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Title: Study of the response of Fenugreek Seedlings (*Trigonella foenum-graecum* L.) to some Plant Growth Regulators and Their effect on the production of the active Compound (Trigonelline)

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Abstract

The experiment was conducted during the 2022–2023 academic year in the laboratories of the Department of Biology, Faculty of Education for Girls, University of Kufa, to investigate the effects of varying concentrations of gibberellic acid (GA_3) — specifically 0, 2, and 3 ppm — and salicylic acid (SA) (0, 2.5, 5 ppm) as well as the interaction between them on the germination percentage and some growth traits of fenugreek seedlings and the production of the active compound Trigonelline. The results showed that treating fenugreek seeds with gibberellin at a concentration of 3 ppm and spraying the seedlings with salicylic acid at a concentration of 5 ppm gave the highest rate of germination percentage, number of leaves, shoot length, root length, fresh and dry weights of the seedling, reaching (86.82%, 5.54 leaves, 7.40 cm, 4.00 cm, 0.64 mg, 0.42 mg) respectively. Also, the results showed the highest accumulation of the alkaloid Trigonelline, reaching (0.99 µg) at the 5 ppm compared to the rest of the other treatments.

These findings suggest that the combined application of GA_3 and SA may enhance both early growth and secondary metabolite production in fenugreek. Such treatments could be beneficial for improving the medicinal value and agronomic performance of this important plant.

Keywords: Fenugreek, Trigonella foenum, gibberellic acid, Trigonelline, Germination.

Introduction

Fenugreek (Trigonella foenum-graecum L.) belongs to the legume family Fabaceae. It is rich in proteins, fats, carbohydrates and vitamins, in addition to containing many effective medical and pharmaceutical compounds, including trigonelline alkaloids with a medical effect and influence [1]. Fenugreek is classified according to color into yellow and red fenugreek, and according to morphological characteristics into Erect (T. *Foenumg-raecum*) and creeping fenugreek (T. Cerulea graecum). Its seeds are characterized by containing colloidal and gelatinous materials, soapy and colored materials, and nicotinic acid, which has an effective effect [2]. Fenugreek seedlings respond to growth regulators which act as stimulants in plants that improve their physiological and reproductive efficiency, enhance gene expression for efficient sucrose transport, and increase dry matter decomposition and partitioning for seed production [3]. Growth regulators affect all vital processes within the plant, which is reflected in its morphological and physiological characteristics, such as respiration, photosynthesis, and others[4].Gibberellic acid is one of the most important growth stimulating substances that clearly improves the phenotypic characteristics of plants such as stem length, vegetative growth, flowering initiation, accelerating ripening, and improving fruit quality in various crops [5] . [6] found that pre-treated seeds with GA3 promoted seed germination in hemp plants and that the optimum concentration of GA3 for hemp seeds was 400 mg/L and 600 mg/L. , also results of their study showed that seeds pre-treated with an appropriate concentration of GA3 enhanced the activity of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) and osmotic adjustment capacity. That's why treating the seeds with GA3 helped the stems and roots grow longer. Generally, soaking seeds in GA_3 at concentrations between 50 and 150 ppm worked best for many plants. Applying GA_3 from outside the plant helped trigger the production and activation of enzymes like protease and α-amylase, which in turn improved the seeds' ability to germinate. [7] Salicylic acid is another major plant hormone that regulates plant immunity and can regulate many different responses, such as abiotic stress tolerance, plant growth and development, and other responses. It is an effective, environmentally and plant-friendly protector and plant growth regulator, but the effective concentrations of salicylic acid for plants and the intended purpose of its use must be determined. SA is synthesized by chorismate plants through two metabolic pathways, the first is based on the enzyme isochorismate synthase and the second is based on the enzyme phenylalanine ammonia lyase. Salicylic acid (SA), a well-known plant hormone, not only plays a key role in plant immunity but also helps regulate plant growth. It plays a vital role in regulating cell division and expansion by influencing molecular mechanisms—binding to certain genes and altering the transcription of key ones involved in the cell cycle and cell wall breakdown—thereby controlling cell growth and overall plant development [8]. Plant growth regulators play a significant role in boosting the production of secondary metabolites, whether used individually or in combination. Researchers have used both in vitro and in vivo methods to boost the production of various secondary metabolites in different plant species. Under controlled laboratory conditions, plant growth regulators have been used successfully to produce numerous medicinal compounds [9]. Trigonelline (TRG) is a key secondary metabolite and a natural hydrophilic alkaloid found in plants like green coffee beans and fenugreek seeds. Trigonelline (TRG) has notable pharmacological and medicinal properties, offering therapeutic effects for a range of conditions, including chronic metabolic diseases, age-related disorders, neurological diseases, diabetic peripheral neuropathy, cardiovascular issues, skin diseases, diabetes, and liver and kidney injuries [10]. Given the medical significance of fenugreek, this study aimed to use plant hormones to enhance the production of the alkaloid trigonelline from various parts of fenugreek seedlings, while also examining the plant's growth characteristics.

