



## Phytohormones priming: Potential strategy for improving seed germination, Yield and Fatty acids content in *Ammi visnaga* L.

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The medicinal herb *Ammi visnaga* has a low seed germination rate, as a result breaking seed dormancy and raising germination percentage warrant a lot of attention and curiosity. The goal of this study was to promote the germination of *A. visnaga* seeds by priming them with gibberellic acid (GA3), kinetin (KN), and naphthaleneacetic acid (NAA) for 12 hours and then tracking their long-term effects on seed yield and fatty acid content. It has the potential to help with the problem of low germination and seed quality in *Ammi* species. Seed priming with KN at 300 and 400 mg/L resulted in maximum increase of germination rate up to 84 %, compared to 40 % for untreated seeds. GC-MS was used to determine the fatty acid profile of the seeds generated. Seed priming with (GA3, KN, and NAA) has a significant impact on the quantity and quality of fatty acids produced in seed yield.

**Keywords:** *Ammi visnaga*; Fatty acids; Germination percentage; yield; Phytohormones.

### INTRODUCTION

Crop development and growth have been hampered in many regions all over the world as a result of recent global warming. Drought, salinity, heat, cold, and heavy metals can be considered among the environmental variables that might have an impact on plant growth and production. It might affect the seed germination and seedling establishment during the early phases of plant growth (Vishal and Kumar, 2018; Yadav et al. 2020). *A. visnaga* and several genera of the Umbelliferae were recorded in various studies with low seed germination and noticed that the ungerminated seeds had a normal seed coat and endosperm (Bourioug et al. 2020).

*A. visnaga* is a medicinal plant that belongs to the family Apiaceae, an annual or biennial herbaceous plant. The inflorescence is a compound umbel of white flowers, and the fruits are compressed structures of oval shapes around 3 mm in length. The plant has its origin in the warm Mediterranean climate. *A. visnaga* is endogenous to North Africa (i.e., Egypt), North America, Argentina, West Asia, as well as a wide part of the European Mediterranean (Beltagy and Beltagy, 2015). The fruits are the main used part in the plant, which turn light brown with a distinctive smell and bitter taste (Batanouny et al. 1999). In folk medicine in Egypt, *A. visnaga* fruits were commonly used many years ago for kidney stones treatment by drinking its powdered fruit tea (Günaydin\* and Beyazit,

2004). Additionally, the seeds are the main source of furocoumarin, giving the plant great medicinal importance, and increasing its demand in the pharmaceutical industry. The seeds of *A. visnaga* contain various classes of chemical components such as pyrones, flavonoids, saponins, and essential oils (Hashim et al. 2014b). Khellin and visnagin are the major components among the  $\gamma$ -pyrones in the fruit, for the commercial formulations. Both are capable of suppressing spasms, suggesting that a calcium channel blocking mechanism is involved (Rauwald et al. 1994). Furthermore, *A. visnaga* has been widely used for colic and gastrointestinal cramps, diabetes, and kidney stones (Hashim et al. 2014a).

Recently, much emphasis has been given to finding new approaches to alleviate the limits imposed by abiotic stresses on seed germination. A variety of physiological and non-physiological techniques for improving seed germination and alleviating abiotic stresses have been proposed for that purpose. Seed priming induces seed germination and boosts plant growth and development in abiotic environments (Eisvand et al. 2010; Jisha et al. 2013). Growth and various stages of plant development are strictly regulated by several classes of plant hormones (Li et al. 2016; Santner et al. 2009). Phytohormones are playing a key role in the growth of plant flowering, increasing seed yield and, as a result, plant oil yield. Several studies have increased interest in oilseed crops

because of their value in the production of oil (Bennett et al. 2011). Hormonal seed priming is currently a widely used technique for improving seed germination, seedling growth, and crop yield in challenging conditions (Hu et al. 2013; Hussain et al. 2019).

Essential oil of *A. visnaga* is known for its cardiovascular disease and bronchial asthma properties (Satrani et al. 2004). Consequently, the new proposal forms part of an inquiry aimed to determine the fatty acids content of oil derived from *A. visnaga* seeds (Cherif et al. 2008; Tomaino et al. 2001).

Therefore, the main aims of the present study are to explore the effect of *A. visnaga* seed priming with various concentrations of (GA3, KN, and NAA) on improving seed germination, seed productivity, and the yields of oil-seed crops.

