

## Combined effect of silicon dioxide nanoparticles and plant-derived smoke solution on physiological and biochemical parameters of pea plant (*Pisum sativum* L.)

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### Abstract

Nanomaterials exhibit distinctive advantages due to their characteristic size range falling within 1-100 nanometers, which can easily penetrate through plant cell membranes. The application of plant-derived smoke (PDS) solutions is also recognized for its beneficial impact on seed germination and growth of diverse plant species. In this context, in the present study, we investigated the effects of silicon dioxide nanoparticles (SiO<sub>2</sub> NPs), PDS, and their combined application on pea seeds, and thereafter evaluated a spectrum of morphological and biochemical growth parameters. The results demonstrated that SiO<sub>2</sub> NPs significantly enhanced pea seed germination, seedling length/weight, secondary root formation, as well as key biochemical indicators including photosynthetic pigments, total soluble sugars, and protein content. Notably, the PDS solution also exerted a significant positive influence on all growth parameters in comparison to that of SiO<sub>2</sub> NPs. However, the combined application of SiO<sub>2</sub> NPs and PDS exhibited superior effects on the morphological and biochemical growth characteristics as compared to their individual applications. From these findings, it can be concluded that both SiO<sub>2</sub> NPs and PDS solutions, whether used independently or in combination, impart beneficial effects on the morphological and biochemical parameters of pea plants. This research highlights the potential of SiO<sub>2</sub> NPs and PDS solutions as promising tools for enhancing plant growth and seedling development. Future studies could further explore the underlying mechanisms and optimize application methods for maximizing the beneficial effects of these materials, thus contributing to sustainable agricultural practices and improved crop yields.

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## Introduction

Nanotechnology advancements have enabled the creation of specific nanoparticles with particular characteristics and a broad range of practical uses (Rai et al., 2018). The use of nanotechnology in modern agriculture has advantages for the ecosystem as well. As an interdisciplinary area of science, nanotechnology is being used as a potent method to empower the agriculture industry by increasing

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crop production and reducing the use of pesticides (Chinnamuthu and Boopathi, 2009; Mousavi and Rezaei, 2011; Tudi et al., 2021). Plant nanoparticles are rapidly being used as ingredients in novel fertilizers and products to extend the life of fresh flowers (Song et al., 2021). Nanomaterials have been proposed as possible bio-stimulators for improving plant multiplication and development (Saha and Dutta-Gupta, 2018; Thangavelu et al., 2018). Using nanomaterials in the agricultural and horticultural sectors could have a number of benefits, but it also raises some concerns due to their unfavorable environmental effects (Feregrino-Perez et al., 2018).

Silicon (Si) is an important element for growth of plants, particularly for those belonging to families Gramineae and Cyperaceae (Rajput et al., 2021). Furthermore, it may alleviate the negative effects of biotic and abiotic stresses, and thus actively or passively improving the plant's response to environmental challenges. For example, it stimulates root elongation and relieves saline stress by lowering salt accumulation (Liu et al., 2015; Siddiqui et al., 2020). Silicon is essential for many physiological and metabolic activities in crops (Bao-Shan et al., 2004). For example, in one study, application of Si-based nanoparticles (Si-NPs) improved seed germination and photosynthetic pigments in *Zea mays* (Yuvakkumar et al., 2011). Si-NPs emerged as a unique Si source that can improve plant stress tolerance. Furthermore, the size, form and other features of Si-NPs play a differential role in plants (Rastogi et al., 2017). In terms of performance, soil-applied Si-NPs were proven to be more efficient than foliar-applied Si-NPs (Suriyaprabha et al., 2014). For example, *Cymbopogon citratus* grew faster and produced more oil after being treated with Si-NP (Mukarram et al., 2021). It promoted *Avena sativa* growth and lignification in plant tissues (Asgari et al., 2018). *Helianthus annuus* seed priming and seed soaking in Si-NPs increased shoot and root lengths, mass, and vigour index (Janmohammadi and Sabaghnia, 2015).

However, there is still room for development, particularly in terms of expanding attempts to boost food crops in order to satisfy the rising demands of an ever-growing population. Application of plant-derived smoke water (PDS) plays a beneficial role in plant growth. For example, PDS can enhance seed germination and plant growth (De-Lange and Boucher, 1990). For example, smoke solution improved seed germination and germination rate in a variety of agricultural and horticultural crops (Antala et al., 2019; Khatoon et al., 2020). This technique is an excellent alternative to conventional agricultural practices in promoting crop growth, because it is less expensive, easily accessible, and effective in obtaining improved yields. Thus, the major goal of this study was to examine how and up to what extent SiO<sub>2</sub> NPs and PDS individually and in combination affected seed germination and the initial growth phase of pea.

## Materials and Methods

### Collection of nanoparticles

Silicon dioxide nanoparticles (SiO<sub>2</sub> NPs) were taken from the Department of Chemical Sciences, University of Lakki Marwat, KP, Pakistan. The size of nanoparticles ranged 10-20 nm, with a surface area of 70 m<sup>2</sup>/g and purity 99.5%.

