Using Composite Nano silica/Activated Carbon to Alleviate Salt Stress during Seed Germination of Fenugreek

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Abstract

Seed germination reflects an important stage of the whole plant life cycle. So, the main purpose of this experiment was to study the impact of five concentrations of composite nano silica/Activated Carbon**,** sodium silicate (100, 300, 500, 700 and 1000 mgl^{-1}) to compare their effects on alleviating salt stress during seed germination of *Trigonella foenum-graecum*. L under Na^+ stress (100 mM NaCl). From results it was illustrated that both of 300 and 500 mg l^{-1} SiO_2/AC induced final germination% by (45%) compared with untreated seeds. While in case of 300 mg l^{-1} SiO_2/AC significantly increased vigor index, fresh, dry weight of seedlings, and germination index, conversely, the average germination time was lowered by 56,60%. On other side, root shoot ratio was induced with 500 mg l^{-1} SiO_2/AC by 59 and 57 comparing with control.

Keywords: Salinity, fenugreek, germination percentage, SiO_2/AC, Na2SiO_3

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Introduction

Water scarcity is the major concern in various countries across the world, particularly in arid and semi-arid regions (Saad *et al*., 2023). Low-quality water must be used in agriculture due to water constraint, population growth, and agricultural expansion. However, there are a number of dangerous issues with wastewater use. Water with elevated levels of Ec and Na+ can be elevated by the development of novel approaches aimed at enhancing its quality or optimizing its utilization by plants(Paranychianakis & Chartzoulakis, 2005). Salinity has a harmful effect on maximizing or sustainability of agriculture by causing various changes in the cell membrane, the water status, synthesis of enzymes and protein besides gene expression to plants (Adil *et al*., 2023). So, in recent years, researchers used some substances as silica to alleviate salinity stress. Silica considerably alleviates an abiotic stress by preserving water status, regulating necessary nutrients and phytohormones levels, adjust osmotic pressure, reduction ROS by improving antioxidant compounds in addition to stimulate expression of several stress-related genes (Bhardwaj *et al*., 2023). Sodium silicate (Na_2SiO_3) considers one of the conventional source of silica and had ability to alleviate salt stress and exhibited an enhancement in wheat plants productivity that grown under salt stress (Mushtaq *et al*., 2022). Now nano-particles can be used under biotic or abiotic conditions in the agriculture field (Raza *et al*., 2023). Using numerous of bioactive nanoparticles can protect plants from salt stress, improve growth, yield parameter, chemical constituents, seed germination and root anatomy. While the molecular mechanisms of this phenomenon are still unknown, but it has exposed that an application of nanoparticles can adjust some gene expressions related with salt stress (Sarkar & Kalita, 2023). On other hand, nanoparticles have various features like high surface area and solubility, so they have ability to change their physiochemical properties and enhance the plant tolerance to various disease, heavy metals, salt and drought stress (Wang *et al*., 2022). SiNPs reduce both abiotic and biotic stresses by enhancing some secondary metabolites as, ascorbic acid, proline, glutathione, phenolic compounds and phytohormones (Naaz *et al*., 2023), also it have a number of physiological criteria, such as a high surface area, an accumulation, a reactivity, a penetrating efficiency, nano size, and the structure, which enable the particles to penetrate cell wall and adjust the metabolic processes. NPs might penetrate plant cell wall and reach the plasma membrane easily. After nanoparticles accumulate and translocate, some changes in cellular and plant physiological functions were produced (Mittal *et al*., 2020). Activated carbon derived from agricultural waste materials is highly valuable in replacing non-renewable commercial activated carbon in a range of applications due to its affordability, availability, and efficiency (Elkholy *et al*., 2023). Because active carbon increases the availability of nutrients, it benefits a variety of plants, soil pH and promoting plant growth and yield (Dawar *et al*., 2023). Using of composite nano silica/activated carbon (SiO_2/AC) might be a novel way to deal with problem of salinity where pores formation in an activated carbon considers an active site that having a vital role in creation of SiO_2/AC. So, nano silica particles dispersed on surface of the activated carbon and form spherical silica particles in an average of diameter 70 – 80 nm (Maha *et al*., 2022).The stage of seed germination considers an important part of the whole plant life cycle, because of the seeds are the significance of plant being mechanisms and it is the material base for plant productivity (Sun *et al., 2021)*. Plant growth is connected with the various growth conditions that occurred during seed germination (Chenyin *et al*., 2023). Application SiNPs promoted seed germination than conventional silica sources and enhanced seeds germination, fresh and dry weight in the tomato plant, SiNPs gave resistance to NaCl stress (Tina *et al*., 2023).