## MATERIALS AND METHODS

### 2.1. Germination experiment

The seeds of *A. visnaga* used in this study were provided by the Faculty of Agriculture, Menoufia University (Egypt). The seeds were surface sterilized by soaking in 10 % sodium hypochlorite for 5 min with continuous shaking and subsequently rinsed thoroughly several times with sterilized distilled water. The seeds were primed for 12 hours in five concentrations of various plant growth hormones at 100, 200, 300, 400, and 500 mg/L of GA3, KN, and NAA. The control treatment was primed in water for 12hr. as well. Six replicates of 9 cm diameter Petri dishes of (25) seeds in each plate were then prepared for germination on sterilized wetted filter paper. The seeds were kept in an incubator at dark conditions for 23 days at  $22\pm 2^{\circ}\text{C}$ . Radicle emergence was considered as a criterion for germination. The number of germinated seeds was recorded every day and the seed quality parameters were recorded and calculated according to the following equation:

#### Germination percentage:

Germination percentage [%] = (Number of germinated seeds/Total number of seeds)\*100

#### Speed of germination:

Speed of germination (SG) was calculated as described by (Anjum and Bajwa, 2005) based on the formula:

$$SG = (n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots + n_i/d_i)$$

where n is the number of germinated seeds and d is the number of days.

#### Germination Energy:

Germination energy (%) was recorded on the 6th day of the experiment. It is the percentage of germinated seeds 6 days after the start of the experiment relative to the total number of seeds tested (Ruan et al. 2002).

### Vigor Index:

The vigor index was calculated according to (Islam et al. 2013) and based on the formula:

$$(VI) = [\text{Total seedling length (cm)} \times \text{germination percentage (\%)}]$$

### 2.2. Pot experiment and experimental design

The seeds of *A. visnaga* were sterilized as mentioned before and divided into two main groups. The seeds were pre-soaked for 12 hours with various doses of each of the plant growth regulators (GA3, KN, and NAA) at concentrations of (100, 200, and 300 mg/L). While the control seeds were soaked in distilled water for the same time. The pretreated seeds were sown in each pot in the mid of October. Each pot was filled with 12 kg of sand: clay soil 50:50. Six replicates of pots were conducted from two biological replicates in the greenhouse of the Faculty of Science, Menoufia University (Egypt) during season 2018/2019. Fertilizers were added monthly to all pots as a nutrient solution of nitrogen, phosphorus, and potassium (NPK). The mature inflorescences were collected and dried in the oven at 50 °C for 7 days.

### 2.3. Gas chromatography-mass spectrometry (GC-MS) analysis for fatty acids detection

#### 2.3.1. Oils extraction

Trans-methylation of lipids and extraction of oils methyl esters of *A. visnaga* seed yield was carried out according to (Garcés and Mancha, 1993). A definite weight of 1 g dry seed powder was added to 10 mL of reagent mixture consisting of methanol: heptane: toluene: H<sub>2</sub>SO<sub>4</sub> (39:34:25:2 by volume). The mixture was incubated in a water bath for two hours at 80 °C. The mixes were cooled and homogenized again before being set aside for several minutes. Two phases were separated and the essential oils methyl esters were carefully collected, weighed, and estimated as mg/g tuber d.wt. The oil ester samples were analyzed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) according to the following technique:

#### 2.3.2. (GC-MS) analysis

The fatty acids profiles of all studied treatments of *A. visnaga* seeds were performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The temperature of the column oven was initially held at 50°C and then increased by 5°C /min to 250 °C hold for 2 min and increased to the final temperature 300°C by 30°C /min and hold for 2 min. The injector and MS transfer lines were held at 270°C and 260°C, respectively, and helium was utilized as the carrier gas at a constant flow rate of 1 mL/min. The solvent delay was 3 minutes, and 1 µL diluted samples were injected automatically using an Autosampler AS1300 and a split mode GC. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650. The

temperature of the ion source was fixed at 250 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

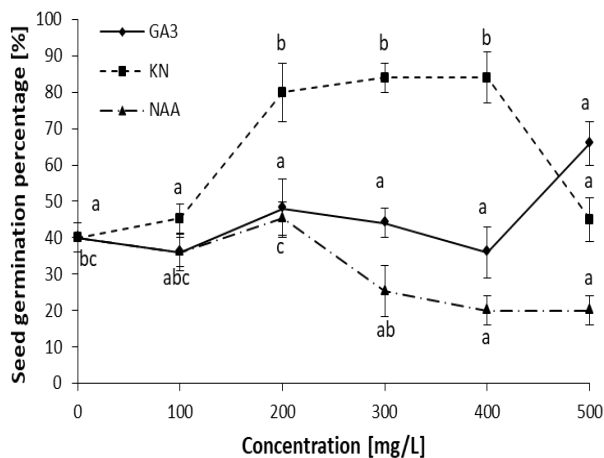
#### 2.4. Statistical analysis

The data are expressed as mean  $\pm$  SD and the significant values were considered at  $p < 0.05$ . The analysis was done using SPSS software version 16. One-way analysis of variance (ANOVA) was used for evaluating the difference between the mean values of the studied treatments. Heat map plots were generated by NG-CHM BUILDER. The Program of PanPlot diagram software was used to visualize fatty acids profiles.