Materials and Methods

Experimental site and experimental design.

A laboratory experiment was conducted during the autumn season of 2022 in the laboratories of the Biology Department of - College of Education for women / University of Kufa, on fenugreek (*Trigonella foenum - graecum* L.), an Iraqi variety registered by the Ministry of Agriculture, using Petri dishes as experimental units according to the Complete Randomized Design (C.R.D) with three replicates. The averages were compared according to Duncan's multiple range test at a probability level of 5%. The seeds were placed in plates containing filter paper as a medium to fix the seeds, with 6 seeds in each plate which was considered an experimental unit and was repeated three times, with a total of 27 treatments. Three levels of gibberellin were used, as the seeds were soaked in gibberellic acid at concentrations of (2 and 3 ppm) for 24 hours, in addition to the control treatment soaked in water only [11], with three levels of salicylic acid sprayed, namely (0, 2.5

and 5 ppm). The first spray was one week after the start of the experiment, while the second spray was after 14 days.

Studied Traits

Germination Percentage (%)

The germination percentage was calculated after 10 days, with seeds considered germinated when their radicle length reached 2-5 mm (Figure 1). The results were then converted into a percentage using the equation from [12]:

Germination percentage = (Number of germinated seeds / Total number of seeds) × 100

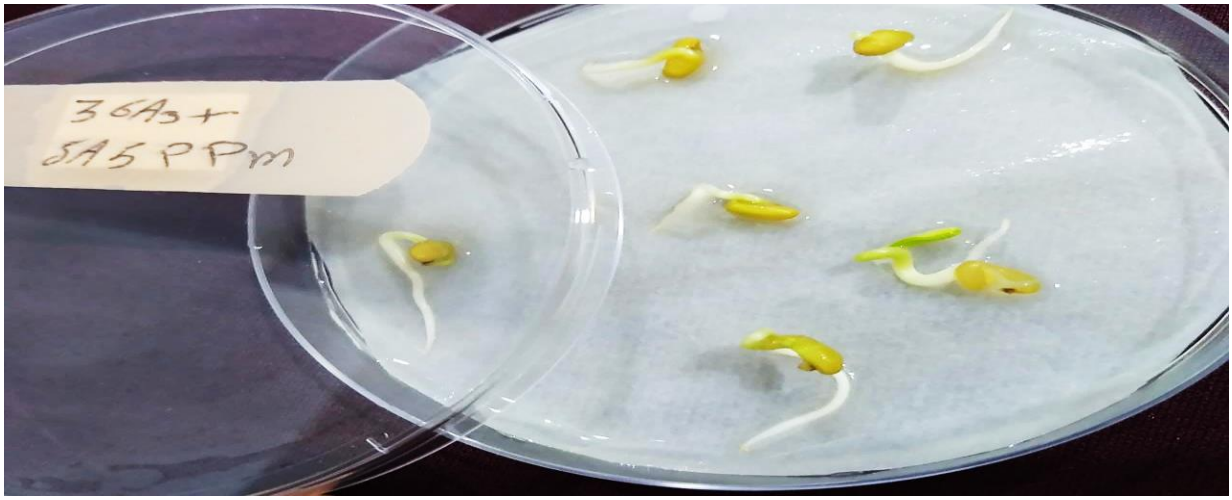

Figure (1). Germinated seeds

Morphological traits, fresh and dry weights of seedlings

During the laboratory experiment, data were recorded regarding the number of leaves (leaf. seedling-1) by calculating the total number of leaves for each seedling, the length of the hypocotyl (cm. seedling-1) from the point of its connection to the radicals to the tip using a graduated ruler , the length of the radicle (cm. seedling-1) using a graduated ruler, the fresh weight (mg. seedling-1) using a sensitive balance, and the dry weight (mg. seedling-1) after the samples used to measure the fresh weight were dried in an electric oven at a temperature of 65°C until the weight was fixed and the dry weight was calculated using a sensitive balance.

Extraction and estimation of trigonelline alkaloid (micromg.mg-1)

