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Titanium Dioxide Nanoparticles mitigates the adverse effects of salinity stress on Grass pea (*Lathyrus sativus* L.) Germination and Seedling development

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ABSTRACT

In the current examination, we assessed the impacts of titanium dioxide nanoparticles (TiO2 NPs) for ameliorating salinity in Grass pea (*Lathyrus sativus* L.), a useful legumes crop. Experimental salinity was controlled in terms of dSm^{-1} at levels of 0 (control), 4, 8 and 12 dSm^{-1}, have been researched in a factorial experiment based on randomized complete block design with three replications. The impacts of four distinctive TiO2 NPs concentrations (0, 20, 40 and 60 mg L^{-1}) were contrasted with deference with plant development and stress reactions. Germination and seedling emergence percentage was fundamentally affected by 12 dSm^{-1} of NaCl. Presentation to NaCl stress essentially decreased length of grass pea seedling organs (root and shoot) comparatively, the substance of proline expanded for the most part in the roots from moderate saltiness conditions. In any case, foliar of nano-TiO2 conquered pernicious impacts of salt pressure and improved the vast majority of these parameters. The best outcomes were found at 20 mg L^{-1} nano-TiO2. Generally, utilization of Tio2 was useful in expanding saltiness resistance in Grass pea seedlings and their application may incite a reasonable strategy to ensure plants against sodium harmfulness.

INTRODUCTION

Around the world, extra than forty 5,000,000 hectares of watered land have been harmed by utilizing salt, and 1.5 million hectares are removed from creation every year because of extreme saltiness levels in the dirt (Munns and Analyzer 2008). Expanding saltiness resilience of the world's principal nourishment crops is an indispensable objective of plant researchers as the total populace is expanding extra rapidly than the place that is known for agricultural land to help it (FAO 2010).

Grass pea (*Lathyrus sativus* L.) is a multipurpose durable grain legume crop (Almeida et al. 2015). It has a high healthy benefit (protein content beginning from 25 to 30%), being essential each for human nourishment and creature feed (Almeida et al. 2015). Notwithstanding its uses as nourishment and feed, advantageous interaction with rhizobia allows in an effective nitrogen obsession inside the dirt, bringing down the information sources required in crop pivot and making them reasonable to be utilized as unpracticed excrement in economical cultivating frameworks (Hanbury et al. 2000). The grass pea is blessed with numerous properties that join to make it an engaging nourishment crop in dry spell stricken, downpour took care of locales wherein the dirt quality is poor and extreme natural conditions win (Palmer et al. 1989).

Nanoparticles (NPs) are wide class of materials that incorporate particulate substances, which have one measurement under 100 nm in any event (Laurent et al. 2010). NPs are not basic atoms itself and consequently made out of three layers for example (a) the surface layer, which might be functionalized with an assortment of little particles, metal particles, surfactants and polymers. (b) The shell layer, which is artificially unique material from the center in all angles, and (c) The center, which is basically the focal part of the NP and typically alludes the NP itself (Shin et al. 2016).

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NPs with various structure, size, and fixation, physical/ chemical properties have been accounted for to impact development and advancement of different plant species with both high-caliber and negative impacts (Ma et al. 2010). Nanoparticles were embroiled in agribusiness for improving harvests (Hojjat and Kamyab 2017; Hojjat and Hojjat 2015; Hojjat 2015; Hojjat and Hojjat 2016).