## RESULTS

### 3.1. Germination percentage of *A. visnaga* seeds

The impact of *A. visnaga* seed priming for 12h with GA3, KN, and NAA at 100, 200, 300, 400, and 500 mg/L on germination percentage were investigated and summarized in **Figure 1**.



**Figure 1: Effect of *A. visnaga* seed priming with GA3, KN, and NAA on germination percentage [%].**

It was revealed that seed priming with KN attributed the highest stimulation of seed germination percentage than GA3 and NAA. Particularly, KN at concentrations of 200, 300, and 400 mg/L recorded the highest germination percentage compared to other tested plant growth regulators. The maximum recorded germination percentage is 84% and giving rise to an enhancement by more than double fold over to control treatment. While high levels of NAA (400 and 500 mg/L) recorded the

lowest germination percentage and decreased germination percentage by 50% than control seeds. The concentration of 200 mg/L for GA3 and NAA was the most effective dose for germination percentage in comparison to other tested doses. As its induction percentage was improved by 20% and 12.5%, respectively compared to control seeds. Moreover, the highest concentration of GA3 (500 mg/L) increased germination percentage by 66% compared to control seeds. Generally, the application of other tested doses of GA3 and NAA attenuated the enhancement of seed germination and decreased germination percentage.

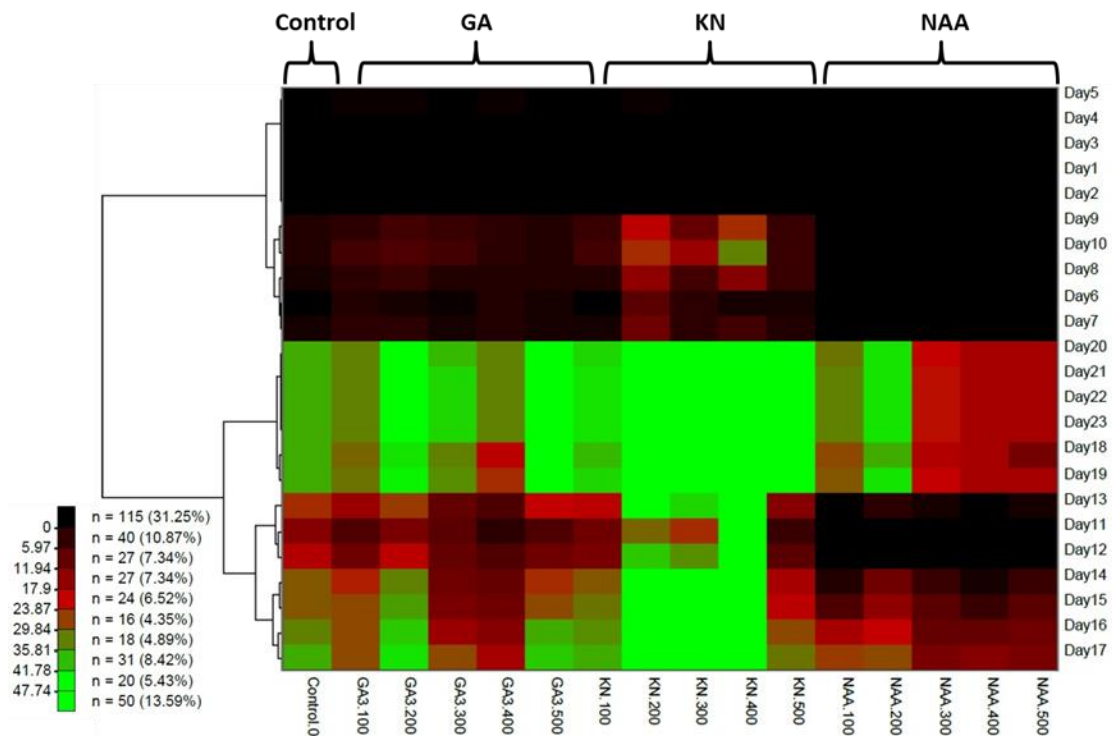
#### 3.1.1. Heat map analysis

The heat map chart summarizes the results of seed germination percentage of *A. visnaga* and the diurnal germination influenced by seeds priming with various concentrations of GA3, KN, and NAA over 23 days as shown in **Figure 2**.