The method of [13] was used in the extraction of trigonelline alkaloid and the method of [14] in the separation technique from fenugreek seedlings (Table 1) using HPLC technique. the method is summarized as follows: 0.5 mg of fresh leaves and stems were weighed for each treatment, and placed in a 100 ml glass beaker, to which 50 ml of T.D.A solution (25 ml tetrahydrofuran, 15 ml distilled water, and 10 ml acetonitrile) was added. The mixture was placed in an electric blender for 5 minutes, and then it was filtered using Wattmun paper. A rotary evaporator was used at 35 °C. To dissolve the precipitate, 20 ml of methanol was added and the pH of the extract was adjusted to 10.5. The extract was centrifuged at 4000 rpm-1 at 4°C for 15 min. The supernatant was discarded and the precipitate was stored in the refrigerator at 4°C until reading.

Table 1: Conditions for separating trigonelline alkaloids using HPLC.

Column Type	Separation Conditions
Separation Column	C-18 (50 X 4.6 mm ID)
Mobile Phase	TetraHydroFuran:Water: Acetonitrile (55:8:37 V/V)
Detector Type	UV Set 215 nm

Flow Rate	1.4m1.min-1
Separation Temperature	25ºC
Sample Volume	25µl
Speed of recording paper on calculator	10 cm/min

Results

Germination percentage (%)

Table (2) This indicates the effect of gibberellic acid, salicylic acid, and their interaction on the germination percentage. The germination percentage rates increase with increasing concentrations of gibberellic acid and salicylic acid. The concentration of 3 ppm of gibberellic acid recorded the highest germination percentage of (78.31%) compared to (49.82%) in the control treatment. A significant positive change is also observed when treating with salicylic acid, as the percentage rates increased to reach (75.76%) at a concentration of 5 ppm compared to the comparison, which recorded the lowest germination percentage of (49.58%). The same table shows the interaction between growth regulators, which enhanced the increase in germination percentage rates, as the percentage rose significantly to (86.82%) in the (3ppm GA3 + 5 ppm SA) treatment compared to the rest of the treatments. As for the seeds to which growth regulators were not added, the germination percentage was found to be the lowest rate of (36.00%).

Number of leaves (leaf.seedling^{-1}).

The results in Table (3) This indicates the number of leaves in fenugreek seedlings treated with various concentrations of gibberellic acid and salicylic acid. A significant increase in the number of leaves was observed when seedlings were treated with 3 ppm of gibberellic acid, recording the highest value of 4.13 compared to the control group, which recorded the lowest value of 2.84 .Treatment with salicylic acid also had a significant effect on seedling growth, recording the highest concentration of 5 ppm of 4.29 compared to the control group, which recorded the lowest rate of leaf number of 2.50 . As for the interaction between gibberellin and salicylic acid, the same table shows that the treatment (3ppm GA3 + 5 ppm SA) recorded the highest rate of leaf number, which amounted to 5.54 , while the lowest rate was in the control group 2.00 .

Hypocotyl length(cm).