### Preparation of PDS solutions

The protocol devised by Tieu et al. (1999) was used to treat aqueous smoke solutions of *Cymbopogon jwarancusa* leaves with slight modifications. Shade dried leaves weighing 333 grams were burnt up in the forge chimney. To provide heat, an electric heater was used, which slowly burned the *C. jwarancusa* leaves. The resulting smoke was gathered in a glass beaker containing 1 liter of pure water after being transmitted through the chimney conduit. This smoke stalk solution was diluted to 1:500 for further use.

### Preparation of SiO<sub>2</sub> NPs suspensions in plant-derived smoke solution

To avoid particle aggregation, 40 mg/L SiO<sub>2</sub> NPs concentration was prepared by suspending them directly in diluted plant-derived-smoke solution (1:500). Nanoparticles were dispersed with a high speed homogenizer for 10 minutes at 5000 rpm speed, and stored them at 4 °C.

### Experimental procedure

The PDS was prepared by burning semi-dried *Cymbopogon jwarancusa* leaves in a furnace. The Arid Zone Research Institute, Rata Kulachi, D.I. Khan, Khyber Pakhtunkhwa, Pakistan, provided seeds of cv. Climax-PF-400 of pea. The pea seeds were subjected to surface sterilization by immersing them in 10% sodium hypochlorite solution for 5 min, and then they were gently rinsed with distilled water. The surface sterilized pea seeds were sown in Petri dishes and treated with SiO<sub>2</sub> NPs and PDS both individually or in combination for 10 days. The experiment was carried out in a laboratory at relative

humidity of 50-60% and a temperature of  $25 \pm 2$  °C. The following four treatments were used: T1 (seedlings treated with DW), T2 (seeds treated with SiO<sub>2</sub> NPs 40 mg/L), T3 (seeds treated with PDS), and T4 (seeds treated with a combined solution of PDS and SiO<sub>2</sub> NPs).

### Measurements of morphological parameters

Morphological characteristics of the pea plants from each treatment were measured. The seeds of pea were tested for seed germination and seedling growth after 10 days of treatment with PDS and/or NPs. Shoot and root lengths were measured, and then shoots and roots of the seedlings were separated, and their fresh weights recorded. In addition, the number of leaves and secondary roots were recorded.

### Leaf chlorophyll contents

Photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids) were estimated using the procedures of Bruinsma (1963). A proportion of 0.2 g of fresh leaf was homogenized in 10 mL of ice-cold acetone using a pestle and mortar. Plant extract was then filtered through a filter paper (Whatman No. 1). The absorption spectrum of the plant extract filtrate was quantified at different wavelengths (645, 652, and 663 nm). The amount of photosynthetic pigments was determined as mg per g fresh mass.

### Biochemical attributes

#### *Soluble sugar content*

Total soluble sugars were extracted from the leaves of pea plants of each treatment in 80% ethyl alcohol. The plant extract was treated with acidic anthrone to quantify total soluble sugars (Liu et al., 2022). Dry leaf material was ground into fine powder and an amount of 0.5 g dry leaf powder was mixed with 10 mL of 80% ethyl alcohol in a test tube and heated at 80 °C in a water bath for one hour. In each of the test tubes, 0.5 mL of the extract was treated with 0.5 mL of distilled water and one mL of 18% phenol, and the mixture heated at 25 °C for one hour. The absorbance of the treated mixture was measured at 490 nm using a spectrophotometer.

#### *Protein content*

The Bradford (1976) method was used to determine the total soluble protein content in leaves, with bovine serum albumin (BSA) as the standard. A spectrophotometer with software (Version 3.0.25.0) was used to assess the protein content.

### Detection of cell membrane injury

Cell membrane injury was measured using the standard protocol of Yang et al. (1996). The shoot and root tissues were cut into small pieces (approximately 1 cm each) and placed in 20 mL of distilled water in each of test tubes. The test tubes were incubated at 10 °C for one day, after which time electrical conductivity "EC-1" of the extracts in the test tubes was noted with a conductivity meter (UTECH CM-645D). All samples in test tubes were autoclaved and then placed at room temperature for 10-15 minutes. Electrical conductivity "EC-2" was again measured in all samples. Cell membrane injury was then calculated using the following formula:

$$EC-1/EC-2 \times 100$$

### Statistical analysis of data

The data obtained from different assays were subjected to a one-way analysis of variance (ANOVA) using a statistical computer package GenStat 8<sup>th</sup> edition version 8.1 software. The means of each treatment were computed and then compared using the Duncan's Multiple Range test (DMR) at the 5% level of significance.

## Results

### Effect of SiO<sub>2</sub> NPs and PDS on seed germination of pea

The present study demonstrated that a 1:500 dilution of PDS had a more significant effect on pea seed germination compared to that of 40 mg/L of SiO<sub>2</sub> NPs, resulting in percent changes of 60% and 25%, respectively (Figure 1). Furthermore, the combination of smoke with SiO<sub>2</sub> NPs significantly increased the pea seed germination rate after 24 and 48 h (Figure 1). The results indicated that the combined solution of PDS + SiO<sub>2</sub> NPs exhibited the highest germination rate, followed by that by PDS alone and SiO<sub>2</sub> NPs alone, with increases of 33.5%, 27.3%, and 11.3% after 24 h, and 50%, 45.4%, and 29.3% after 48 h, respectively, compared to their respective controls.