Fenugreek is an annual plant under *Fabaceae* family. It is one of the most significant medicinal plants in different countries. It is affected by different an environmental stress (Maha *et al.*, 2022). It has various nutritional benefits beside their biological and pharmacological properties for people. Where, it used to improve hyperglycemia, adipocyte differentiation, an inhibition of inflammation. As fenugreek contains numerous chemical contents as carbohydrates, minerals, proteins, amino acids, lipids, alkaloids such as trigonelline, flavonoids, β-carotene, various vitamins, nicotinic acid, fixed and the volatile oils (Ahmad *et al*., 2016). Moreover, Fenugreek seeds has anticancer, antimicrobial ,antioxidant effects declining pancreatic and renal damage (Al Mosawi, 2021).

The present research investigated the effects of different concentrations from sodium silicate and composite Nano silica/activated carbon on seed germination of *Trigonella foenum-graecum* L. under Na^+ stress (100 mM NaCl).

Materials and methods

Obtaining and characterization of materials

- Fenugreek seeds were got from Horticultural Research Institute, Agricultural research center, Egypt.
- Equivalent volumes of sodium chloride (NaCl) (from Sigma-Aldrish) were dissolved in deionized water to create Na+ solution.
- Sodium silicate (Na_2SiO_3) was obtained from Sigma-Aldrich.
- Composite Nano silica/Activated Carbon was used due to high efficacy in alleviate salt stress based on a previous study (Maha *et al*., 2022). For composite SiO2/AC, Zeta potential analysis, Dynamic light scattering was determined. Concerning with TEM, silica particles are scattered over the surface of the activated carbon in the composite nano silica/active carbon and have a tendency to form a spherical silica particle with an average diameter of 70 to 80 nm.

Germination of fenugreek seeds

To compare the effects of 5 different concentrations of sodium silicate, and composite nano silica/Activated Carbon (100, 300, 500, 700, and 1000 mgl^{-1}) on fenugreek seed germination under Na^+ stress, a completely randomized random design with 3 replicates was employed, to compare their effects on seed germination of fenugreek under Na^+ stress (100 mM NaCl) according to (Qados, 2015) and (Adil *et al*., 2023) and arranged in three replicates.

Ten seeds of fenugreek were washed per each replicate carefully with distilled water then immersed in the solution of hypochlorite 10% for five minutes to clean fenugreek seeds' surface before the experiment. Fenugreek seeds were first soaked in suspension (according to each treatment) for 2 hours before transplanting. Then, the seeds were equally dispersed on petri dishes with a filter paper in a 15 cm diameter to let seeds to germinate at the same distance from each other; next each petri dish were received with 15 mL of Na^+ solution amended with five different concentrations of composite Nano silica/Activated Carbon and sodium silicate with (100, 300, 500, 700 and 1000 mgl^{-1}) according to treatment in addition to control that received 15 mL of Na^+ solution. All petri dishes were good sealed to prevent any drying and maintained moist and wet across 14 days that is the experiment period. All petri dishes were put in a dark place with a room temperature of 23 ±2 C.