Table (4) shows a significant effect of GA3 and SA in the length of the Hypocotyl in fenugreek seedlings, as the results of the treatment with GA3 showed the highest value of 6.36 cm when treated with 3 ppm, while the lowest values were in the control treatment which was 4.24 cm. The effect of the treatment with SA was also observed, as the seedling treatment with 5 ppm recorded the highest value in this trait, recorded 6.33 cm, compared to the control group, which recorded the lowest value of 4.48 cm. As for the combination in the same table, it indicates that the highest rate in the length of the shoot was at the combination (3 GA3 and 5 ppm SA) and recorded 7.40 cm, while the lowest value was recorded by the seedlings not treated with both growth regulators and recorded 3.20 cm.

Radical length (cm).

Table (5) shows the significant effect of GA3 and SA on the radical length in fenugreek seedlings. It was noted from the results that the treatment with GA3 gave the highest rates, which recorded 3.51 cm at a concentration of 3 ppm, compared to the untreated seedling, which gave the lowest value, and recorded 1.37 cm. The same table indicates the presence of a significant difference in the radical length when treating with SA at a concentration of 5 ppm, which gave the highest value to record , 3.18 cm, compared to the control t , which recorded the lowest value 1.93 cm, While the interaction gave a clear significant differences, recording the

highest value at the interaction (3 GA3 + 5ppm SA), which did not differ significantly from the interaction (3 GA3 + 2.5ppm SA) in this trait and recorded 4.00 cm and 3.97 cm , respectively, compared to the lowest value recorded by the control which was 1.00 cm.

Seedling Fresh weight

From the results shown in Table 6, we note the effect of both GA3 and SA and the interaction between them in the fresh weight of fenugreek seedlings. It was noted that with increasing GA3 concentrations, the fresh weight increase, as the concentration of 3 ppm recorded the highest value of (0.43 mg, compared to the lowest value recorded by the control of (0.15 mg. SA also had a significant effect by giving the highest value at the concentration of 5 ppm of (0.45 mg compared to the control, which recorded the lowest value of (0.16 cm . The same table showed the effect of the two-factor interaction, giving the highest value at the interaction (3 GA3 + 5 ppm SA) which recorded 0.64 mg, while the lowest value was given by seedlings not treated with both hormones which recorded 0.08 mg.

Seedling Dry weight

Table (7) shows that there were significant differences in the dry weights of fenugreek seedlings in the presence of growth regulators GA3 and SA. The results showed a clear increase in dry weight when seedlings were treated with 3 ppm ofGA3 and recorded the highest value (0.28 mg compared to the control, which recorded the lowest value 0.08mg .As for the effect of SA, seedlings treated with concentration 5 ppm recorded the highest value (0.29 mg, while the control recorded the lowest value of 0.09 mg. The same table shows the effect of the interaction between the two factors, giving the highest value in the treatment (3 GA3+5 ppm SA)of 0.42 mg, while the lowest value was in the control which is recorded 0.03 mg.

Trigonelline alkaloid (µg.mg-1)

Figure 2 showed the significant effect of GA3 and SA and their interaction on the quantitative content of trigonelline alkaloid in seedlings. It was found that the rates increase with increasing concentrations of gibberellin and salicylic acid, especially the concentration of 3 ppm GA3, which recorded (0.81 µg.mg-1) compared to the control, which recorded the lowest value (0.46 µg.mg-1). The treatment with salicylic acid (SA) at a concentration of 5 ppm recorded the highest rate at 0.86 µg/mg, while the control showed the lowest value at 0.41 µg/mg. As for the interaction between GA3 and SA, it gave the highest value in the (3 GA3+5 ppm SA) treatment, which recorded (0.99 µg.mg-1) compared to the rest of the treatments, while the control recorded the lowest value of trigonelline content, recorded (0.29 µg.mg-1).

Table 2: The effect of GA3 and salicylic acid concentrations and the interaction between them on the germination rate.

GA3	SA			Average
	Control	2.5 ppm	5 ppm	
control	36.00 i	50.33 h	63.15 f	49.82 c
2 ppm	58.22 g	70.00 d	77.31 c	68.51 b
3 ppm	67.42 e	80.65 b	86.82 a	78.31 a
Average	49.58 c	67.00 b	75.76 a	

* Rates sharing the same letters within the main factors or their interactions are not significantly different from each other, as determined by Duncan's multiple range test at the 0.05 probability level.

