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INFLUENCE OF EXOGENOUS CALCIUM SULPHATE ON SEED GERMINATION AND GROWTH OF *RAPHANUS SATIVUS* L. (RED VARIETY)

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Abstract

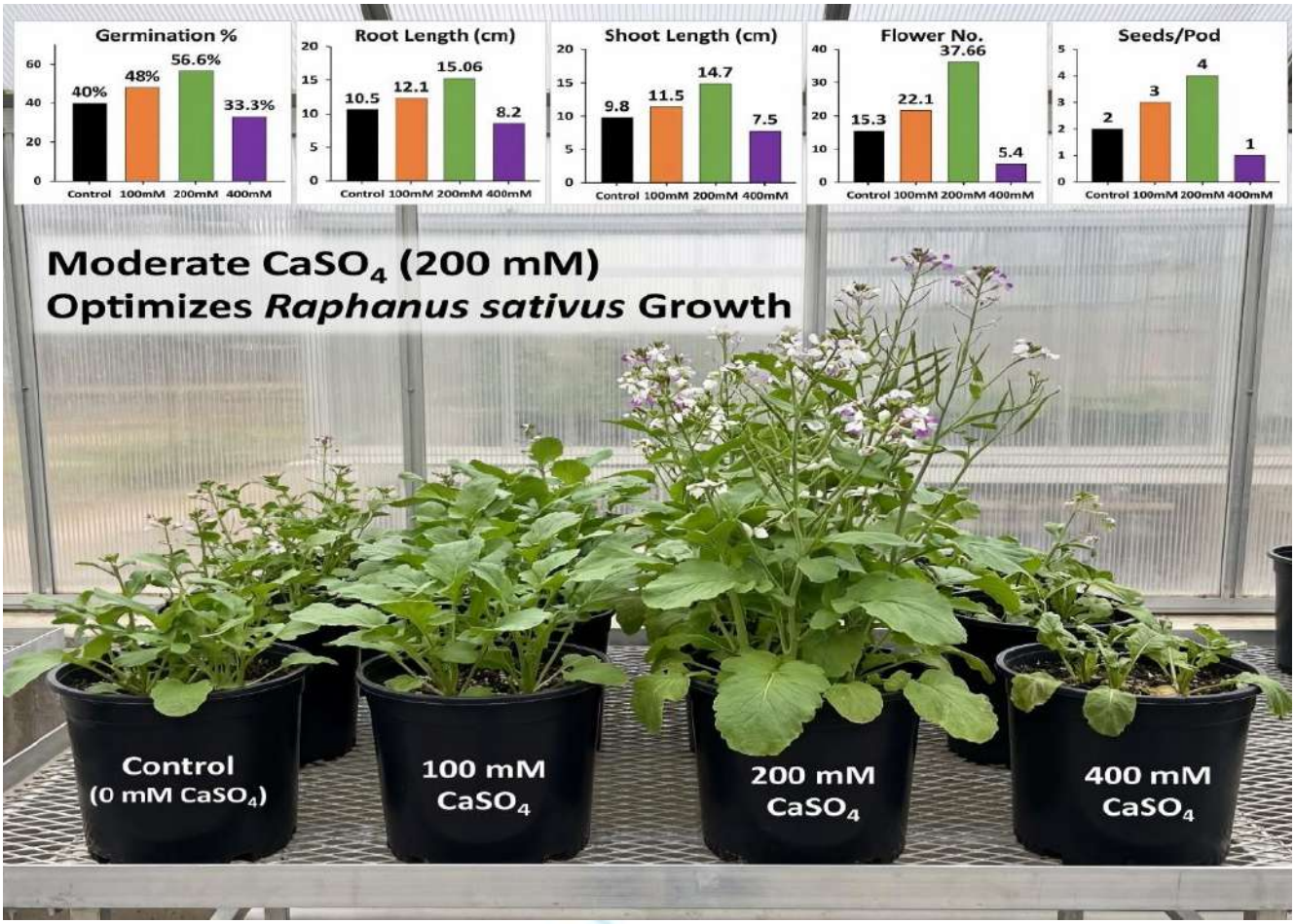
Calcium is an essential secondary macronutrient involved in membrane stabilization, cell wall development, and intracellular signaling in plants. The present study investigated the concentration-dependent effects of calcium sulphate ($CaSO_4$) (100 mM, 200 mM, and 400 mM) on seed germination and growth performance of Raphanus sativus under pot conditions. The experiment was conducted using a completely randomized design with three replicates per treatment. Germination percentage and morphological parameters were recorded at 15-60 days intervals. Results revealed that 200 mM $CaSO_4$ significantly enhanced germination (56.6%) compared to control (40%) and 400 mM (33.3%). Radicle length (2.33 cm), plumule length (5.6 cm), root length (15.06 cm), and shoot length (14.7 cm) were highest at 200 mM treatment. Similarly, leaf number (7.66), flower number (37.66), and seed production (4 seeds per pod) were markedly increased at 200 mM compared to control plants. In contrast, 400 mM $CaSO_4$ reduced most growth parameters, indicating possible osmotic imbalance at higher concentration. The findings demonstrate that moderate calcium supplementation (200 mM) improves vegetative and reproductive performance of R. sativus, whereas excessive calcium levels adversely affect plant growth. Optimized calcium management may therefore enhance radish productivity under controlled cultivation conditions.