This diagram used NG-CHM Heat Map and the color of the chart [Red (low) to green (high)] was related to the percentage of seed germination. Based on a heat map analysis, we deduced that the germination percentage of *A. visnaga* seeds was significantly affected by seed priming with GA3, KN, and NAA. Hierarchical clustering analysis expresses the visual depiction of accessions in different groups based on similarity and the correlation with different features.

The heat map shows two-way trait and accession classification; seed germination percentage and day to germination, which were significantly varied by different levels of GA3, KN, and NAA.

NAA delays time to germination until day 12 at all measured concentrations. In the present study, the percentage of seed germination daily for 23 days with three kinds of growth hormones at different levels was distributed into three clusters. The cluster heat map shows that cluster 3 starts from day 11 to day 17 where the active and KN at (200 and 400 mg/L) recorded the highest germination percentage (84%) while GA3 and NAA recorded the less percentage. Cluster 2 start from day 18 to day 23 was active for GA3 and NAA but still not more than KN, while cluster 1 start from day 1 to day 10 and recorded the lowest germination percentage at all tested growth hormones.



**Figure 2:** Heat map distribution of the percentage of *A. visnaga* seed germination and the day to germination under the effect of the three studied plant growth regulators (GA3, KN, and NAA), the color distributed from Red (low germination percentage) to green (high germination percentage).

### 3.2. Seed quality parameters

The *A. visnaga* seeds quality parameters were significantly influenced by priming seeds with (GA3, KN, and NAA) for 12 h and represented in Table 1.

#### 3.2.1. Vigor Index

The seed vigor index, as shown in (Table 1), is a valuable indicator that needs to be valued for supplement germination. Priming seeds with a high concentration of GA3 (500 mg/L) attributed the maximum value of vigor index which increased by 43 % over the control treatment. Similarly, priming seeds with a medium concentration of KN (200 mg/L) had the highest vigor index, with a 90.7% over control seeds. While NAA at 500 mg/L causes the lowest seed vigor index compared to other examined growth hormones.

#### 3.2.2. Speed of germination

Priming seeds with KN improved the speed of germination at all levels tested as shown in (Table 1). Particularly, 400 mg/L yielded the highest speed of germination two-fold over the control seeds. Moreover, GA3 promotes the speed of germination by 22% compared to the control at low concentrations (100 mg/L). However, priming seeds with NAA reduces the speed of germination at all studied doses compared to the control seeds.

#### 3.2.3. Germination energy

Seed priming with GA3 and KN for 12h at various levels enhanced germination energy of *A. visnaga* seeds as shown in (Table 1). In particular, KN at 200 mg/L increased germination energy of the seeds to the maximum level by three folds over the control seeds. However, NAA suppressed germination energy at all previously investigated levels, as evidenced by germination delays till day 13.

#### 3.2.4. Seedling length

The germinated seeds were observed, as well as the seedling length was measured and recorded after 23 days of seed sowing as represented in Figure 3. Priming seeds with the previously mentioned concentrations of GA3, improved seedling length at 400 and 500 mg/L by 8 and 2.3%, respectively compared to control seeds. On the other hand, seedling length was negatively influenced by all tested concentrations of KN and NAA and showed a downward trend when the concentrations were increased.

Table 1: *A. visnaga* seed quality parameters as influenced by seed priming with various plant growth regulators (GA3, KN, and NAA) at (100, 200, 300, 400, and 500 mg/L).

Treatment	Concentration (mg/L)	Vigor Index	Speed of germination	Germination energy (%)
Control		311.9 ±1.8 i	27.76 ±0.74 e	0.14 ± 0.03 b
GA3	100	333.47 ±1.0 j	34.21 ±1.53 f	0.28 ± 0.06 c
	200	392 ±0.4 k	24.02 ±1.98 d	0.21 ± 0.09 bc
	300	311.13 ±0.9 i	20.81 ±0.88 c	0.14 ± 0.03 b
	400	305.1 ±0.8 h	13.48 ±0.98 b	0.16 ± 0 b
	500	446.87 ±0.7 m	33.86 ±0.88 f	0.14 ± 0.03 b
KN	100	297.73 ±0.2 g	27.55 ±0.69 e	0.11 ± 0.01 b
	200	595 ±0.6 n	44.06 ±0.71 g	0.60 ± 0.07 d
	300	430.53 ±0.6 l	57.80 ±0.81 h	0.15 ± 0.02 b
	400	312.40 ±0.8 i	59.85 ±0.51 h	0.32 ± 0 c
	500	124.00 ±0.8 d	28.64 ±0.79 e	0.16 ± 0 b
NAA	100	143.07 ±1.6 e	13.92 ±1.35 b	0 ± 0 a
	200	206.53 ±0.6 f	15.89 ±0.19 b	0 ± 0 a
	300	36.87 ±1.6 c	15.11 ±0.77 b	0 ± 0 a
	400	31.33 ±1.1 b	8.55 ±0.68 a	0 ± 0 a
	500	28.20 ±0.5 a	6.94±0.74 a	0 ± 0 a

Values are means ± SD (n = 3). Different letters indicate significant differences under different treatments at the 0.05 probability level according to Tukey's HSD test.