Table 3: The effect of GA3 and salicylic acid concentrations and the interaction between them on the leaves Number

GA3	SA			Avarage
	Control	2.5 ppm	5 ppm	
control	**2.00 h**	**3.00 f**	**3.52 d**	**2.84 c**
2 ppm	**2.55 g**	**3.20 e**	**4.10 b**	**3.28 b**
3 ppm	**2.95 f**	**3.91 c**	**5.54 a**	**4.13 a**
Average	**2.50 c**	**3.37 b**	**4.29 a**	

*Rates with similar letters within the main factors or their binary interactions are not significantly different from each other according
to Duncan's multiple range test at the 0.05 probability level.

Table 4: The effect of GA3 and salicylic acid concentrations and the interaction between them on the hypocotyl length.

GA3	SA			Avarage
	Control	2.5 ppm	5 ppm	
control	3.20 g	4.32 f	5.20 d	4.24 c
2 ppm	4.99 e	5.55 c	6.40 b	5.65 b
3 ppm	5.25 d	6.44 b	7.40 a	6.36 a
Average	4.48 c	5.44 b	6.33 a	

*Rates with similar letters within the main factors or their binary interactions are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 5: The effect of GA3 and salicylic acid concentrations and the interaction between them on the radical length.

GA3	SA			Avarage
	Control	2.5 ppm	5 ppm	
control	1.00 h	1.31 g	1.80 f	1.37 c
2 ppm	2.21 e	3.10 d	3.75 b	3.02 b
3 ppm	2.57 c	3.97 a	4.00 a	3.51 a
Average	3.18 a	2.79 b	1.93 c	

*Rates with similar letters within the main factors or their binary interactions are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 6: The effect of GA3 and salicylic acid concentrations and the interaction between them in the seedling fresh weight.

GA3	SA			Avarage
	Control	2.5 ppm	5 ppm	
control	0.08 g	0.14 f	0.23 e	0.15 c
2 ppm	0.15 f	0.32 d	0.48 b	0.32 b

3 ppm	0.27 e	039 c	0.64 a	0.43 a
Average	0.16 c	0.28 b	0.45 a	

*Rates with similar letters within the main factors or their binary interactions are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

Table 7: The effect of GA3 and salicylic acid concentrations and the interaction between them in the seedling dry weight**.**

GA3	SA			Avarage
	Control	2.5 ppm	5 ppm	
control	0.03 h	0.09 fg	0.13 e	0.08 c
2 ppm	0.10 ef	0.20 d	0.33 b	0.21 b
3 ppm	0.14 e	0.27 c	0.42 a	0.28 a
Average	0.09 c	0.18 b	0.29 a	

*Rates with similar letters within the main factors or their binary interactions are not significantly different from each other according to Duncan's multiple range test at the 0.05 probability level.