Germination indicators were be calculated:

Final germination percentage (FGP) be calculated as percentage using the equation (Santana & Ranal, 2006):

$$\textbf{Final germination percentage (FGP)} = \frac{\text{Total n. of germinated seeds}}{\text{total n. of planted seeds}}$$

Mean germination time (MGT) be calculated with (Mauromicale & Licandro, 2002):

$$\textbf{MGT}=\sum\frac{ni*ti}{ni}$$

Where, ni = number of fenugreek germinated seeds on day; ti = days number during the germination period (0 and 14 days)

Germination index (GI) was calculated according to (Islam *et al*., 2009) by subsequent formula:

$$\textbf{GI}=\frac{\text{number of seedlings emerging on day}}{\text{day after planting}}$$

Vigor Indexes:

vigor indexes of fenugreek seed germination (Emamverdian *et al*., 2021)

Vigor Index I= Germination percentage ×seedling length (mm)

Seed Vigor II = Germination percentage ×seedling dry weight (g)

Vegetative parameters:

Plumule length, Radicle length: On the 14th day of germinate, seedlings were randomly selected to measure plumule length and radicle length

Root-shoot ratio**:** The root to shoot ratio from each treatment for each seedling was calculated as follows: **Root to Shoot Ratio=**$\frac{Radicle\ length\ (mm)}{Plumule\ length\ (mm)}$

Fresh and dry weight:

On the 14th day of germination, 5 seedlings were randomly selected from each replicate to record Fresh weight and dry weight. For recording dry weight, the seedlings were kept in an oven at 60^{0}c, till constant weight was obtained.

Statistical Analysis

With three replicates, the 11 treatments were be arranged in a fully randomized block design. Software for statistical analysis, Costate (2005), was used to analyze the data. Analysis of variance was applied to every multiple comparison (ANOVA). Duncan's multiple range test was used to compare means at the P level of 0.05.

Results and discussion

Final germination percentage (FGP)

Data in Figure (1) exhibited the final germination percentage of fenugreek seeds after treating with various concentrations from composite nano silica/Activated Carbon and sodium silicate recorded the higher FGP values comparison to untreated seeds (control). Though, the treatments of 300 and 500 mg L^{-1} SiO_2/AC achieved 95% FGP, while control seeds were germinated by 65.50% Figure (2). Our recent data agree with those reported by (Almutairi, 2016), (Alsaeedi *et al*., 2019) (Liu *et al*., 2022) and Kumar *et al*., 2023). Salt stress alleviation by nanoparticles, through its entering the cytoplasm by some unknown membrane channels and its interaction with biomolecules to regulates some gene expression and also improves antioxidant activity of some enzymes (Sarkar and Kalita, 2023). Similarly, this may indicate that the increasing Na+ reduce a relative water content in the seedlings. NaCl delay an initiation of the seed germination of fenugreek seeds and exhibited a decreation in its FGP (Cokkizgin, 2012). In similar condition, (Kaymakanova, 2009) found that a negative impact of NaCl on germination of common bean seeds might be due to an inhibition of water absorption by seeds an effect of increment the osmotic potential of a solution and/or ionic effect in which Na^+ and Cl^- concentrate in the plant tissues, lead to an inequity in the uptake of various nutrients and poisonous impact and improve water permeability in the seed coats, cell walls, or organelle membranes by nano materials (Kumar *et al*., 2023).