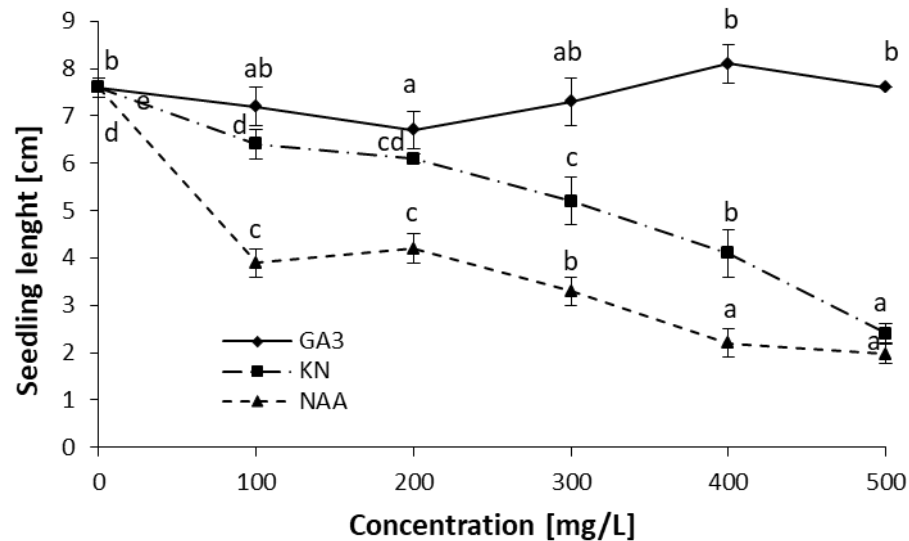


Figure 3: Effect of *A. visnaga* seed priming with various concentrations of GA3, KN, and NAA on seedling length [cm].

### 3.3. Seed yield productivity

The current study investigates two approaches: 1) the effects of *A. visnaga* seed priming with plant growth regulators (GA3, KN, and NAA) on seed yield. Seed yield productivity of *A. visnaga* is affected greatly by seed priming with GA3, KN, and NAA at (100, 200, and 300 mg/L) as shown in Figure 4. Priming seeds with GA3 at 100 mg/L yielded the maximum increase of seed yield by 88.5% over the control seeds. On contrary, 200 and 300 mg/L decreased seed productivity by 47 and 52%, respectively. Furthermore, priming seeds with low concentrations of KN (100 and 200 mg/L) decreased seed yield by 46.4 %, while high concentration (300 mg/L) improved seed productivity by 66 % over the control seeds. Moreover, seed priming with auxin expressed in NAA reduced seed yield at all examine doses (100, 200, and 300 mg/L) by 38.4 %, 47%, and 19.4 %, respectively than the control.

### 3.4. Fatty acids content in the produced seed yield

The fatty acids profile of the produced yield of *A. visnaga* seeds which were pre-treated before cultivation with (GA3, KN, and NAA) were estimated by GC-MS and represented in Figure 5. The synthesis of fatty acids in *A. visnaga* seeds was greatly affected and induced by priming them with various doses of (GA3, KN, and NAA). GA3 promoted the accumulation of petroselinic acid (Omega-12) in the produced seeds by 8.07 % at concentration of 100 mg/L over the control seeds. It was interestingly remarked that eicosanoic acid increased at 200 mg/L GA3 priming compared to control seeds. The maximal amount of linolenic acid (Omega-3) released was 19.13 % higher than the control treatment at 300 mg/L of GA3 application. Some fatty acids not detected in untreated seeds but induced at high concentrations of all GA3 treatment (100, 200 and 300 mg/L) such as octadecatrienoic acid, margaric acid, paullinic acid,

propionic acid, butanoic acid, and montanic acid in concentrations of 0.78, 0.75, 0.74, 0.48, 0.77, and 0.23%, respectively. Priming *A. visnaga* seeds with GA3 may inhibit or reduce the induction of some fatty acids such as tetradecanoic acid, behenic acid, oleic acid, and arachidonic acid even though they exist in low amounts in control seeds at concentrations 1.2, 2.43, 0.3, and 0.59 %, respectively.