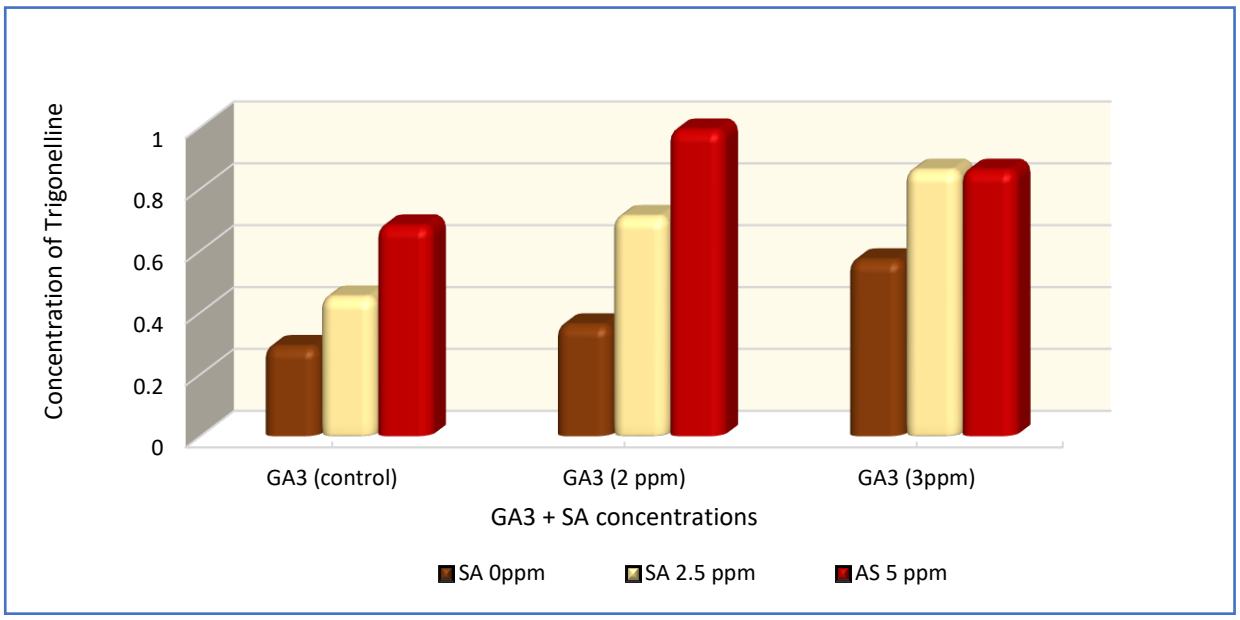

Fig 2. The effect of GA3 and SA concentrations and the interaction between them in t Content the seedlings of the compound Trigonelline

Discussion

Seed germination results from the balance of internal plant hormones in the seed with the wavelength of incident light and low temperature, which stimulates the decomposition of the seed through interconnected molecular pathways that ultimately lead to the rupture of the seed coat and its germination [15]. Gibberellins have a significant effect in breaking seed dormancy by reprogramming plants for higher growth and greatly mitigate the harmful effects of unnatural in the environment [16]. The results obtained in this study can be explained by the role of plant growth regulators in increasing seed germination rates and plant growth, as gibberellic acid is important in germination processes because it improves and increases the ability of seeds to settle and grow within a wide range of environmental conditions suitable for their germination, and it also affects the aleurone layer, which is the surface layer of protein-rich cells located around the endosperm, so the function of the aleurone is to act as a storage tissue before germination and as a source of decomposition

enzymes that are secreted to digest the endosperm reserve during the germination process, which begins its activity after the seeds absorb water..it also enhances the physiological functions of the plant, including its responsibility for stimulating seed germination by controlling the synthesis of enzymes necessary for germination and its effect on amylase in seeds, thus facilitating the germination process [17]. When treating these seeds with gibberellin, it will cause an increase in the activity of the amylase enzyme, which works to decompose the food stored in the endosperm of starch and sugars, thus increasing the percentage and speed of germination [18,19]. These results are consistent with what was stated by [20] that the use of high concentrations of gibberellic acid gives positive results in increasing the germination rate and improving seedling growth. It was also found that gibberellin helps in increasing the rate of cell wall formation and promote changes in lignin content and composition and increasing its division, which leads to increasing the efficiency of the photosynthesis process and the transfer of nutrients, and thus increasing the length of the plant and increasing the length of the frond by activating the elongation of the subapical cell region, in addition to increasing the length of the root, the number of leaves and the fresh and dry weights as a result of improving the overall vital processes of the seedling [21-24] . GA3 increases the elongation of the radical and hypocotyl shoot through its role in increasing cell division and elongation [25]. The results of the studied traits showed a positive effect of SA on the seedling of fenugreek plant, especially at a concentration of 5 ppm, as it works as a plant regulator and a hormonal mediator, as it plays a fundamental role in regulating various physiological processes, including plant growth, ion absorption, and photosynthesis [26]. SA affects the content of photosynthetic pigments and photosynthesis in the seedling, which is positively reflected by increasing the number of leaves, the length of the shoot, and the fresh and dry weights through the response of the entire plant or parts of it to a change in the effectiveness of some enzymes to increase the speed of reactions in all vital processes that occur within the plant, thus increasing the efficiency of growth [27]. In addition to SA, it has a role in increasing the level of auxins and cytokinins, which work to increase cell division and elongation, thus increasing biomass and its superiority in giving the highest rate of fresh and dry weight, which represents a reflection of the increase in the construction of alkaloids. The increase in the total content of the alkaloid trigonelline in the vegetative group may be attributed to the increase in the number of leaves and the fresh and dry weights of the seedling, which is due to the increase in nitrogen, the basic component for building amino acids, which is also followed by an increase in those acids, as alkaloids are produced through a series of biosynthetic processes for essential amino acids, which are considered as initiators or raw materials for their production, and growth regulators (gibberellins) play a role in the enzymatic activity of cells and the production of those acids. Therefore, both GA3 and SA together affect the increase in nitrogen required for building alkaloids, in addition to all other vital processes, thus improving growth characteristics. The studied results are consistent with what was reached by [28,29].