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Graphical Abstract



INTRODUCTION

Calcium plays a central role in plant growth, development, and physiological regulation. It functions as a structural component of cell walls, stabilizes cellular membranes, and acts as an important secondary messenger in intracellular signaling pathways. Calcium is classified as a secondary macronutrient, yet it directly influences many metabolic and developmental processes in plants. Adequate calcium availability supports cell division, cell elongation, and tissue differentiation (Kour et al., 2023). Deficiency or excess of calcium disrupts physiological balance and can impair plant growth and productivity (Weng et al., 2022; Jing et al., 2024).

The family Brassicaceae includes several economically important vegetable crops cultivated worldwide for food and nutritional purposes. These crops are known for their high growth rate, nutritional value, and adaptability to diverse agroecological conditions. Among them, *Raphanus sativus* L. (radish) is a widely cultivated root vegetable consumed in many regions due to its nutritional richness and short growth cycle. Radish contains important vitamins, minerals, antioxidants, and bioactive compounds that contribute to human health (Zhu et al., 2007; Zhang et al., 2024; Jankowski, 2025). Its rapid germination and fast vegetative development make it an ideal model crop for studying nutrient dynamics and plant physiological responses (Mahto et al., 2024).

Plant growth and productivity are fundamentally dependent on balanced mineral nutrition. Calcium (Ca^{2+}) is classified as a secondary macronutrient but plays a primary structural and regulatory role in plant

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development. It is a key component of calcium pectate in the middle lamella, contributing to cell wall rigidity and structural stability. Additionally, calcium stabilizes plasma membranes by maintaining phospholipid bilayer integrity and regulating membrane permeability (Tanveer & Yousaf, 2020; Jing et al., 2024). Beyond its structural functions, calcium acts as a ubiquitous intracellular secondary messenger involved in signal transduction pathways that regulate plant responses to biotic and abiotic stresses (Verma et al., 2022). Fluctuations in cytosolic Ca^{2+} concentrations trigger activation of calcium-binding proteins, kinases, and transcription factors, thereby modulating gene expression and physiological adaptation mechanisms (Koide et al., 2025).

Adequate calcium supply has been associated with enhanced root elongation, improved nutrient uptake, increased leaf expansion, and better reproductive performance. It contributes to meristematic activity and cellular division, particularly in rapidly growing tissues such as root tips and young leaves. However, mineral nutrition follows a concentration-dependent response curve, where both deficiency and excess may negatively affect plant performance (Wilkins et al., 2016). Calcium deficiency often results in impaired cell division, weak cell walls, and reduced growth, whereas excessive calcium availability may disturb ionic balance, interfere with uptake of other essential nutrients such as magnesium and potassium, and create osmotic stress conditions (Ramos et al., 2003). Elevated mineral concentrations in the rhizosphere can reduce water potential, limiting water absorption during seed germination and early seedling establishment (Meng et al., 2009).

Although numerous studies have investigated calcium-mediated stress alleviation under saline or heavy metal conditions, comparatively fewer studies have focused on the direct concentration-dependent effects of calcium sulphate ($CaSO_4$) on germination dynamics and reproductive attributes of vegetable crops. Calcium sulphate serves as a common soil amendment and calcium source, yet its optimal concentration range for maximizing growth without inducing osmotic imbalance remains insufficiently explored in *Raphanus sativus*. Understanding these responses is particularly important for improving nutrient management strategies and enhancing crop productivity under controlled and field conditions.