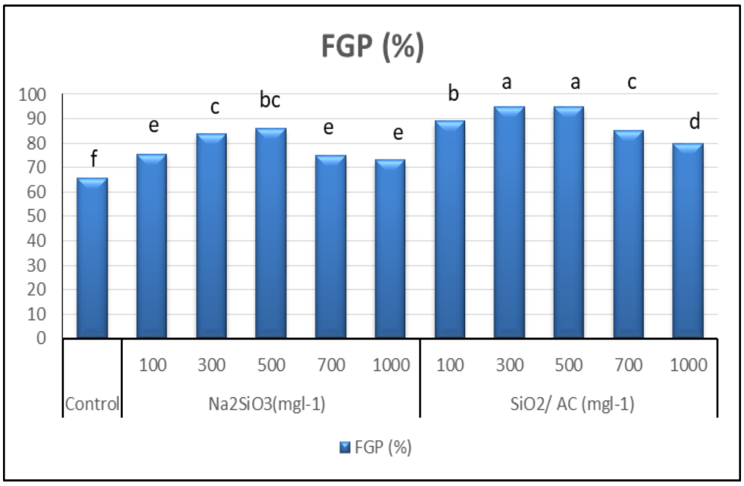

Fig. (1): Effects of Na_2SiO_3, SiO_2/AC on final germination percentage (FGP) in fenugreek seeds under salinity stress (100 mM NaCl).

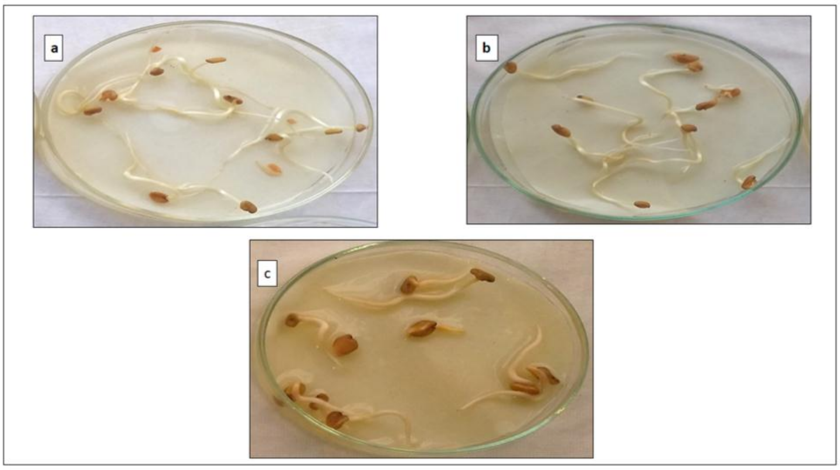

Fig. (2): Germination of *Trigonella foenum-graecum*. L, under under Na^+ stress (100 mM NaCl), where a, b: 300 and 500 mg L^{-1} SiO2/AC, respectively., c: control.

Mean germination time (MGT)

Mean germination time which defined as the time that the seed wants to start and end its germination is represented in Figure (3). The shorter the germination time, the better, therefore there are positive impact observed by adding different concentrations from SiO_2/AC or Na_2SiO_3 that lead to decrease the MGT and this will reflect on all vigorousness of the seedlings and lastly the productivity of plant. Figure (3) showed MGT of fenugreek seeds among the germination. Applying 300 mg L^{-1} SiO_2/AC have a positive effect on reduces the MGT and needed only 2.65 days. On other hand, control seeds required 4,15 days for complete germination. In this current study, adding different concentrations from SiO_2/AC or Na_2SiO_3 induced MGT compared to untreated seeds. Our data are same with Alsaeedi *et al*. (2017), reported that MGT of *Phaseolus vulgaris* seeds was reduced when seeds were treated with NSi decreased MGT at Na^+ concentrations. Shortest mean germination on the same way, the results conducted by Alsaeedi, *et al*. (2019) on cucumber seeds and Kumar *et al*. (2023) on sweet corn.