Conclusion

Through the results obtained in the current study, we found that soaking with different levels of gibberellin, especially at a concentration of 3 ppm, led to an increase in the germination percentage and improved the growth characteristics of the seedlings. In addition, spraying fenugreek seedlings with salicylic acid, especially at a concentration of 5 ppm, improved the studied growth characteristics. As for the results of separation and diagnosis using high-performance liquid chromatography (HPLC) technology, the study concluded that the joint interaction between soaking with gibberellin and spraying with salicylic acid, especially at the level, caused a significant increase in the plant content of the medicinal compound (trigonelline alkaloid) in fenugreek seedlings compared to the rest of the treatments.

References

1. Adhikary P, Tarai P. Effects of temperature and gibberellic acid (GA3) on seed germination of Vicia sativa, Chenopodium album and Physalis minima. Int J Agric Environ Biotechnol. 2013;6(4):629-632.

2. Alam MS. Effect of gibberellic acid on morphology, vegetative growth and seed yield of fenugreek (Trigonella foenum-graecum) MD. Shahin Alam registration no. 12-04883. Department of Agricultural Botany, Sher-E-Bangla Agricultural University, Dhaka-1207, Dr. 2018.

3. AL-Khafaji MA. Plant growth regulators, applications and utilization in horticultural. University of Baghdad, Ministry of Higher Education and Scientific Research, Iraq. 2014. p. 1-225.

4. Bushway RJ, Bureau JL, King J. Modification of the rapid high performance liquid chromatographic method for determination of potato glycoalkaloids. J Agric Food Chem. 1986;34:277-279.

5. Castro-Camba R, Sanchez C, Vidal N, Vielba JM. Plant development and crop yield: The role of gibberellins. Plants. 2022;11(19):2650. doi: 10.3390/plants11192650.

6. Dempsey DA, Klessig DF. How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans? BMC Biol. 2017;15:23. doi: 10.1186/s12915-017-0364-8.

7. Du G, Zhang H, Yang Y, Zhao Y, Tang K, Liu F. Effects of gibberellin pre-treatment on seed germination and seedling physiology characteristics in industrial hemp under drought stress condition. Life (Basel). 2022 Nov 16;12(11):1907. doi: 10.3390/life12111907.

8. Edwards EJ, Cobb AH. Current methods of potato glycoalkaloids analysis: problems and potential. Aspects of Applied Biology. 1998;52:331-337.

9. Falcioni R, Moriwaki T, Oliveira DM, Andreotti GC, Souza LA, Santos WD, Bonato CM, Antunes WC. Increased gibberellins and light levels promote cell wall thickness and enhance lignin deposition in xylem fibers. Front Plant Sci. 2018;9:1391. doi: 10.3389/fpls.2018.01391.

10. Farooq M, Awais W, Ma W, Shen S, Gu A. Underlying biochemical and molecular mechanisms for seed germination. Int J Mol Sci. 2022;23(15):8502. doi: 10.3390/ijms23158502.