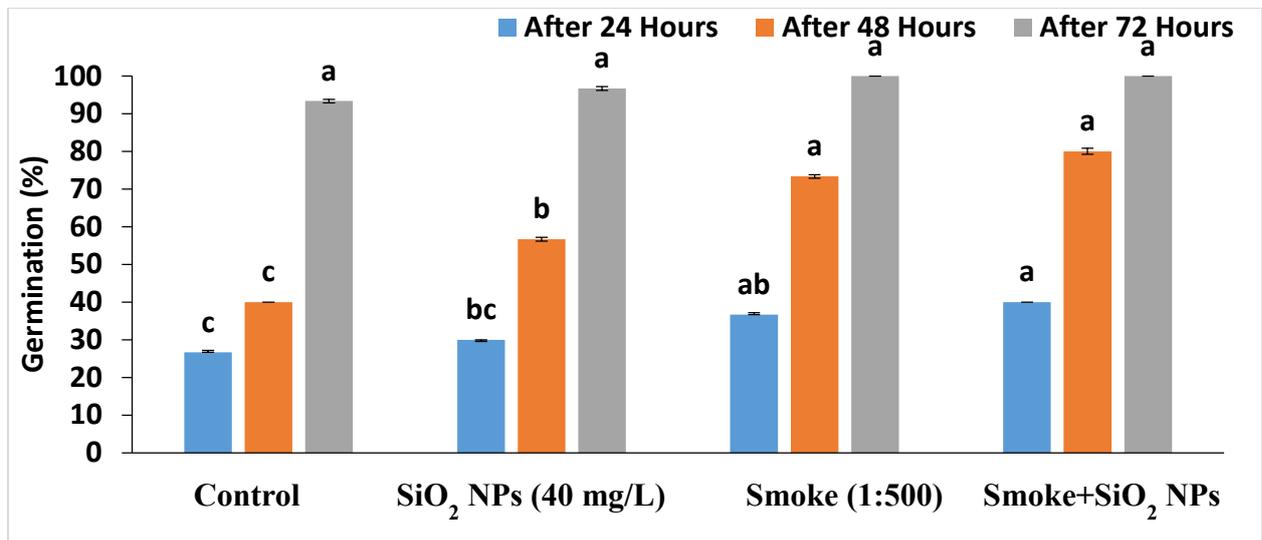


Figure 1. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on pea seed germination rate after 24, 48 and 72 h. The X-axis represents the time intervals (24, 48, and 72 h), while the Y-axis indicates the germination rate. Error bars indicate the variability in the data. Statistical analysis was performed using one-way ANOVA, demonstrating significant differences (*P* < 0.05) among the groups.

**Effect of SiO<sub>2</sub> NPs and PDS on seedling growth of pea**

An increase in shoot and root lengths was observed due to the treatments involving SiO<sub>2</sub> NPs and PDS solutions. Specifically, the combined treatment of SiO<sub>2</sub> NPs + PDS solution resulted in a maximal shoot and root lengths of 7.37 cm and 8 cm, respectively. These lengths represented 35% and 26.46% increase in shoot and root length, respectively, compared to the control group. Similarly, the treatment with PDS + SiO<sub>2</sub> NPs combined solution led to the highest shoot and root weights of 0.34 mg and 0.32 mg, respectively, resulting in 38.23% and 32.25% increase in shoot and root weights compared to the control group (Figures 2-5).

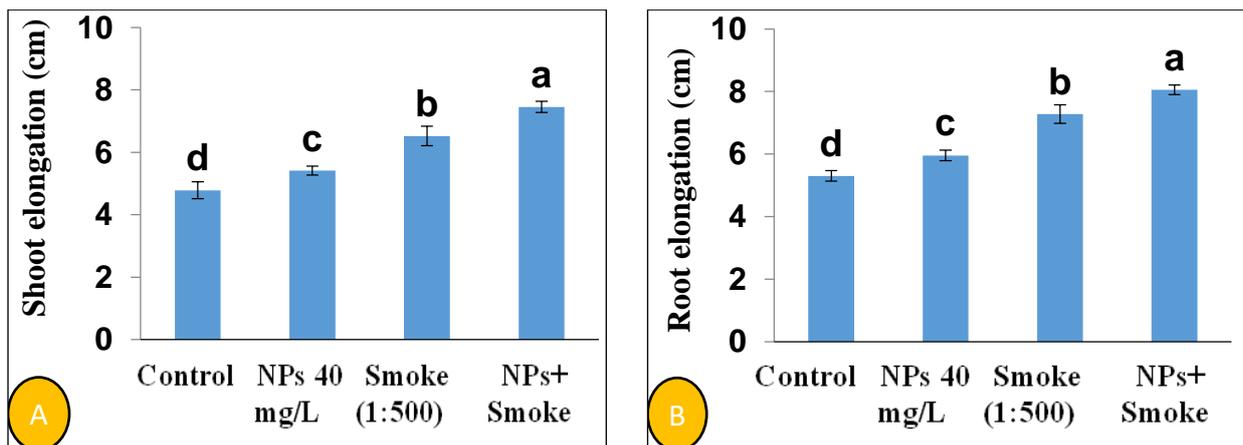


Figure 2. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on (A) shoot and (B) root elongation of pea seedlings. Error bars represent standard deviations, and statistical analysis revealed significant differences through one-way ANOVA (*P* < 0.05).