Nano-TiO2 is an environmentally friendly material. It has wide application esteem in numerous fields because of its incredible basic, optical, and chemical properties. The photocatalytic procedure of nano-TiO2 changes over light vitality into electrical or chemical energy under mellow conditions. As of late, the examination and use of nano-TiO2 in the agrarian sector have gently attracted consideration. Nano-TiO2 uses of corrupting pesticides, plant germination, and development, crop sickness control, and so forth are appropriately viewpoint (Giorgetti et al. 2019). In spite of the fact that the capability of Tio2 NPs in improving saltiness opposition has been accounted for in a few plant groups (Karami and Sepehri 2018; Lin and Xing 2007), its role in the mitigation of saltiness sway and related components is as yet obscure. The expansion of TiO2 particles in the sludge-amended soil, further modified plant development, and achieved oxidative and ultrastructural harms (Karami and Sepehri 2018). NP-TiO2 can invigorate cell reinforcement framework, improve capacities of engrossing and using water, and hurry germination and development in Glycine max (Kim et al. 2010). NP-TiO2has assorted consequences for redox systems oxygen (ROS) within the sight of UV radiation (Feizi et al. 2013). NP-TiO2 has expanded impact on seed germination of fennel (*Foeniculum vulgare*) (Zheng 2005). NP-TiO2 could support and quicken seed germination through helping water assimilation in spinach seeds (Hurby 2002). The examination affirmed that the impacts of TiO2on plants increment chlorophyll in paprika (*Capsicum annuum* L.) and green growth (*Chlorella pyrenoidosa*) (Lenient 2013). The soaking of flax seeds inside the suspensions of anatase nanoparticle has increased affected seed germination and root development (Feizi et al. 2013). NP-TiO2 has expanded effect on seed germination of fennel (*Foeniculum vulgare*). Use of mass TiO2 particles in forty ppm consideration focus enormously constructive outcomes happened as far as shoot dry weight and germination rate (Mahmoodzadeh et al. 2013). A few study of has been done on the impacts of NP-TiO2and TiO2on different plants, for example, *Triticum aestivum*, *Zea mays*, *Salvia officinalis* (Morteza et al. 2013). Utilization of forty ppm nanosized TiO2 treatment improved mean germination time by 31.8% in contrast with the untreated control (Mahmoodzadeh et al. 2013). The use of Nanomaterial in mix improved the germination rate, shoot and root length, seedling new weight and seedling dry weight and seedling dry substance of grass pea seedlings under focused on conditions (Hojjat 2019).Therefore, the principle goal of this work was to consider the Titanium Dioxide Nanoparticles mitigates the antagonistic impacts of salt weight on Grass pea (*Lathyrus sativus* L.) Germination and Seedling advancement.

MATERIALS AND METHODS

Field Location: This examination was conducted under the laboratory conditions of Research Center for Plant Sciences, Ferdowsi University of Mashhad, during 2019 year. The targets of the experiment were to assess the impact of Titanium Dioxide Nanoparticles mitigates the the unfriendly impacts of salt weight on Grass pea (*Lathyrus sativus* L.) Germination and Seedling improvement.

Sodium Chloride Solutions and Characterization: Water saltiness was typically described by electrical conductivity (EC) strategies. High purity sodium chloride [NaCl] was procured from Merck, Darmstadt, Germany or J.T. Baker, USA. To test normal saltiness stress, four centralizations of NaCl arrangements were set up as estimated regarding dSm^{-1}: 0 (control), 4, 8 and 12 dSm^{-1}. Deionized water filled in as the control. The measured electrical conductivity of the DI water was 0 dSm^{-1}.

TiO2Nanoparticle Solutions and Characterization: Commercial powder of TiO2 was purchased from Iranian Nanomaterials Pioneers Inc. (Mashhad, Iran). Titanium Oxide (TiO2, 80 vol% anatase + 20 vol% rutile, Virtue: 99+%, APS (D50): 20 nm, SSA: 10 - 45 m2/g, Shading: white, Mass Thickness: 0.46g/ml, PH: 5.5-6.0). Four centralizations of TiO2Np (0, 20, 40 and 60 mg L-1 by mass). TiO2Np were suspended in DI water and scattered with the guide of ultra-sonication (100 W @ 30 kHz) for an hour. Titanium Dioxide Nanoparticles were assessed by transmission electron magnifying instrument (TEM) procedure and x-beam diffraction (XRD), gave by seller information (Figure 1.).