The present study was designed to evaluate the effects of different concentrations of calcium sulphate on seed germination and growth performance of *Raphanus sativus* under pot conditions. The study specifically aimed to assess the influence of calcium sulphate treatments on germination percentage, early seedling development, vegetative growth traits including root and shoot development, and reproductive parameters such as flower and seed production. Identifying an optimal calcium concentration may help improve nutrient management strategies for radish cultivation and provide insights into the physiological responses of plants to exogenous calcium supplementation.

Research Methodology

Experimental site and plant material

The experiment was conducted in the Botanical Garden of the Department of Botany, Islamia College, Peshawar. Certified seeds of *Raphanus sativus* L. red variety were used for the study. Radish is widely used as a model vegetable crop in nutrient response studies due to its rapid germination and short growth cycle, which allows efficient evaluation of nutrient treatments under controlled conditions (Kanjevac et al., 2022).

Experimental design

The experiment followed a Completely Randomized Design with four treatments and three replicates per treatment. The treatments included control distilled water, 100 mM $CaSO_4$, 200 mM $CaSO_4$, and 400 mM $CaSO_4$. Uniform plastic pots were filled with homogenized garden soil to maintain consistent growth conditions. Ten seeds were sown per pot and thinning was performed after germination to maintain uniform plant density. Pot experiments arranged under CRD are widely used in plant nutrition studies to evaluate the effect of nutrient concentrations on plant growth and physiological responses (Festing, 2020).

Preparation of solutions

Calcium sulphate solutions were prepared by dissolving appropriate quantities of $CaSO_4$ in distilled water to obtain concentrations of 100 mM,

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200 mM, and 400 mM. Solutions were freshly prepared before each application to maintain chemical stability. Each pot received 25 mL of the respective treatment solution at fifteen day intervals starting from the day of sowing. Application of mineral nutrient solutions at regular intervals is a standard method used to evaluate concentration dependent responses of plants to nutrient supply (Shen et al., 2020).

Germination assessment

Seed germination percentage was calculated using the standard formula based on the ratio of germinated seeds to total seeds sown multiplied by one hundred. Radicle and plumule lengths were measured after complete emergence using a digital measuring scale. Germination parameters such as radicle and plumule growth are commonly used indicators to evaluate early seedling vigor and nutrient influence during the germination phase (Mulaudzi et al., 2020; Huang et al., 2017).

Growth measurements

Plant growth parameters were recorded at intervals between 15 and 60 days after sowing. The measured traits included root length, shoot length, number of leaves per plant, leaf length and width, petiole length, number of flowers, and number of seeds per pod. Measurements were obtained from randomly selected plants within each replicate to reduce sampling bias. Morphological and reproductive traits are commonly used indicators for assessing plant response to mineral nutrition and environmental factors in pot experiments (Etejere & Olayinka, 2015).

Statistical Analysis

The experiment was conducted in a completely randomized design (CRD) with three replicates per treatment. Data were expressed as mean ± standard deviation (SD). The data obtained were statistically analyzed using MS Excel 365.

Results and Discussions

The germination rates were higher in 200 mM concentration of $CaSO_4$ with a mean of 5.66 seeds, 5.33 seeds in 100 mM. The effects of $CaSO_4$ on *Raphanus sativus* seed germination demonstrated a clear concentration-dependent pattern. Seeds treated with 200 mM $CaSO_4$ exhibited the highest germination success (5.66 seeds), followed by 100 mM (5.33 seeds), whereas control seeds germinated at a lower mean (4 seeds). The highest concentration (400 mM) resulted in the lowest germination (3.33 seeds) presented in Fig.1. This suggests that moderate calcium supplementation improved early seed viability and radicle emergence. Calcium plays a dual role in plants: as a structural component of cell walls and as an important intracellular signal mediator that influences water uptake and enzyme activities during seed imbibition and germination (Mulaudzi et al., 2020). Changes in cytosolic Ca^{2+} are known to modulate signaling pathways that regulate germination-related gene expression and cellular metabolism, enhancing germination under favorable conditions. However, supra-optimal external Ca^{2+} concentrations can create osmotic imbalances that restrict water influx into seed tissues, adversely affecting germination (Huang et al., 2017; Zhang et al., 2025).