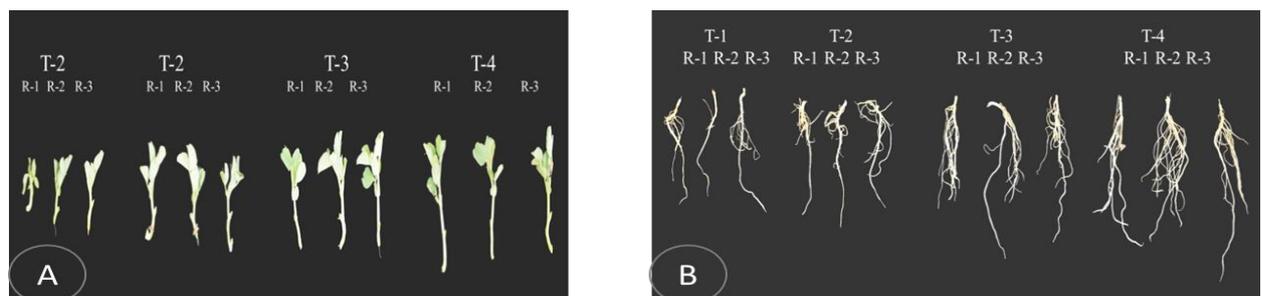


Figure 3. (A) Shoot and (B) root lengths of plants from all four treatments, i.e., untreated control group T1 (seedlings treated with DW), T2 (seedlings treated with SiO<sub>2</sub> NPs 40 mg/L), T3 (seedlings treated with PDS), and T4 (seedlings treated with a combined solution of PDS and SiO<sub>2</sub> NPs).

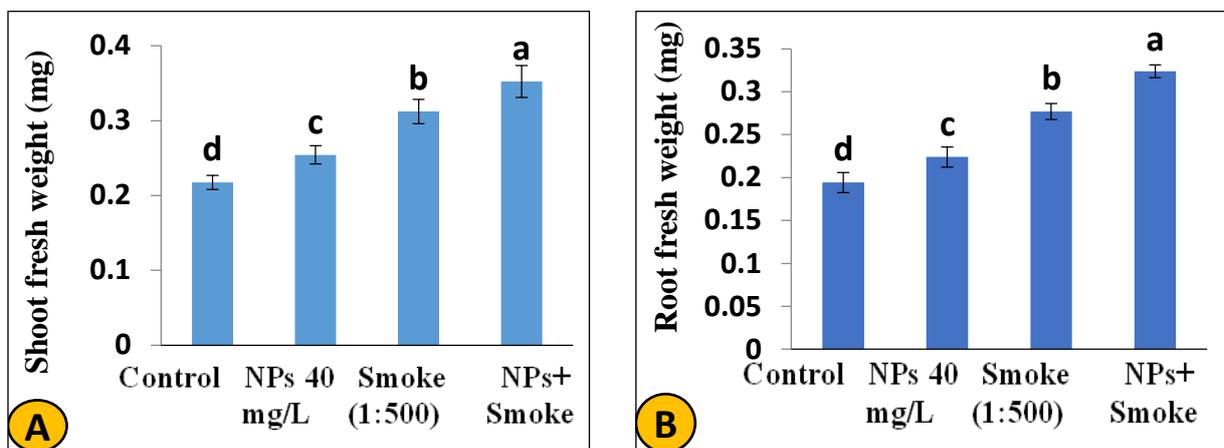


Figure 4. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on (A) shoot fresh weight (B) root fresh weight of pea seedlings. Error bars represent standard deviations. Statistical analysis revealed significant differences through one-way ANOVA ( $P < 0.05$ ).

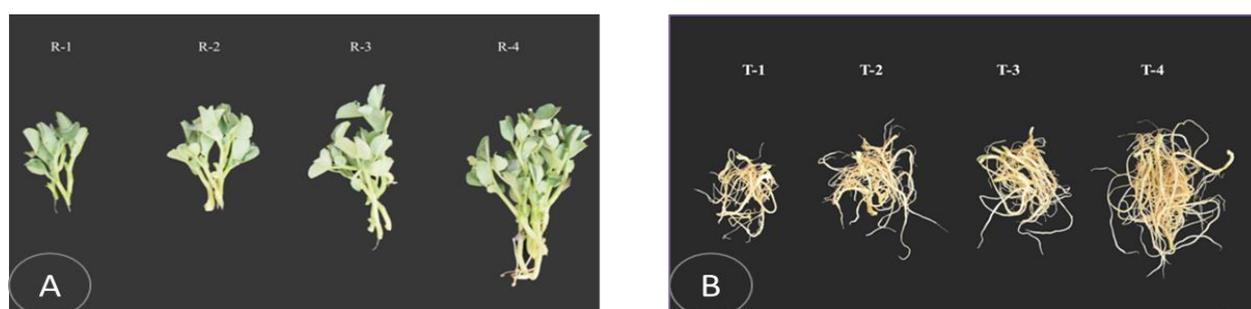


Figure 5. Shoot (A) and root (B) fresh weights of plants from all four treatments, i.e., untreated control group T1 (seedlings treated with DW), T2 (seedlings treated with SiO<sub>2</sub> NPs 40 mg/L), T3 (seedlings treated with PDS), and T4 (seedlings treated with a combined solution of NPs + PDS).