Seeds and Experimental Conditions: Grass pea seeds were obtained from the Seed Bank Research Center for Plant Sciences, Ferdowsi University of Mashhad (Figure 2.). All seeds were first checked for suitability by suspending them in DI water. The seeds that settled to the base of the glass were chosen for additional examination. Along these lines, practical seeds were washed in DI water. Seeds were then disinfected at interims in sodium hypochlorite [NaOCl] 5–wt. % answer for ten minutes (Rehman 1996) and afterward washed multiple times in DI water.

Seeds had been sharpened by the method for placing them in particular test nanoparticle solutions (0, 20, 40, and 60 mg L^{-1}) and mixed for 1 h using a magnetic stirrer. Semipermeable paper (Whatman No. 2) was put in standard scale Petri dishes (100 mm × 15 mm). 25 seeds were set equally over each paper and secured with another layer of Whatman No.2-channel paper forming a sandwich. Five mL of the proper TiO2 NPs arrangement were added to each Petri dish utilizing Air displacement micropipettes. Following situation of the seeds on the filter paper, the filter papers were soaked with the appropriate test saline solutions. Temperature during germination was held at 25 ±1 °C (Marlia et al. 2009). Three reproduces of each testing condition have been accommodated investigation. Nanoparticles were included 0.25 mL portions each other day to the comparing Petri dish for 3 weeks to guarantee newness. Comparing NaCl arrangements were additionally included each other day at the pace of 0.25 mL^{-1} .

Parameters measured in this study are listed as follows: Germination indexes (GI), germination rate index (GRI), Mean germination time (MGT) Root and Shoot Length Seedling Vigor Index I (SVI-I), Fresh and Dry Mass and Proline estimation:

- Germination index (GI) = (20×N1) + (19×N2) + ... + (1×N20); where N1, N2 ...N20 is the number of germinated seeds on the first, second and subsequent days until 20th day and the multipliers (e.g. 20, 19 ...etc.) are weights given to the days of the germination (Al-Mudaris 1998).

- Germination rate index (GRI) = $G1\times1^{-1}+G2\times2^{-1}+.... + Gi\times i^{-1}$; where G1 is the germination percentage on day 1, G2 is the germination percentage at day 2; and so on (Bates 1973) [30].

- Mean germination time (MGT) = $\Sigma Fx\times\Sigma F^{-1}$; where F is the number of seeds germinated on day (Bates 1973).

- Root and Shoot Length: Root length values were gotten by measuring the distance between the hypocotyls and the end tip of the root. Shoot length was estimated from the root–hypocotyl move zone up to the base of the real cotyledons. Root and shoot lengths were assessed following the three-week growth period.

Fresh and Dry Mass: The fresh mass was measured through weighing in precision scale, and the dry mass was resolved through weighing in a precision scale after the perpetual of the material in a kiln with air forced circulation, at a temperature of 70°C, until constant weight. Toward the finish of analysis, radical and plumule length and fresh weight were measured. Each condition was afforded three replicates.

Proline estimation: Proline content was estimated by the technique of (Maas and Hoffman 1977), 100 mg of plant material (shoots or roots) were homogenized in 5 ml of 3 % aqueous sulfosalicylic acid and centrifuged at 4 C for 15 min at 4800 g. 2 ml of extract was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in test tubes. Samples were kept for 1 h at 100 C. The response was terminated in an ice bath. 4 ml of toluene was used for reaction mixture extraction. The absorbance of color reaction product was measured at 520 nm using toluene for a blank. The proline concentration was determined from a calibration curve. Calibrations were made with proline (Sigma-Aldrich Chemie, GmBH, and Steinheim, Germany) as a standard.