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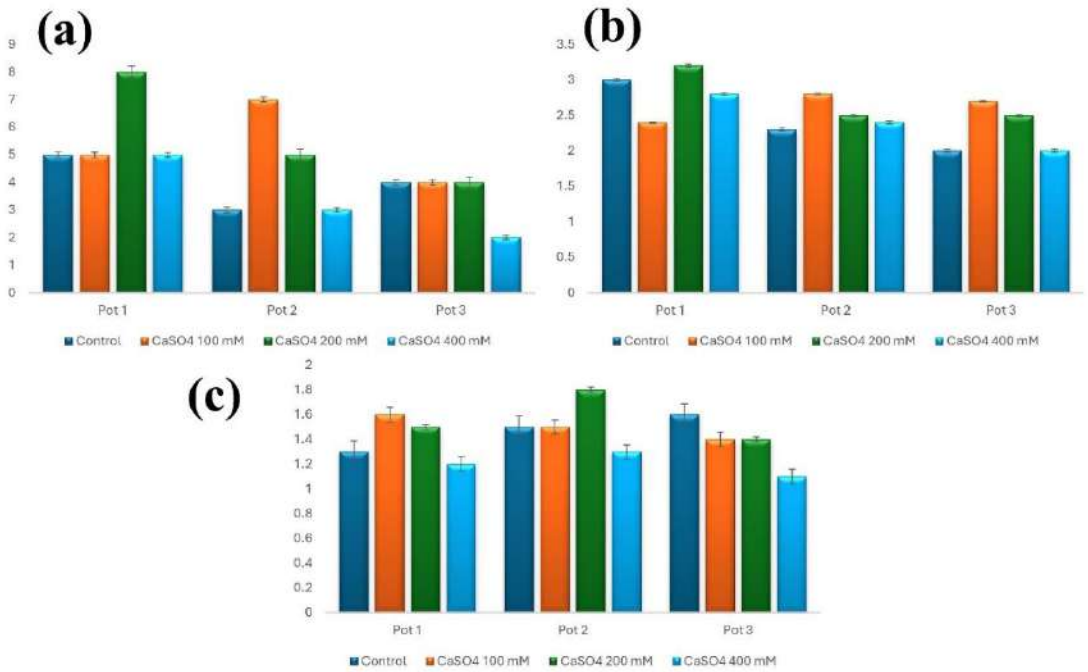


Fig. 1 Effect of different concentrations of calcium sulphate $CaSO_4$ on seed germination percentage of *R. sativus*. Values represent mean of three replicates.

Radicle and plumule development followed similar concentration-dependent trends. Radicle length was greatest at 200 mM (2.73 cm), intermediate at 100 mM (2.63 cm) and control (2.43 cm), whereas 400 mM reduced radicle elongation (2.40 cm) shown in Fig. 2. Early seedling vigor is closely linked to nutrient availability; Ca^{2+} facilitates cell wall cross-linking and cytoskeletal dynamics necessary for cell elongation. Studies have highlighted that calcium functions as a key regulator of both developmental processes and stress signaling, modulating growth responses to external stimuli. Optimal calcium availability supports expanding tissues by maintaining cell wall integrity and by stabilizing membranes, which leads to improved root system architecture (Wang et al., 2023). In contrast, excess calcium may perturb osmotic balance, limiting water and nutrient uptake required for extension growth. Comparable ionic stress responses have been reported under elevated heavy metal exposure, where excessive ion concentrations impaired physiological activity and growth performance (Emamverdian et al., 2015; Jan et al., 2026).