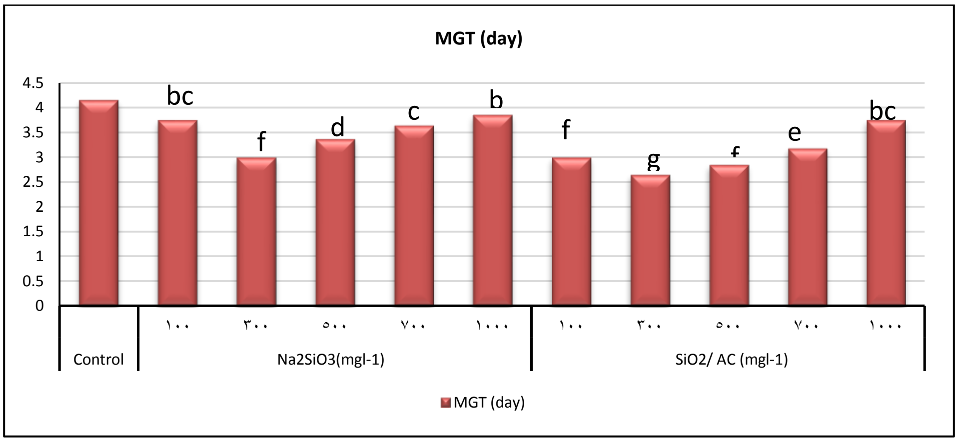

Fig. (3): Effects of Na_2SiO_3, SiO_2/AC on mean germination time (MGT) in fenugreek seeds under salinity stress (100 mM NaCl).

Germination index (GI)
The germination index defined as a role of germination percentage and rate of germination. Low results represent the delay of most fenugreek seeds germinate, while high data represented that majority seeds germinate first with a small time (Al-Mudaris and Jutzi, 1998). Results in Figure (4) illustrated that adding different concentrations from SiO_2/AC or Na_2SiO_3 significantly increased GI compared with control seeds that decreased GI data with 1.57, while when using SiO_2/AC with the concentration of 300 mg L^{-1} increased GI to be 3,58 as the highest value among all treatments. Our results agreed with the findings of (Alsaeedi, *et al*. 2019, Emamverdian *et al*., 2020**;** Kumar *et al*., 2023). Exogenous Si has effects on many vital processes inside the seed as embryo viability, reserve mobilization, activity of hormones and enzymes, the membrane integrity, antioxidant metabolism, and regulation of some gene expression in seed germination (El Moukhtari *et al*., 2023).

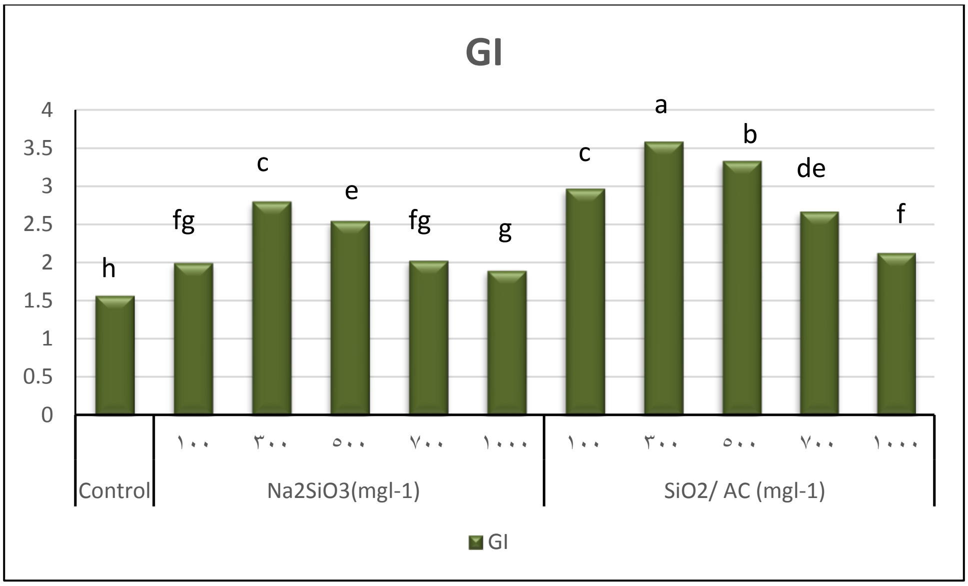

Fig. (4): Effects of Na_2SiO_3, SiO_2/AC on germination index (GI) in fenugreek seeds under salinity stress (100 mM NaCl).