Statistical Analysis: Statistical analysis every treatment was conducted with three repeats and the results were presented as 'mean ± SD (Standard Deviation)'. The results were analyzed by ANOVA by the use of Minitab Adaptation 16.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) shown that saltiness gets to be controlled in expressions of dSm^{-1} at degrees of (control), all (100 %) seeds developed (Table 1). in any case, with creating salt concentration (12 dSm^{-1}), the germination percentage decreased. The GRI, reflecting the rate of germination on each day of the germination period, diminished underneath NaCl, and at the highest concentrations, influenced the dry weights of the complete seedlings. Examining the basis morphology affirmed that the whole root term in all cures was the foremost influenced root parameter, as appeared by using F-ratios (Table 1). Application of salt stress significantly deferred the time to realize 50% germination (Figure 3). Because it might be gathered from Figure 3a, TiO2 NPs (20 mg L^{-1}) essentially advanced seed germination compared to untreated control beneath normal condition. NaCl induced direct (8 dSm^{-1}) and serious (12 dSm^{-1}) saltiness stress resulted in significant decreases in germination percentage.

Among the treatments, 20 g L^{-1} of Tio2 recommended Germination indexes, germination rate index, mean germination time, Seedling Vigor Index. Moreover, TiO2 NPs -intervened compelling results have been concentration-dependent with 20 mgL^{-1} TiO2 NPs being the most successful, while 40 mg L^{-1} showed a middle of the road response and 60 mgL-1was nearly ineffectual

Table 1. The mean comparison of germination parameters of Grass pea influenced by different concentrations of TiO2 nanoparticles under salinity stress

		%Germination		Speed Germination		Fresh weight		Dry weight		Root length		Shoot Length	
Source	DF	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
NaCl	3	99.27	3.07**	5.013	47.3**	0.486	11.08**	0.003	2.86**	159.81	7.78**	334.55	7.49**
TiO2 NPs	3	43.33	1.34*	0.798	7.53**	0.164	3.075**	0.001	0.097*	131.38	6.39**	261.38	5.85**
NaCl*TiO2 NPs	9	40.07	1.24*	0.195	1.85*	0.0643	1.47*	0.0049	4.53**	27.93	1.36*	65.83	1.47*
Error	32	32.29				0.0439		0.001		20.55		44.69	
Total	47												

*, ** Significant at the 0.05and 0.01 probability level, respectively. Ns Not significant.

under both control and saline conditions. As shown in Figure 3, germination energy altogether expanded in non-stressed seeds by adding 20 mg L^{-1} TiO2 NPs to the test medium, but application of TiO2 NPs at 60 mg L^{-1} essentially decreased germination energy in Grass pea seeds beneath control condition. Application of TiO2 NPs at 20 mgL^{-1} significantly decreased the germination rate in Grass pea seeds beneath ordinary conditions. Our results appeared that the application of TiO2 NPs advanced seed germination and early development of Grass pea under control condition and salt stress created by NaCl solutions (Figures 3). Beneath non-stressed condition, as it were the application of TiO2 NPs at 20 mgL^{-1} in combination with SNP essentially abbreviated cruel germination time. But beneath direct and extreme salt stress conditions, the application of TiO2 NPs at 20 decreased mean germination time up to 60% in Grass pea seeds (Figure 3). Salt stress initiated by NaCl solutions essentially diminished the length of roots and shoots.