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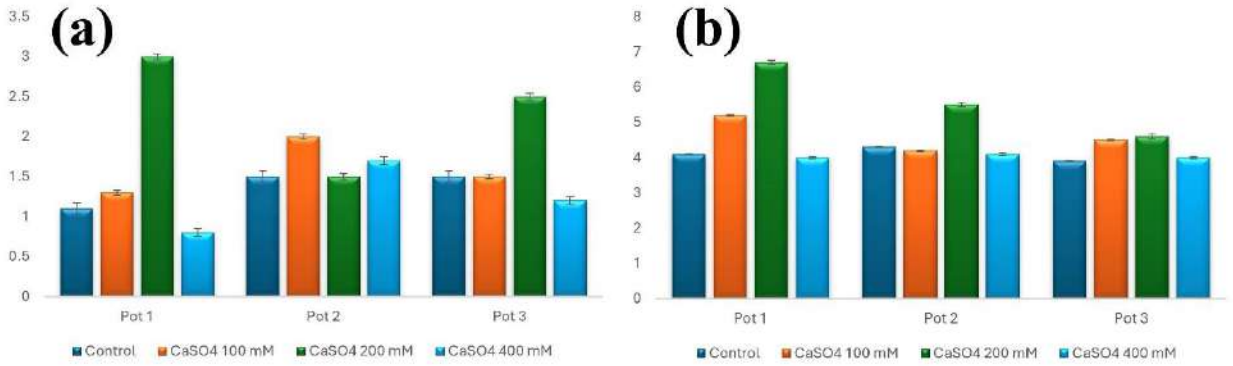


Fig. 2 Effect of calcium sulphate treatments on radicle length and plumule length of *R. sativus* seedlings. Values represent mean of three replicates.

Root length at 200 mM (15.06 cm) and shoot length (14.7 cm) were significantly greater than both control and 400 mM treatments, echoing the positive role of calcium in structural reinforcement and expansion growth (Fig. 3). Calcium is vital for stabilizing cell walls through interactions with pectic substances in the apoplast, strengthening tissues and reinforcing growth potential (Hepler & Mollica, 2024). In addition, appropriate Ca^{2+} nutrition is associated with improved nutrient transport and membrane function that supports both root and shoot development. At 400 mM, reduced root and shoot growth likely reflects negative osmotic and ionic impacts in the rhizosphere, diminishing water uptake and metabolic activity (Park et al., 2026).

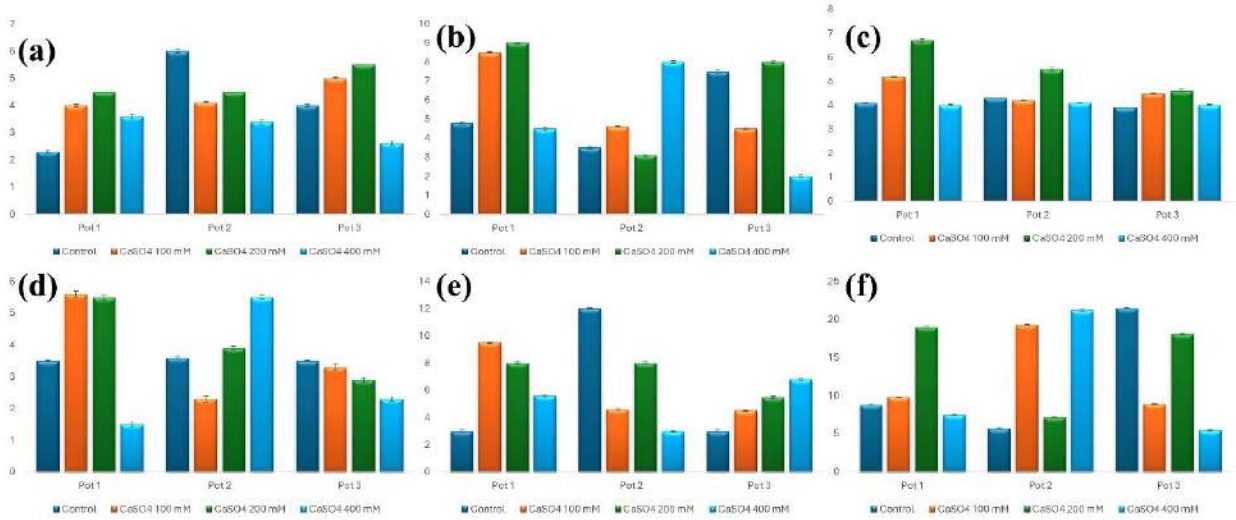


Fig. 3 Influence of calcium sulphate concentration on root length and shoot length of *R. sativus* plants measured during vegetative growth.