Vigor Indexes

Vigor Index (I) and Vigor Index (II)
Results in table (1) showed vigor indexes during seed germination. According to the results, adding different concentrations from SiO_2/AC or Na_2SiO_3 significantly improved seed vigor in fenugreek seeds under Na stress. The values showed that application 300, 500 mg L^{-1} SiO_2/AC improved vigor index (I) and (II) significantly by 180.72 and 145.77 %, respectively, compared with control treatment. If the higher the vigor index, the seedling will be strong. Our results are supported by (Naguib and Abdalla, 2019) and Kumar *et al*. (2023). Vigor Index (I) and (II) are consider an important sign on seed germination, due to its ability to determine if seedlings will be able to grow well or not after germination. Plants exposed to stress, activate many defense mechanisms to increase their tolerance against the stress. Furthermore, various genes are activated and produced several proteins (Wang et al., 2003). NSi might be entered directly or indirectly in the morphological changes and the physiological processes in plants (Almutairi, 2016).

Vegetative parameters

Plumule length, radicle length, and Root-shoot ratio

It was obviously clear in Figure (5) and Table (2) that application of SiO_2/ AC gave positive values for root length, Where at 500 mgl^{-1} reached to 9,15mm. On other hand, the application of 1000 mgl^{-1} from SiO_2/ AC or Na_2SiO_3 had a negative effect on these traits. Referring to plumule length, it was showed that 300 mgl^{-1} SiO_2/ AC induced the length by 68,75% comparing with control. Concerning with, Root-shoot ratio, different concentrations from SiO2/AC or Na_2SiO_3 significantly increased Root-shoot ratio compared with control seeds by 59.57%. It was observed that there is no significant effect between 300 and 500 mg $L^{-1}$$SiO_2$/AC. While, control seeds recorded lowest ratio as shown in figure (6). Our results are in line with (Almutairi *et al*., 2016) that explained tomato seedling have enhancing in root length and fresh weight after application of N-Si under NaCl as N-Si decreased the inhibitory effects of salt stress. It was found that using of SiO_2/ AC has a positive effect on saline tolerance, root growth, yield parameters of fenugreek (Maha *et al.*, 2022).

Table (1): Effects of Na_2SiO_3, SiO_2/AC on Vigor traits in fenugreek seeds under salinity stress (100 mM NaCl)

Treatment		Vigor index I	Seed Vigor II
control		721 h	6.03h
Na_2SiO_3	**100 (mgl^{-1})**	1038 f	7.93g
	300 (mgl^{-1})	1470cd	10.33de
	500 (mgl^{-1})	1505d	9.46ef
	700 (mgl^{-1})	1226e	7.48g
	1000 (mgl^{-1})	913g	7.23g
SiO_2/ AC	**100 (mgl^{-1})**	1691b	12.28c
	300 (mgl^{-1})	1810a	14.82a
	500 (mgl^{-1})	2024a	13.59b
	700 (mgl^{-1})	1585c	10.97d
	1000 (mgl^{-1})	1010e	9.36f

Values are means of 3 replicates. In a row, means with different letters are significantly different according to ANOVA.

