Table 3). For the rest of the treatments root length observed was shorter than the roots recorded at control. The relative decrease ranged between -27.9 % at GA<sub>3</sub> (5 ppm) to -4.0% recorded at 150 ppm of ethereal.

**Fresh seedling biomass (g):** At different levels of GA<sub>3</sub>, FSB ranged between 0.1133 g to 0.2583 g with a mean value of 0.20 g. FSB observed at all concentrations of GA<sub>3</sub> appeared to have better performance than control and relative increase observed ranged between 17.7% (5 ppm GA<sub>3</sub>) to 127.9% (50 ppm GA<sub>3</sub>) with a mean increase of 75.35% over control (Table 3). In case of ethereal, FSB ranged between 0.0777 g to 0.1580 g with a mean value of 0.11 g. FSB observed at two out of five concentrations of ethereal appeared to have better performance than

control and relative increase observed ranged between 6.0% (5 ppm ethereal) to 39.8% (100 ppm ethereal) with a mean increase of 22.93% over control. A relative decrease in FSB observed for three concentrations of ethereal ranged between -9.2 (150 ppm ethereal) to -29.0 (50 ppm ethereal) with a relative mean decrease of -23.2% (Table 3). The proportionate increase in both GA<sub>3</sub> and ethereal did not reveal any symmetrical trend in FSB.

**Dry seedling biomass (g):** Dry Seedling Biomass (g) in carrot revealed significant differences when analysed against various levels of GA<sub>3</sub> and ethereal (Table 2). When different concentrations of GA<sub>3</sub> were applied, FSB ranged between 0.0060 g to 0.0123 g with a mean value of 0.008 g. FSB observed at all concentrations of GA<sub>3</sub> appeared to have better performance than control and relative increase observed ranged between 63.6% (150 ppm GA<sub>3</sub>) to 236.4% (50 ppm GA<sub>3</sub>) with a mean increase of 130.91% over control (Table 2). For ethereal, FSB ranged between 0.0033 g to 0.0067 g with a mean value of 0.005 g. FSB observed at three out of five concentrations of ethereal appeared to have better performance than control and relative increase observed ranged between 27.3% (5 ppm ethereal) to 81.8% (100 ppm ethereal) with a mean increase of 48.48% over control. There was no symmetrical behaviour observed in FSB against increasing concentrations of ethereal.

Seed priming with plant growth regulators has been an efficient method for increasing seed vigour as well as seedling growth under stressful conditions (Yarnia & Tabrizi, 2012). Pre-sowing seed treatment with GA<sub>3</sub> and ethereal also promotes the growth and development of the seedlings. GA<sub>3</sub> enhanced seed germination by stimulating the rate of cell division, and cell elongation activity (Taiz & Zeiger, 2017). These results are similar to our findings in which the maximum germination was recorded in all the concentrations of GA<sub>3</sub> over the control, but the best results were recorded in GA<sub>3</sub> 50% (45.33 cm).

Similar results were shown by Bassi *et al.*, (2011) describing priming with GA<sub>3</sub> 50 ppm enhanced emergence, germination, and speed of germination in soybean as compared to non-primed seed lots. Kumar & Singh, (2013) also reported that bitter melon seeds primed with 100 ppm GA<sub>3</sub> better germination, field emergence, speed of emergence, seedling length and vigour index over the control. Jendrzczak & Smigerska, (2014) results also showed that Amaranthus seeds treated with GA<sub>3</sub> showed more abundant growth than control which contributed to maximum yield. Similar findings were also reported by Soubhagya, (2016) in brinjal and chilli seeds where priming with GA<sub>3</sub> had significantly increased germination as compared to unprime seeds. In the case of ethereal the maximum germination was obtained in ethereal 100%.

**Comparison of cumulative response of invigorants:** For studying the overall invigorative potential of different compounds, mean relative response was determined. Comparing the invigorative potential of KNO<sub>3</sub>, NaCl, KH<sub>2</sub>PO<sub>4</sub>, PEG, ethanol, GA<sub>3</sub> and ethereal when applied alone, it was observed that GA<sub>3</sub> remained at top revealing 91.4%

improved germination over control that was followed by KNO<sub>3</sub> improving 65% germination (Fig. 1). With minor deviations, rest of the parameters also supported the similar pattern. Except ethereal and ethanol, rest of the invigorants when used alone revealed germination improvement ranging between 14.7% (PEG) to 18.9% (KH<sub>2</sub>PO<sub>4</sub>). Ethereal and ethanol did not display invigorative potential in carrot resulting in 75.2% less germination over control. On the other hand, KNO<sub>3</sub> when applied in combination with NaCl, salicylic acid, KH<sub>2</sub>PO<sub>4</sub>, PEG and ethanol, it was noted that its combination with NaCl and KH<sub>2</sub>PO<sub>4</sub>, revealed improved germination revealing 6.7% and 20.4% over control, respectively. Although, the combined use of invigorants showed improved germination over control, but it was either low or near to germination revealed by their single use. Rest of the combination did not perform better than control. It displayed that by combining both chemicals hampered the invigorative potential of both as the two invigorants alone displayed better performance. Salicylic acid alone revealed zero germination and when added with NaCl, also declined the germination.

## Conclusion

According to our findings NaCl (20%), KNO<sub>3</sub> (1%), KH<sub>2</sub>PO<sub>4</sub> (1%), PEG (10%), GA<sub>3</sub> 50ppm, Ethereal 100ppm, provided good results but among all the combinations the combination of 1% KNO<sub>3</sub> with different concentrations of KH<sub>2</sub>PO<sub>4</sub> also provide good results further investigation may be required in this regard. Anyhow improved seed invigoration techniques are well known to reduce emergence time, accomplish uniform emergence and give better crop stand in carrot as well as many other horticultural crops and these includes hydro priming, osmo conditioning, hormonal priming and soaking before sowing. The accurate choice of priming duration and method are important to attain maximum benefits from seed priming techniques. KNO<sub>3</sub> alone and in wide array of combinations proved synergistic, though some positive interactions were also observed. The present study underscores the efficacy of GA<sub>3</sub> (gibberellic acid) and KNO<sub>3</sub> (potassium nitrate) as effective invigorants that significantly enhance the germination process in carrot seeds. This study not only sheds light on the specific benefits for carrot cultivation but also paves the way for potential applications in future studies involving diverse crops. The notable improvement observed in germination rates suggests that GA<sub>3</sub> and KNO<sub>3</sub> hold promise as seed treatment options that can be explored and adapted for optimizing seed performance across various agricultural contexts. This valuable insight contributes to the growing body of knowledge in seed science and has the potential to influence agricultural practices aimed at enhancing crop productivity and overall yield.

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