Leaf number and morphology were enhanced by 200 mM $CaSO_4$ plants showed a higher mean number of leaves, broader leaves and longer petioles compared to control and 400 mM treatments (Fig. 4). Adequate calcium supports leaf expansion by maintaining cell turgor and aiding the structural integrity of expanding leaf tissues. Calcium also influences stomatal functioning and transpiration, which indirectly supports leaf growth by regulating water status and CO_2 exchange (He et al., 2024;

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Kang & Lopez, 2026). Lower performance at 400 mM can be attributed to disrupted cellular water relations and reduced nutrient assimilation due to osmotic stress (Adhikari et al., 2023; Nazari et al., 2023).

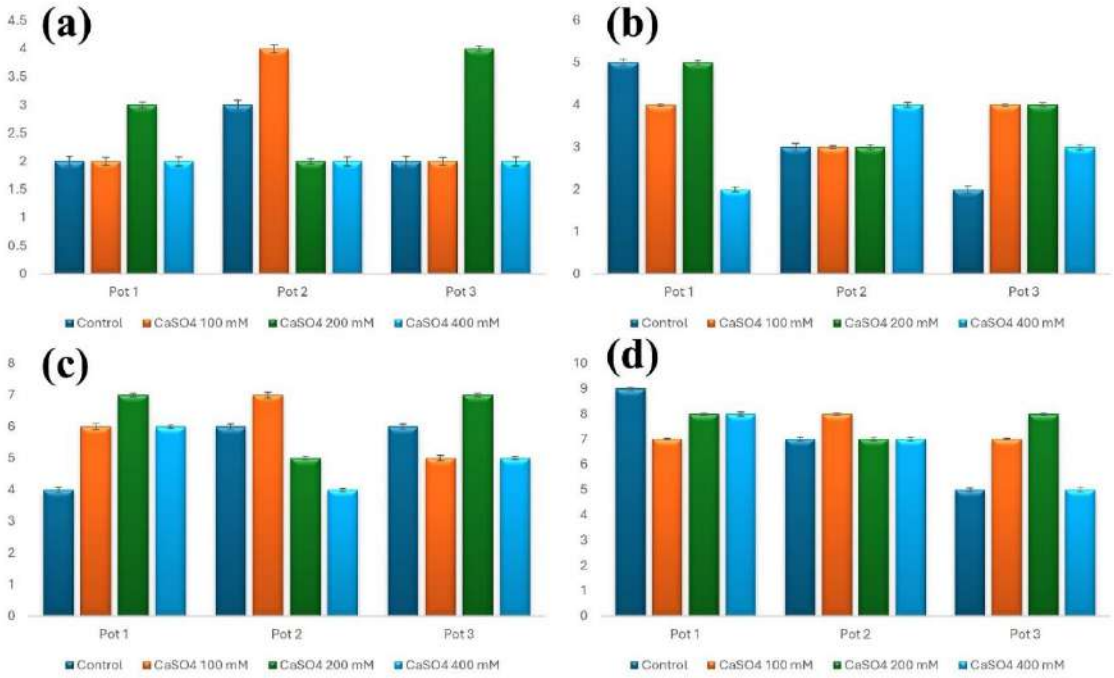


Fig. 4 Effect of calcium sulphate treatments on vegetative traits of *R. sativus* including number of leaves, leaf size, and petiole length under different $CaSO_4$ conditions.

Reproductive parameters (flower and seed number) were also highest at 200 mM $CaSO_4$, with a mean of 37.66 flowers and 4 seeds per pod (Fig. 5). Increased reproductive output under moderate calcium supplementation may result from the role of calcium in pollen tube growth, fertilization, and early seed set, as calcium signaling pathways are critical for successful sexual reproduction in plants. Calcium signals generated upon fertilization influence downstream developmental programs that govern embryo and seed development. Emerging green nanotechnology approaches demonstrate that nano engineered mineral delivery systems can regulate ion release and reduce osmotic toxicity. Controlled release systems may provide calcium at optimal physiological thresholds without inducing ionic stress (Fang et al., 2010; Khan et al., 2025; Khan et al., 2026). Conversely, the lowest flower (22) and seed numbers (1.66) at 400 mM suggest reproductive development is compromised when external calcium concentration exceeds optimal levels, likely due to resource allocation shifts towards stress responses rather than reproductive investment (Arumugam et al., 2025; Siani et al., 2026).