The 20 mgL^{-1} TiO2 NPs application essentially expanded shoot length, leaf range and root dry weight of plants beneath ordinary conditions (Figure 3). In later a long time, different analysts have considered the impacts of nanomaterials on plant germination and development to advance the utilization of nanomaterials for agricultural applications (Dehkourdi and Mosavi 2013). Comes about gotten in this study shown that interaction of salt × TiO2 had a noteworthy impact on development indices in all the cases [$p<0.01$]. In any case, the application of distinctive concentrations of TiO2 NPs essentially improved seed germination rate up to 10% and 33% beneath moderate (8 dSm^{-1}) and (12 dSm^{-1}) salt push invigorated by NaCl arrangements (Figure 3). Under control condition, the application of TiO2 NPs at 60 mg L^{-1} had a significant negative impact on root length, but the application of TiO2 NPs at 20 mg L^{-1} application of diverse concentrations of TiO2 essentially improved root length. In the nearness of direct and serious salt stress created by NaCl solutions, root length significantly increased by up to 20% when seeds were treated by TiO2 NPs (Figure 3). Under the non-stressed conditions, the application of TiO2 NPs at 20 mg L^{-1} significantly increased shoot length. Shoot new weight altogether progressed up to 60% and 75% respectively, under seeds were treated by TiO2 NPs. Under control condition, application of TiO2 NPs at 40 and 60 mg L^{-1} had no significant positive affect on vigor file but TiO2 NPs at 20 mg L^{-1} essentially improved vigor index in Grass pea seeds. Improved seed germination and seedling development have been detailed in plants upon presentation to TiO2 NPs (Faraj and Sepehri 2019; Feizi et al. 2012; Mahmoodzadeh and Aghili, 2014). A significant increment within the proline substance of Grass pea plants was watched within the reaction of TiO2 and NaCl. Grass pea plants proline contributes to stabilizing subcellular structures in cell cytosol and adapt with cell lack of hydration by amassing of proline beneath saltiness push. Concurring to the two-way interaction impact of TiO2 and NaCl, with the rise of saltiness concentration, the proline substance expanded. Application of TiO2 at 20, 40 and 60 mg L^{-1} improved proline substance by almost 22.45, 8.65 and 6.12%, respectively under 12 dSm^{-1} NaCl. Plants differ significantly in their tolerance to salinity, as reflected of their different growth responses. The comprehensive overview of salt resilience of crops and field species distributed by the US Saltiness Laboratory (USDA-ARS. 2005; Rahnama et al. 2010), presents a threshold saltiness underneath which there's no reduction in yield and after that a straight decrease in yield with expanding saltiness. Saltiness diminishes the rate of leaf extension, and closes stomata and in this manner Limiting Factors in photosynthesis, through the soil, water deficit caused by the osmotic stress (Munns and James 2003). Upon presentation to soil saltiness, plants gather poisonous concentrations of Na^+ in leaves, which force an extra restriction to development by diminishing the longevity of photosynthetic tissues (Greenway and Munns 1983). The control of Na^+ transport and its successful exclusion from the mesophyll cells of leaves is subsequently an imperative requirement for saltiness tolerance. Salinity inhibits seed germination and changes the physiology and anatomy of the plants coming about in diminished crop yield.

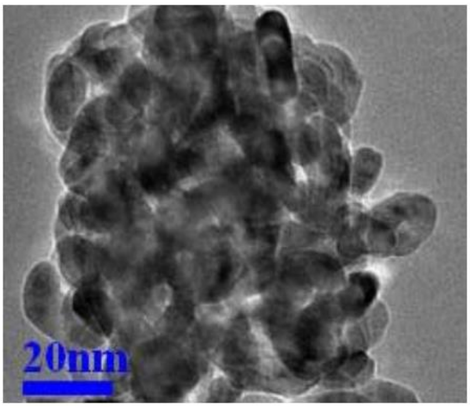

Figure 1. Titanium Oxide (TiO_2, 80 vol% anatase + 20 vol% rutile), Purity: 99+%, APS(D50): 20 nm SSA: 10 - 45 m2/g, Color: white, Bulk Density: 0.46g/ml, PH: 5.5-6.0, Loss of weight in drying: 0.48%, Loss of weight on ignition: 0.99%.