11. Naqi ZJ, Yahya WH, Abdulhussain AK, Nasir B. A study of the effect of alcoholic extract of celery seeds on the concentration of sex hormones in male white rats treated with depakine. Al-Harf J. 2025;24(1):March 2025.

12. Haq MZ, Hossain MM, Huda MS, Zamal SS, Karim MR. Response of foliar application of GA3 in different plant ages for seed production in black cumin. Eco Friendly Agric J. 2013;6:150-155.

13. Hayat S, Ali B, Ahmad A. Salicylic acid: Biosynthesis, metabolism and physiological role in plants. In: Salicylic Acid: A Plant Hormone. Dordrecht, Netherlands: Springer; 2007. p. 14-27.

14. Jamwal O, Bhattacharya S, Puri S. Plant growth regulator mediated consequences of secondary metabolites in medicinal plants. J Appl Res Med Aromatic Plants. 2018;9:26-38.

15. Jones RL, Carbonell J. Regulation of the synthesis of barley aleurone α-amylase by gibberellic acid and calcium ions. Plant Physiol. 1984;76:213-218. doi: 10.1104/pp.76.1.213.

16. Khaeim H, Kende Z, Balla I, Gyuricza C, Eser A, Tarnawa Á. The effect of temperature and water stresses on seed germination and seedling growth of wheat (Triticum aestivum L.). Sustainability. 2022;14(7):3887. doi: 10.3390/su14073887.

17. Koo YM, Heo AY, Choi HW. Salicylic acid as a safe plant protector and growth regulator. Plant Pathol J. 2020 Feb;36(1):1-10. doi: 10.5423/PPJ.RW.12.2019.0295.

18. Li A, Sun X, Liu L. Action of salicylic acid on plant growth. Front Plant Sci. 2022;13:878076. doi: 10.3389/fpls.2022.878076.

19. Mishra P, Dubey RS. Effect of aluminium on metabolism of starch and sugars in growing rice seedlings. Acta Physiol Plant. 2008;30:265-275.

20. Abdul-Jabbar ZS. The effect of vitamin A dose for different periods on the spleen of white rats: A histological study. Omni Sci: A Multi-disciplinary J. 2025;15(2):8-15.

21. Nguyen V, Taine EG, Meng D, Cui T, Tan W. Pharmacological activities, therapeutic effects, and mechanistic actions of trigonelline. Int J Mol Sci. 2024;25:3385. doi: 10.3390/ijms25063385.

22. Ramawat KG. Plant Biotechnology. New Delhi, India: S. Chand and Company Ltd; 2004. p. 1-265.

23. Reddy PP, Hore JK. Role of growth regulators on fenugreek (Trigonella foenum-graecum L.). Int J Curr Microbiol App Sci. 2020;9(7):25-32. doi: 10.20546/ijcmas.2020.907.004.

24. Selvaraju P, Krishnasamy V. Effect of gibberellic acid on breaking seed dormancy in rice variety ADT38. Modran Agric J. 2005;92(10-12):735-737.

25. Shariatmadari MH, Parsa M, Nezami A, Kafi M. The effects of hormonal priming on emergence, growth and yield of chickpea under drought stress in glasshouse and field. Biosci Res. 2017;14:34-41.

26. Vazirimehr MR, Rigi K. Effect of salicylic acid in agriculture. Int J Plant Animal Environ Sci. 2014;4(2):291-296.

27. Verpoort R, Alfermann AW. In: Metabolism, Engineering of Plant Secondary Metabolism. Kluwer Academic Publishers; 2000. p. 3-8.

28. Hasan KA, Maysoon KA, Taiban ZK. Assessment of vitamin D levels among Hashimoto and hypothyroidism disease in Iraq: A comparative study. Res Rev Life Sci. 2024;14(3):.

29. Xu H, Mo D, Zhang X, Li F, Tao J, Ge P, Yang Y, Wang Z, Zhang Y. Gibberellin treatment accelerates starch decomposition and seed germination in sticky nightshade (Solanum sisymbriifolium Lam.). Horticulturae. 2024;10:1342.