#### Effect of SiO<sub>2</sub> NPs and PDS on number of secondary roots and leaves of pea plants

Total number of secondary roots were substantially greater in plants that had been exposed to a combined treatment of SiO<sub>2</sub> NPs and PDS. In the combined treatment of PDS and SiO<sub>2</sub> NPs, the increase in secondary roots was 37.5% (Figure 6); this treatment also resulted in more secondary roots than those of the control and other treatments.

#### Effect of SiO<sub>2</sub> NPs and PDS on photosynthetic pigments of pea leaves

In all treatments, the levels of chlorophyll a, chlorophyll b, and carotenoids were higher than those in the controls. The treatment NPs + PDS exhibited the highest amounts of chlorophyll a, chlorophyll b, and carotenoids, with increases of 35.5%, 37.83%, and 42.8%, respectively, compared to the control.

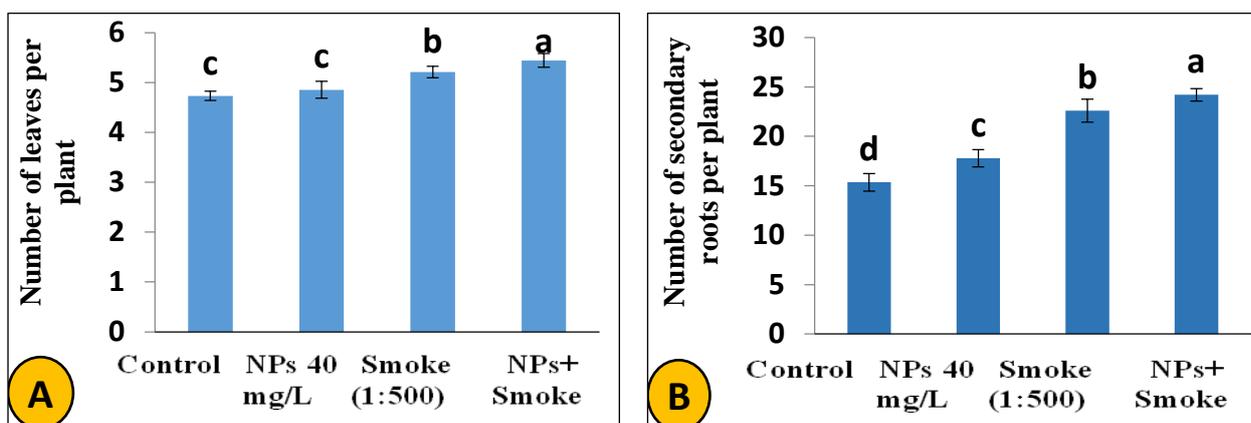


Figure 6. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on total number of leaves per plant (A) and number of secondary roots per plant (B) of per pea seedlings. Statistical analysis was performed using one-way ANOVA, which revealed a significant difference among the groups at  $P < 0.05$ .

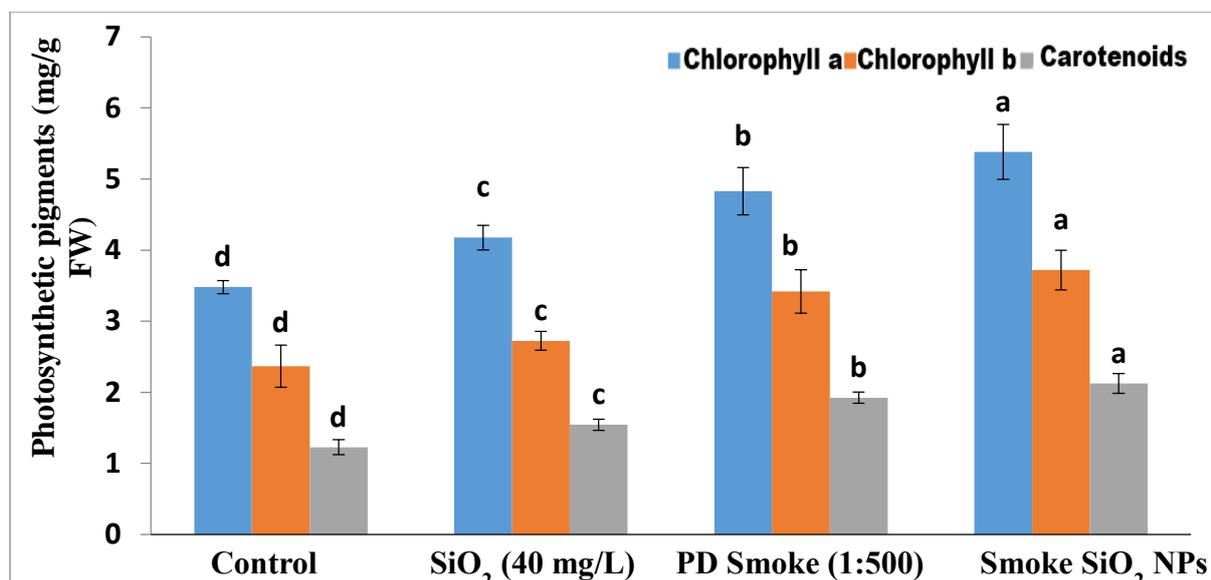


Figure 7. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on photosynthetic pigments of pea plants. Statistical analysis was performed using one-way ANOVA, which revealed a significant difference among the groups at  $P < 0.05$ .