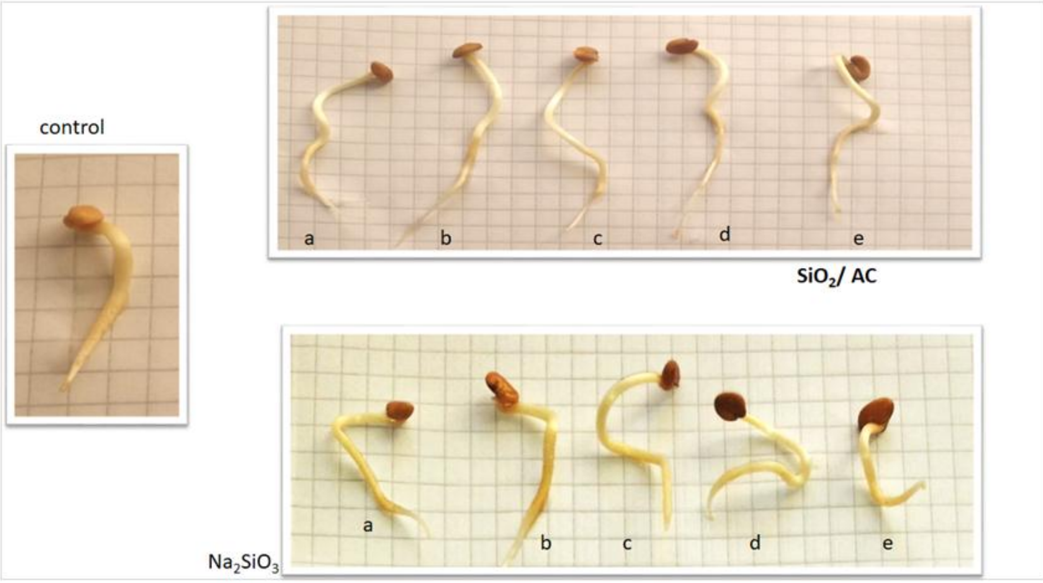

Fig. (5): Seed germination of *Trigonella foenum-graecum*. L after 4 days, under con. (100, 300, 500, 700 and 1000 mgl^{-1}) for a, b, c, d, e respectively.

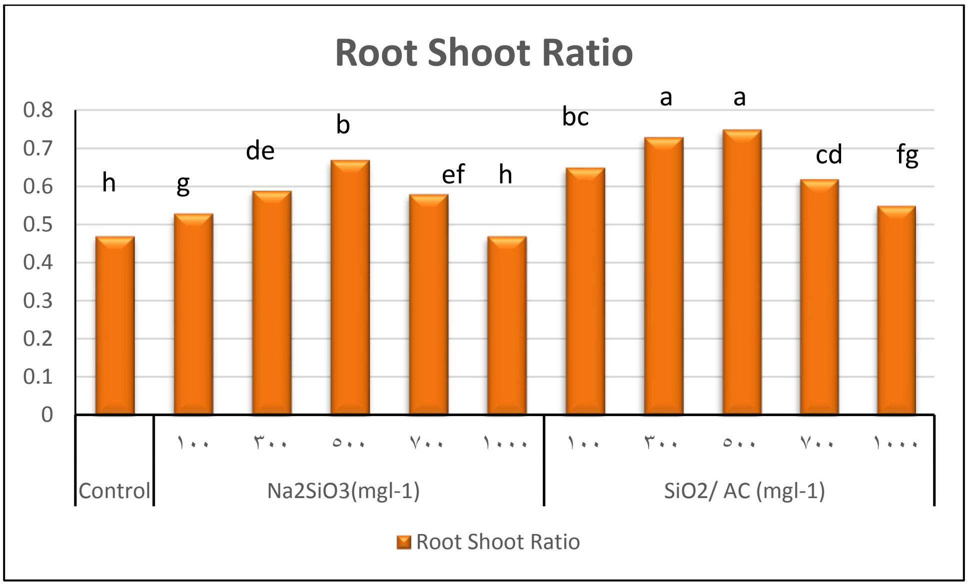

Fig. (6): Effects of Na_2SiO_3, SiO_2/AC on root shoot ratio in fenugreek seeds under salinity stress (100 mM NaCl).

Fresh weight and Dry weight

Values in table (2) represented that, as concentration up to 300 mgl^{-1} from SiO_2/ AC increased fresh and dry weight of seedling by (66.66 and 69.56) compared with control. These results are matched with (Almutairi *et al*., 2016; (Farhangi-Abriz & Torabian, 2018). To alleviate stress, the systemic signals pathways as electricity, calcium, ROS, and hormones are be changed (Fichman & Mittler, 2020) as the surface area of SiO2/AC is 660.33 m^{2}g^{-1} as discussed before (Maha *et al*., 2022). Hence , composite nano silica activated carbon enhance seedling tolerance toward salt stress improving growth by decrement ROS production (

Wang et al., 2022), improving different antioxidant enzymes activities (Naguib and Abdalla, 2019), increasing K^+ uptake and decreasing Na^+ uptake (Shahzad *et al*., 2012). Furthermore, the activated carbon has appositive effect on the callus induction and regeneration of tissue culture medium of rice, because it had randomly pores on the surface (Chutipaijit & Sutjaritvorakul, 2018).