The maximum amount of petroselinic acid and palmitic acid were improved by priming seeds with KN at 100 mg/L and recorded an increase of 60.16 and 22.38%, respectively over the control seeds. High concentrations of KN (300 mg/L) promoted linolenic acid induction by 4.37 % were recorded. Some fatty acids such as paullinic acid, margaric acid, and heneicosylic acid, not detected in the control seeds but induced at 100, and 200 mg/L of KN in quantities of 0.59, and 1.08 % respectively. Medium and high tested concentrations of KN improved the accumulation of polyunsaturated Adrenic fatty acid was higher than the control seeds.

Seed priming with all previous three tested doses of NAA improved the accumulation of palmitic acid by 8.75, 19.52, and 30.95 %, respectively over the control seeds. Furthermore, seeds pre-treated with a low concentration of NAA (100 mg/L) recorded the lowest amount of petroselinic acid (7.29%) over the control seeds.

In comparison to all other examined plant growth regulators, seeds pre-treated with NAA 100 and 200 mg/L yielded the highest amount of linolenic acid and oleic acid improvement over the control.

Priming seeds with low and medium concentrations of NAA, induced accumulation of the polyunsaturated fatty acid adrenic acid by 28.57 % as compared to the control seeds. Contrary, the high concentration (300 mg/L) inhibits adrenic acid induction. Also, NAA application inhibits the induction of tricosanoic acid at 100 and 200 mg/L.

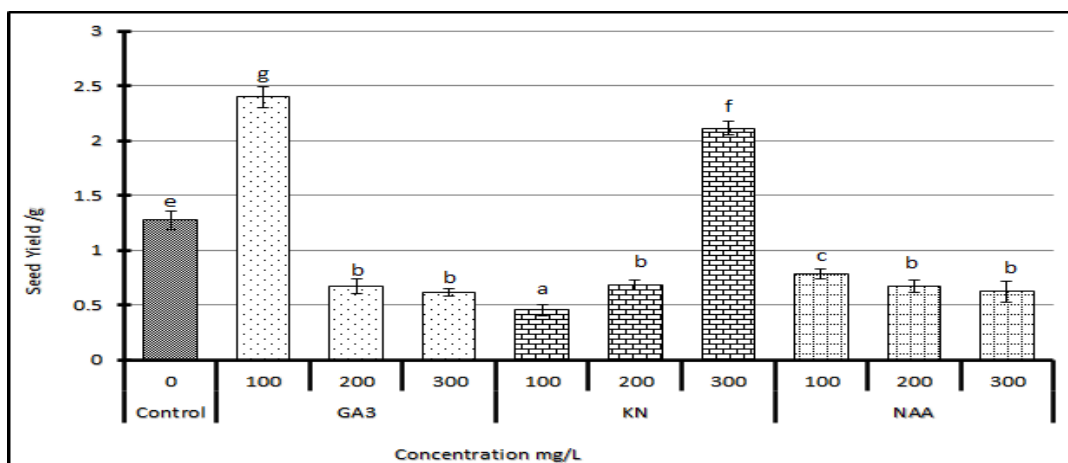
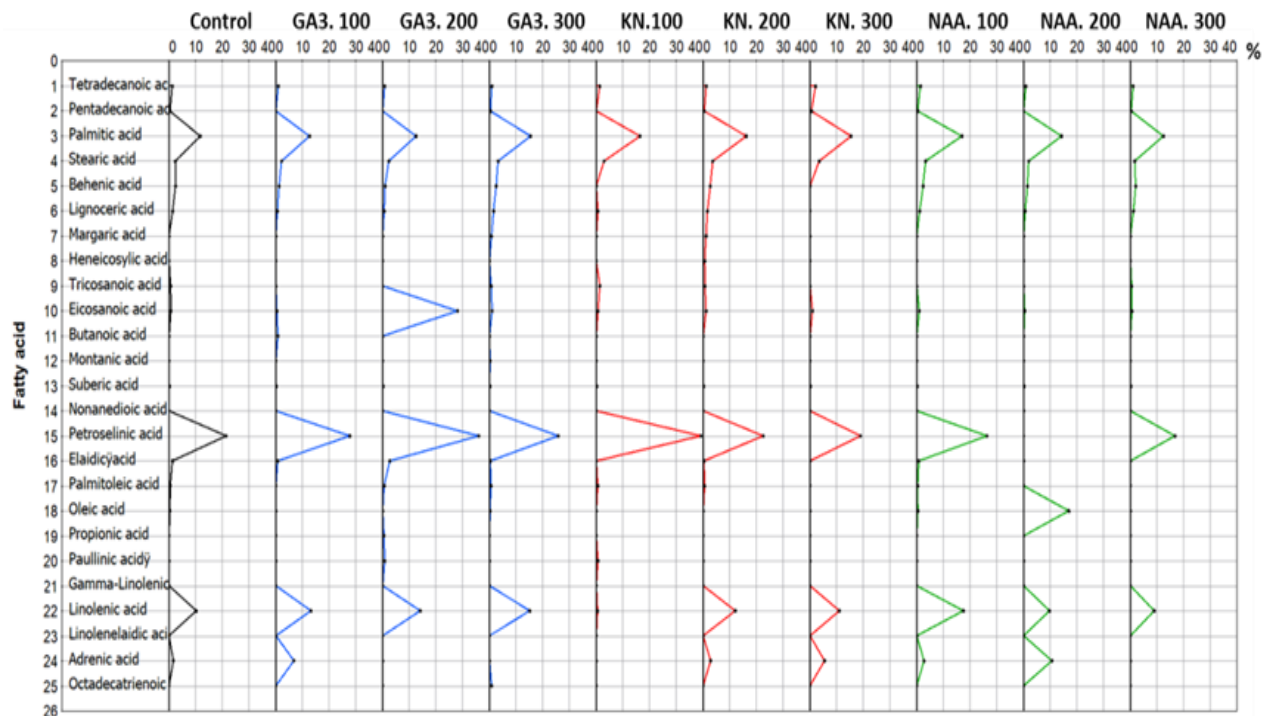