#### Effect of SiO<sub>2</sub> NPs and PDS on total soluble protein and sugar content

Total soluble protein content differed greatly across all treatments applied to the pea seedlings. In the 10-day old plants, total soluble protein contents were found 0.77, 0.67, 0.52 and 0.47 mg/g FW in the treatments PDS + SiO<sub>2</sub> NPs, PDS alone, SiO<sub>2</sub> NPs alone, and control, respectively (Figure 8). Similar pattern was found for total soluble sugar contents. Total soluble sugars were found as 0.707, 0.65, 0.52 and 0.46 mg/g FW in PDS + SiO<sub>2</sub> NPs, PDS alone, SiO<sub>2</sub> NPs alone and control, respectively (Figure 8).

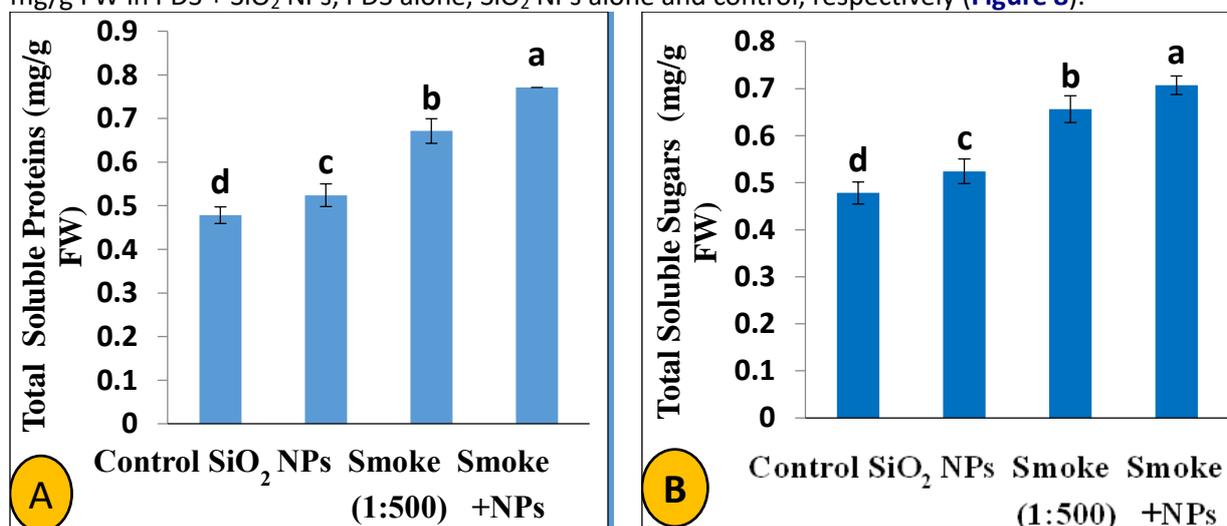


Figure 8. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on soluble protein content (A), and soluble sugars (B) of pea plants. Statistical analysis was performed using one-way ANOVA, which revealed a significant difference among the groups at  $P < 0.05$ .

#### Effect of SiO<sub>2</sub> NPs and PDS on cell membrane injury of pea plants

This study clearly showed that all the treatments applied to the pea seedlings had no major effect on cell membrane injury. The cell membrane injury test findings suggested that PDS in combination with SiO<sub>2</sub> NPs had no adverse effect on the vigor of pea seedlings. Despite the lack of statistical significance, cell membrane injury level of the pea seedlings treated with SiO<sub>2</sub> NPs and PDS alone and in combination were slightly lower than that of the control seedlings (Figure 9).