Table (2): Effects of Na_2SiO_3, SiO_2/AC on vegetative parameters of fenugreek (at 14 days after germination) under salinity stress (100 mM NaCl)

Treatment		Radicle length (mm)	plumule length (mm)	Fresh weight (g)	Dry weight (g)
Control		3.50g	8.00g	0.138g	0.092i
Na_2SiO_3	**100 (mgl^{-1})**	4.75e	10.00ef	0.157ef	0.105gh
	300 (mgl^{-1})	6.50d	12.00bc	0.176d	0.123de
	500 (mgl^{-1})	7.00cd	10.50de	0.161e	0.11fg
	700 (mgl^{-1})	6.00d	10.40de	0.146fg	0.10ghi
	1000 (mgl^{-1})	4.00fg	9.50f	0.140g	0.099hi
SiO_2/ AC	**100 (mgl^{-1})**	7.50bc	12.50b	0.198b	0.138bc
	300 (mgl^{-1})	8.05b	14.00a	0.230a	0.156a
	500 (mgl^{-1})	9.15a	13.50a	0.218a	0.143b
	700 (mgl^{-1})	7.15c	11.50c	0.190bc	0.129cd
	1000 (mgl^{-1})	4.50ef	10.85d	0.178cd	0.117ef

Values are the means of 3 replicates. In a row, means with different letters are significantly different according to ANOVA.

Conclusions

Seed germination has a vital role in plant growth and development, as the most critical stages in the life cycle of the plant. So, this work illustrated that the use of SiO_2/AC enhanced the salinity tolerance in fenugreek seeds germination. Application of 300, 500 mg L^{-1} SiO_2/AC have significant effect on FGP, GI and vigor index with reduced germination time.

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إستخدام مركب النانو سيليكا /الكربون المنشط لتخفيف إجهاد الملح أثناء إنبات بذور الحلبة

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الملخص العربي

يعكس إنبات البذور مرحلة مهمة من دورة حياة النبات بأكملها. لذا أجريت هذه التجربة لدراسة تأثير خمسة تراكيز من مركب النانو سيليكا/الكربون المنشط وسيليكات الصوديوم (١٠٠، ٣٠٠، ٥٠٠، ٧٠٠ و١٠٠٠ ملغم$^{-١}$) مقارنة بالكنترول لمقارنة تأثيرها في تخفيف الإجهاد الملحي أثناء إنبات بذور الحلبة باستخدام ١٠٠ ملي مولار كلوريد صوديوم. من النتائج تبين أن كلا من ٣٠٠ و ٥٠٠ ملجم لتر$^{-١}$ من مركب النانو سيليكا/الكربون حفز نسبة الإنبات النهائي بنسبة (٤٥%) مقارنة بالبذور غير المعاملة. في حين أن معدل ٣٠٠ ملغم/لتر$^{-١}$ من نفس المركب أدي إلى زيادة معنوية في مؤشر النشاط والوزن الطازج والجاف للشتلات ومعامل الإنبات، وعلى العكس من ذلك فقد أدى إلى إنخفاض متوسط زمن الإنبات بنسبة ٥٦.٦٠%. من جهة أخري حفز معدل الجذر للسويقة بإستخدام ٥٠٠ ملجم لتر$^{-١}$ من مركب النانو سيليكا/الكربون مقارنة بالكنترول بنسبة٥٩.٥%.

الكلمات الدالة: الملوحة، الحلبة، نسبة الإنبات، النانو سيليكا/الكربون المنشط وسيليكات الصوديوم.