Figure 4: Effect of *A. visnaga* seed priming with various concentrations of GA3, KN, and NAA on seed yield [g].



**Figure 5: Panplot diagram for GC-MS analysis of fatty acids profile of *A. visnaga* seeds content after priming seeds with (GA3, KN, and NAA) at various concentrations of (100, 200, and 300 mg/L).**

## DISCUSSION

Plants are subjected to all abiotic stresses that cause complex responses, resulting in reduced growth and crop yield. Hence, more strategies for improving crop productivity should be provided. Seed priming is a low-cost, high-impact physiological and biochemical process, leading to higher germination percentages, as well as improving seedling growth and crop yield. Plant hormones influence a variety of plant functions, including seed dormancy and germination (Graeber et al. 2012; Jisha et al. 2013). This could explain the major difference in seed priming outcomes with different levels of GA3, KN, and NAA.

Cytokinins are plant hormones that control a variety of functions in plants, including seed germination (Chiwocha et al. 2005; Nikolić et al. 2006; Riefler et al. 2006). This was demonstrated by presoaking *A. visnaga* seeds for 12 hours with KN, resulting in a maximum germination percentage up to two-fold over the control seeds. These results are in line with other studies that reported seed priming of *Arachis hypogaea* with CKs (150 ppm) improved germination and seedling proportions by increasing antioxidant enzyme activities and reducing oxidative damage (Sepahri and Rouhi, 2017). However, the exact mechanisms by which priming with CKs reduces abiotic stress have not been investigated yet.

Similarly, Priming *A. visnaga* seeds with GA3 at

various concentrations increased seed germination percentage. These findings are in agreement with other studies that reported that seed priming with GAs also improved the percentage and rate of seed germination, as well as the growth, and various crops yield (Shineeanwarialmas et al. 2019; Toklu, 2015). However, further future studies are highly needed to determine the mechanisms of GA priming in the mitigation of abiotic stress. GA3 beneficial effects may be due to its signaling pathways, which can promote seed germination by releasing coat dormancy, weakening endosperm, and embryo cell growth (Voegelé et al. 2012).

However, auxins appear to have no influence on seed germination in *Arabidopsis* and tomato mutants lacking GA3 (Koornneef and Karssen, 1994). Although it is unknown if auxins have a function in *Arabidopsis* germination, while exogenous GA increases the early expression of numerous auxin-related genes (Ozga et al. 2003). These findings were also validated by our study during priming *A. visnaga* seeds with NAA. Which revealed that it had no influence on seed germination percentage, and recorded the less percentage compared to other tested phytohormones.

GA3, rather than KN or NAA, was the most effective phytohormone for increasing seedling length. This increase in seedling height with GA3 treatment could be owing to improved osmotic nutrient uptake, resulting in cell elongation and consequently higher shoot length (Shanmugavelu, 1966). Similar findings were observed in

jackfruit (Maiti, 2003) and tamarind (Vasanthi et al. 2014).

Seed vigor index is an important quality that needs to be valued to supplement germination. At 500 mg/L GA<sub>3</sub>, the vigor index reached its maximum value, which was 34% higher than the control. As well as KN priming seeds recorded a high amount of vigor index. These data are in line with (Afzal et al. 2008; Akbari et al. 2007) who reported that seed priming with an appropriate concentration of phytohormones, improves germination and seedling vigor by enhancing nutrient intake through increased physiological activities and root formation.