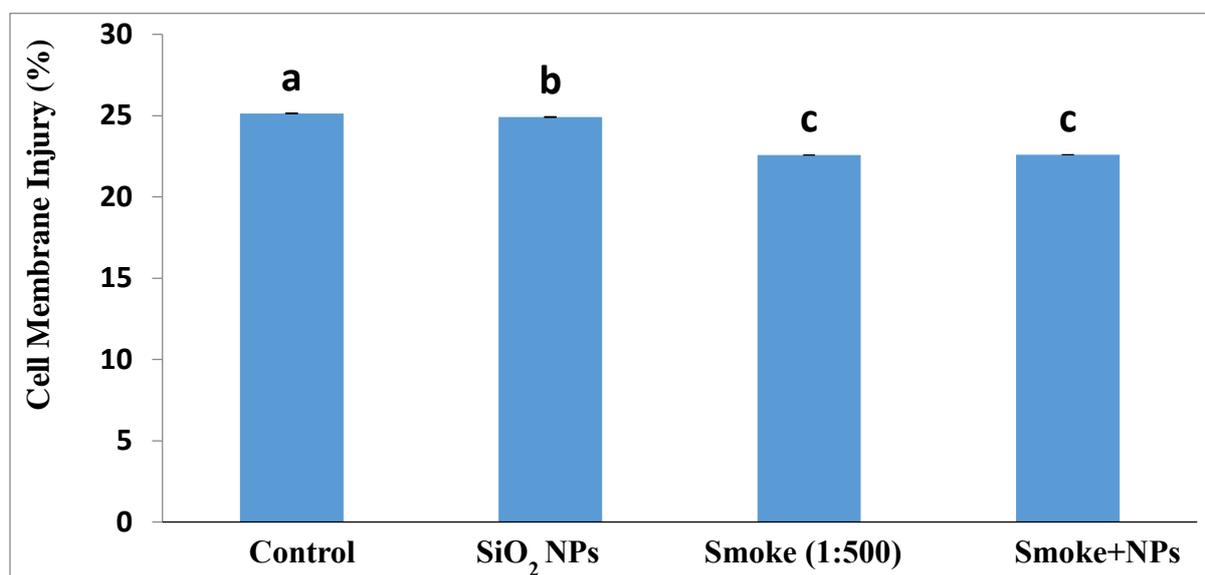


Figure 9. Effect of SiO<sub>2</sub> NPs alone and in combination with PDS on cell membrane injury of pea plants. Statistical analysis was performed using one-way ANOVA, which revealed a significant difference among the groups at  $P < 0.05$ .

## Discussion

### Effect of SiO<sub>2</sub> NPs on morphological, physiological and biochemical characteristics of pea

In comparison to the control, SiO<sub>2</sub> NPs treatments greatly boosted the seed germination rate of pea. Similar results have been recorded in an earlier study in which SiO<sub>2</sub> nanoparticles significantly improved the seed germination of tomatoes (Siddiqui and Al-Whaibi, 2014). Further investigations, like those conducted by other scientists (Lee et al., 2010; Wang et al., 2011; Ahmadi et al., 2021) have shown that seed germination of different crops was significantly improved by the treatment with SiO<sub>2</sub> nanoparticles. Similarly, fresh masses of pea seedling shoots and roots grew markedly at 40 mg/L of SiO<sub>2</sub> nanoparticles and number of secondary roots increased dramatically at the same treatment. Silicon oxide's positive properties could be attributed to its hydrophilic nature (Romero-Aranda et al., 2006). Tahir et al. (2010) also observed that silicon treatment boosted wheat biomass considerably. Furthermore, under the influence of NPs, water uptake and transportation have been reported to be facilitated in plants (Karimi and Mohsenzadeh, 2016), which could play a vital role in improving growth of plants.

Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in pea seedlings in response to SiO<sub>2</sub> nanoparticle treatment increased as compared to those in the control plants. In the presence of SiO<sub>2</sub> nanoparticles, there was a notable increase in chlorophyll a, b, and carotenoid concentrations in the pea seedlings exposed to SiO<sub>2</sub> nanoparticles compared to those in the control plants. Such findings were in agreement with those of other research studies (Sabaghnia et al., 2015; Karimi and Mohsenzadeh, 2016). Under stress conditions, cellular membranes undergo changes such as increased permeability and decreased specificity, leading to increased electrolyte leakage (Blokhina et al., 2003). The observed increase in electrolyte leakage under SiO<sub>2</sub> nanoparticles suggests cellular membrane injury, as noted in the study by Campos et al. (2003). However, it was noted that SiO<sub>2</sub> nanoparticles did not fully overcome this negative effect, especially in the context of pigment-related processes. Total soluble proteins and sugars increased with the treatment of SiO<sub>2</sub> nanoparticles, particularly in comparison to the control samples (Figure 8). In an earlier study, Xie et al. (2014) reported increased chlorophyll content in maize plants treated with SiO<sub>2</sub> NPs. In Jujube, Yu et al. (2012) reported that enhanced protein content, total free amino acids, nitrogen (N), phosphorus (P), potassium (K), POD and SOD activities and other biochemical processes were positively associated with plant growth stimulated by SiO<sub>2</sub> NPs.

### Effect of PDS on the morphological, physiological, and biochemical attributes of pea

The treatment of seeds or plants with plant-derived smoke is a good initiative towards sustainable agricultural practices (Bhuvaneshwari et al., 2019). The smoke generated by the partial combustion of plant matter is beneficial in promoting morphological development. The need for this kind of organically produced agro-chemicals for sustainable agricultural systems has increased. In the current study, pea plants showed an improvement in morphological characteristics like seed germination, root/shoot length, fresh weight, and total number of secondary roots (Figure 2, Figure 4, and Figure 6). The presence of butenolide, which has a stimulatory impact on plants, may have an ability to increase physio-

biochemical parameters related to growth (Iqbal et al., 2018). The smoke produced from plant waste material elicited growth of wheat (*Triticum aestivum* L.) by improving morphological, physiological and biochemical activities. Promising effects of smoke treatments on plant roots and shoots of different crops have been earlier demonstrated (Iqbal et al., 2005; Zou et al., 2005; Khatoon et al., 2020). This improvement in root elongation might have been attributable to a rise in duplicated DNA (Jain and van Staden, 2007), cell growth, and cell division (Soós et al., 2009), and gibberellin contribution (Long et al., 2010). Ghebrehiwot et al. (2012) reported that aerosol plant-derived smoke treatments significantly enhanced the dry biomass of Tef grass; moreover, plant-derived smoke resulted in a substantial increase in number of wheat leaves, as observed in their study. Aremu et al. (2012) explored the positive effects of plant-derived smoke on leaf area increase in the banana variety Williams. All these findings underscore the diverse impacts of plant-derived smoke on various plant attributes.