The presence of salts within the soil solution decreases the potential of the plant to soak up water, and this leads to a decrease in the growth rate, referred to as the osmotic or water-deficit impact of saltiness. On the off chance that an excessive sum of salt enters the plant within the transpiration stream, it causes damage to cells within the transpiring takes off, coming about in encouraging diminishment in plant development. Usually called the salt-specific or ionic impact of saltiness (Khot et al. 2012). TiO2 nanomaterials can initiate active oxygen, counting superoxide and hydroxide anions, within the photocatalytic process, which increments the seed stress resistance and water and oxygen admissions. Zheng et al. reported the impacts of TiO2 photocatalysts on the development of spinach seeds (Pais 1983). They demonstrated that the nano-TiO2-treated seeds that have been produced from plants that had a higher dry weight, higher photosynthetic rate, and expanded chlorophyll formation. This proposed that TiO2 nanomaterials advanced the absorption of inorganic nutrients and expanded the photosynthetic rate (Tune et al. 2012). In addition, moo concentrations of TiO2NPs advanced plant development of Lemna minor, whereas tall concentrations, inhibited it. (Gl et al. 2012). TiO2 nanoparticles stimulated plant development at moo concentrations but hindered plant development at tall concentrations (Yang et al. 2007).

Moreover, the fresh weight, dry weight, and protein in spinach were clearly increased (Raliya et al. 2015). These results demonstrated a critical increment in plant growth for plants that were treated with TiO2 nanoparticles. Within the control, plants exposed to TiO2 nanoparticles appeared significant improvements in shoot length and root length (Larue et al. 2012). An increase in spinach dry mass and mung bean (*Vigna radiata* L.) shoot

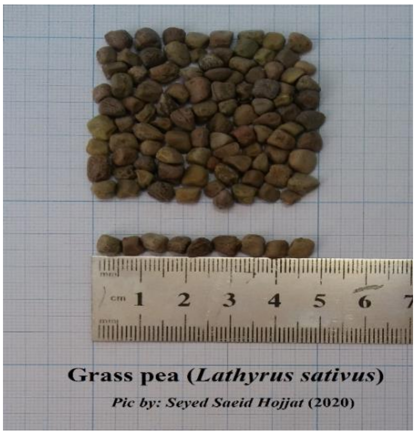

Figure 2. Grass pea seeds

and root length in reaction to TiO2 NPs has been detailed(Larue et al. 2012). Under saltiness stress conditions, plants treated with 20 ppm TiO2 NPs had more proline and much less control (12 dS/m) substance as compared to untreated plants. It has appeared that foliar connected NPs can enter the leaves through stomatal openings and after that are translocated to various tissues by means of the symplast and/or apoplast (cell divider and intercellular space) pathways (Gao et al. 2008). When found interior the photosynthetic cells, TiO2 could increment Rubisco activity and indeed its mRNA expression and advance photosynthesis rate, the possible reasons behind increment in shoot dry mass of treated dragonhead plants (Zhang et al. 2008; Raliya et al. 2015). Moreover, TiO2 NPs in step with se may also act as nano-nutrient fertilizer to improve biomass production by invigorating plant metabolic activities (Hojjat 2019). In expansion, NPs can sequester nutrient components on their surface and serve as a nutrient stock to the plants (Karami and Sepehri 2018). TiO2 NPs may actuate oxidation-reduction reactions by the superoxide ion radical amid germination, coming about in rummaging free radicals within the sprouting seeds and oxygen produced in such preparation might moreover be expended in the respiration, which would advance enhance germination (Khot et al. 2012).

It appears that TiO2 NPs may regulate plant development and play a role similar to plant hormones such as cytokinin and gibberellin as demonstrated by the capacity to induce plant cell division and cellular improvement (Sauret-Güeto et al., 2012). The shoot and root lengths of L. sativa had been moved forward as much as were 49% and 62%, respectively, at the treatment of soil with