Priming *A. visnaga* seeds with the plant hormones (GA<sub>3</sub>, KN, and NAA) affect greatly seed yield productivity. The enhancement of seed yield by GA<sub>3</sub> at 100 mg/L is probably because GA<sub>3</sub> could regulate signal pathways and plant growth as well (Cavusoglu and Sulusoglu, 2015). Also, it may stimulate the development of xylem and phloem which resulted in increased assimilation product flow and deposition in seeds (Islam et al. 2021). Several researchers have observed that GA<sub>3</sub> at various concentrations significantly increased seed yield in a variety of crops, including (Sarkar et al. 2002) for soybeans and (Tiwari et al. 2011) for rice.

The high yield of seeds with KN (300 mg/L) can be interpreted by cytokinins that have been related to cell division and differentiation, shoot and root growth, fruit and seed formation, and many other aspects of plant development (Jameson and Song, 2016). On the other hand, low concentrations of KN slightly decrease seed yield. This could be due to the antagonistic effect between natural auxin and cytokinin (KN) which influence the plant development since auxin restricts the action of cytokinin at low concentrations (Kurepa et al. 2019). Moreover, our results about the reduction effect of NAA on seed yield are compatible with the study of (Saifuddin et al. 2009) which indicated the negative effect of NAA on plant growth.

Phytohormones also play a key function in the development of oilseed crops through inhibition or stimulation of enzymatic activity and also regulating physiological activities in the plant (Fan et al. 2011; Fu et al. 2014; Iqbal et al. 2014). Those previous findings were also obvious in priming seeds with GA<sub>3</sub> at varied concentrations, which increased the accumulation of eicosanoic acid and linolenic acid (Omega-3) in the produced seeds. Similarly, our results are in line with other studies that reported endogenous gibberellins in plants could increase oil content in *Origanum majorana* (Arafa, 2017).

*A. visnaga* seed priming with KN produces the maximum amount of petroselinic acid (Omega-12). These results are in line with studies that reported cytokines to serve a vital role in cell proliferation and lipid embedding. Zeatin supplementation enhances cell growth and division by stimulating critical factors and enzymes involved in nitrogen metabolism (Cerutti and Delatorre, 2013). KN priming seeds promote induction of some new fatty acids that not were detected in untreated seeds such as

paullinic acid, margaric acid, heneicosylic acid. This finding is significant because it shows that the plant growth regulators can alter the fatty acid composition in plants and stimulate fatty acid production (Aly et al. 2008).

NAA priming with *A. visnaga* seeds increases the accumulation percentage of polyunsaturated fatty acids linolenic acid which is thought to provide health advantages due to its ability to lower blood cholesterol and prevent cardiovascular disease (Wu et al. 2014). These findings are contrary to the study of (Stearns Jr and Morton, 1975), who reported that auxin treatment increased saturated fatty acid concentration while the reduced polyunsaturated fatty acid level in soybeans.

Furthermore, seeds pre-treated with different concentrations of NAA developed the accumulation of numerous fatty acids such as petroselinic acid, oleic acid, palmitic acid, linolenic acid. These findings were confirmed by (Aly et al. 2008) who reported that using varying concentrations of NAA, promising results on the fatty acids profile with greater oil content in callus of *J. curcas* generated in vitro compared to oil found naturally in field-collected seeds.

## CONCLUSION

*A. visnaga* seed priming with phytohormones (GA<sub>3</sub>, KN, and NAA) has emerged as a promising strategy in breaking physiological seed dormancy and increasing germination percentage. Priming seeds with KN was superior to GA<sub>3</sub> and NAA and increase germination percentage twofold than untreated seeds. Seed germination could be a convenient step to start studying plant growth. Furthermore, a low concentration of GA<sub>3</sub> and a high concentration of KN produce the highest yield of seed yield. Seed priming with (GA<sub>3</sub>, KN, and NAA) have a significant impact on the fatty acids content of *A. visnaga* seeds using GC-MS technique. The resulting accumulation of polyunsaturated fatty acids and new compounds of fatty acids were induced that were not discovered in untreated seeds.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conceptualization, D.G.; Methodology, M.A. and D.G.; Software, D.G.; Validation, M.A., D.G., M.Z., M.E.; Formal analysis, M.A.; Investigation, D.G., M.E., M.A., M.Z.; Writing—original draft preparation, D.G. and M.A.; Writing—review and editing, D.G., M.A., M.E., M.Z. All authors have read and agreed to the published version of the manuscript.



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