Physiological properties of pea plants, such as chlorophyll a, b, and total chlorophyll content, were significantly increased by plant-derived smoke treatments compared to those of the plants treated with SiO<sub>2</sub> NPs, while no significant changes were noted in the case of membrane stability. Such findings are parallel to those of other investigations (Sabaghnia and Janmohammadi, 2015; Karimi and Mohsenzadeh, 2016). Plant -derived smoke, according to van Staden et al. (2000), interacts with endogenous plant growth hormones to positively alter physiological parameters.

Plant membrane stability and integrity are critical for the proper functioning of plant systems. Biological membranes maintain their integrity under normal conditions, and plant growth factors play a part in maintaining membrane stability (Rawat et al., 2021). The PDS also plays a crucial part in this situation due to the existence of a chemical called butenolide, which acts as a plant growth hormone. In the current study, plants showed an increase in the membrane stability index in response to PDS exposure as has earlier been observed elsewhere (Aslam et al., 2017). According to Rao et al. (2012), increased chlorophyll content contributes to higher plant growth and productivity. Chlorophyll a/b proportion, for instance, is a marker of the functioning of pigment apparatus and light adaptation, while total chlorophyll concentration represents the greenness characteristic of crops (Netto et al., 2005). Aremu et al. (2012) also found a comparable rise in chlorophyll a/b levels in banana plants treated with PDS. Total soluble protein and sugar levels were markedly enhanced with the treatment of *Cymbopogon*-derived smoke (Figure 8). Plant-derived smoke might cause protein synthesis by improving uptake of essential nutrients (Akhtar et al., 2017). In the current study, total soluble sugar contents were higher in the PDS-treated plants than those in the plants treated with SiO<sub>2</sub> NPs.

### Combined effects of SiO<sub>2</sub> NPs and PDS on pea morphological, physiological, and biochemical attributes

Appropriate agricultural and advanced techniques related to resource preservation are viable options for increasing crop yield. SiO<sub>2</sub> NPs and PDS show enhanced seed germination or other growth parameters, that are good examples of initiatives towards sustainable agriculture methods. As a consequence, there has been a rise in demand for such naturally produced agrochemicals for sustainable agricultural practices (Sun et al., 2016). On the other hand, the application of PDS, which has a stimulatory impact on plants, may have contribution in initiatives towards sustainable agricultural methods. As a result, smoke generated by the partial combustion of plant materials is beneficial in promoting morphological growth. The smoke treatment in the current study was also found to have a good impact on plant shoot and root growth. The pea seedlings treated with PDS also showed an improvement in morphological characteristics like seed germination speed, root and shoot lengths, fresh weight, and total number of secondary roots. It has been reported that PDS solutions contain key nutritional ingredients such as calcium, magnesium, potassium, iron, manganese, and copper (Akhtar et al., 2017); smoke may well be associated with improved protein synthesis by improving uptake of growth nutrients. As with PDS, silicon NPs also had a significant influence on all growth and morpho-physiological parameters of pea plants appraised in the current study. This could have been due to the fact that silicon is known to have the capability of increasing plant stability and responsiveness when applied in combination with other growth-promoting techniques (Rastogi et al., 2019).

### Conclusion

Nanomaterial treatments can promote rapid seed germination and increase plant yield. To the best of our information, it was the first study on the impacts of SiO<sub>2</sub> nanoparticles and PDS alone and in combination on morphological and biochemical parameters related to growth of pea. Based on the results of this research, it is concluded that combination of SiO<sub>2</sub> NPs and PDS is a feasible alternative for increasing pea growth. The most effective combination was the use of SiO<sub>2</sub> NPs 40 mg L<sup>-1</sup> plus PDS. The

combined treatment of SiO<sub>2</sub> NPs and PDS produced better results than both treatments applied singly. This suggested that integrated application of nutrients is an appropriate technique for achieving enhanced crop growth and vegetative performance.

## **Author(s), Editor(s) and Publisher's declarations**

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### **Supplementary material**

No supplementary material is included with this manuscript.

### **Conflict of interest**

The authors declare no conflict of interest.

### **Source of funding**

Declared none.

### **Contribution of authors**

Conceptualization and designing of the study: AM, RN, MJ, AK. Conduction of experiment and collection of data: AM, MJ. Analytical work: AM. Written first draft of the manuscript: AM, RN, MJ, SUR, AK. Helped to prepare figures and tables: AM, RN, MJ. Statistical analysis of data: AM, RN, MJ. Final draft reviewed and read by all authors.

### **Ethical approval**

This study does not involve human/animal subjects, and thus no ethical approval is needed.

### **Handling of bio-hazardous materials**

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

### **Availability of primary data and materials**

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

### **Authors' consent**

All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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### **Declaration of Generative AI and AI-assisted technologies in the writing process**

It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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