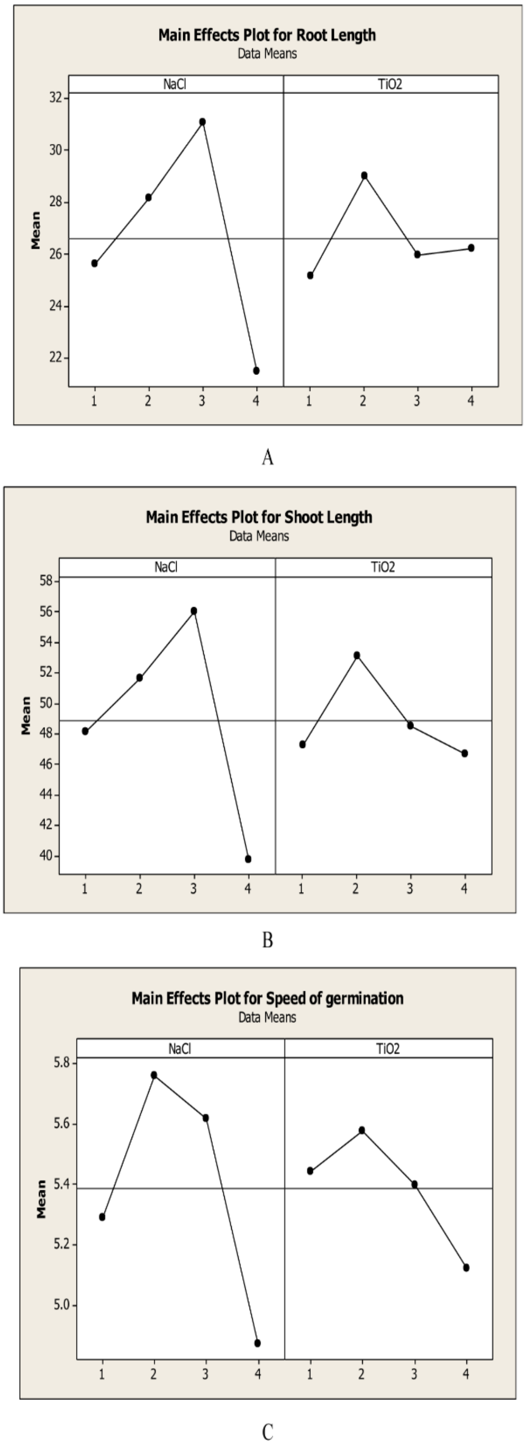

Figure 3. Interaction Effects (Nano Silver & NaCl) on Grass pea (*Lathyrus sativus* L.) Seed.
A) effects on the Root length (mm); B)effects on the shoot length (mm); C) effects on the Speed of germination

TiO_2 NPs of sizes less than 65 nm at concentration 100 mg TiO_2 NPs kg^{-1} soil, as compared to the control treatment. Titanium applied at low dosages can be considered as valuable the detail that progresses different physiological characteristics such as plant biomass and yield, Chl substance (Carvajal et al. 1994; Hrubý et al. 2002) and basic component contents, in spite of the fact that at higher concentrations it is phytotoxic (Hrubý et al. 2002; Zheng et al. 2005). The acceptance of proline amassing in response to n-TiO_2 may be due to an actuation of proline synthesis through the glutamate pathway. There are a few reports that affirmed proline content was advanced in plants treated with n-TiO_2 (Mohammadi et al. 2016).

CONCLUSIONS

Grass pea (*Lathyrus sativus* L.) may be a multipurpose strong grain legume crop tall nutritional value being important both for human food. In arranging to get it the conceivable positive impacts of applying Titanium Dioxide Nanoparticles mitigates the adverse impacts of salt stress on Grass pea Germination and Seedling improvement, it is imperative to analyze the penetration and transport of nanoparticles in plants. To the most excellent of our knowledge, this study is the first report illustrating TiO_2 NPs have invigorating impact on salinity in Grass pea plants. Our results showed that seed germination and early seedling growth of Grass pea were adversely influenced by NaCl-stimulated salt stress in a dose-dependent way. In any case, the application of TiO_2 NPs altogether improved Grass pea germination and growth beneath salt push. In conclusion, it seems that the application of TiO_2 NPs can altogether reduce the adverse impacts of salt stretch on seed germination and early seedling development of Grass pea.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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