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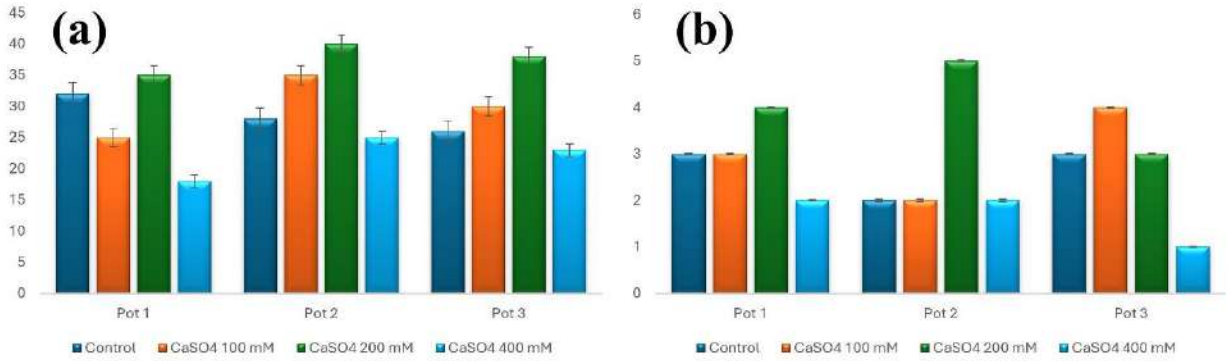


Fig. 5 Effect of calcium sulphate treatments on reproductive traits of *R. sativus* including number of flowers and seeds per pod under different $CaSO_4$ treatments.

The concentration-dependent responses observed in this study are consistent with the multifaceted role of Ca^{2+} in plant physiology. Calcium acts not only as a structural macronutrient but also as a critical signaling entity that decodes environmental and developmental cues. Calcium signals are perceived and transduced by calcium sensors (such as calcium-dependent protein kinases, calmodulins, and calcineurin B-like proteins), which regulate gene expression and enzymatic activities related to growth and stress adaptation. This signaling capacity enables plants to optimize growth under moderate calcium supply while activating protective responses under stress conditions. Excessive calcium, as seen at 400 mM, may overstimulate stress pathways or disrupt ionic homeostasis, leading to reduced growth and reproductive performance (Kang et al., 2023).

Future studies should explore advanced nutrient delivery systems to improve calcium utilization and minimize ionic imbalance in crop plants. Controlled release fertilizers and nanocomposite based formulations can regulate nutrient availability and maintain optimal ion concentrations in the rhizosphere (Ullah et al., 2025; Khan et al., 2025). Nanotechnology driven fertilizers improve nutrient use efficiency and reduce nutrient loss in soil systems. Integration of calcium nutrition with nano enabled delivery platforms may enhance germination, root architecture, and reproductive performance in vegetable crops. Recent progress in nanocomposite fertilizers and green nanotechnology shows strong potential for improving agricultural productivity through precise nutrient management and stress mitigation (Khan et al., 2026; Ullah et al., 2026).

Another important direction involves the interaction between mineral nutrition and beneficial microorganisms in the rhizosphere. Plant growth promoting bacteria and endophytes regulate nutrient solubilization, phytohormone production, and stress tolerance in plants. Studies should examine how optimized calcium levels influence microbial diversity, root colonization, and plant microbe signaling pathways. Beneficial genera such as *Paenibacillus* and other rhizospheric bacteria may improve nutrient uptake and suppress phytopathogens when combined with balanced mineral supply (Khan et al., 2025; Khan et al., 2026). In addition, endophytic bacterial inoculation has shown promising effects on plant growth promotion and stress resilience in different crops (Iqbal et al., 2026). Future research should integrate microbial biofertilizers, nutrient management, and phytoremediation strategies to develop sustainable agricultural systems that improve crop productivity while maintaining soil health (Maqbool et al., 2025; Jan et al., 2026).

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Conclusion

The present study demonstrates that calcium sulphate exerts concentration-dependent effects on the germination and growth of *Raphanus sativus*. Moderate supplementation (200 mM $CaSO_4$) significantly enhanced germination percentage, vegetative growth parameters, and reproductive traits compared to control plants. In contrast, higher concentration (400 mM) negatively affected plant development, likely due to osmotic imbalance and disruption of ionic homeostasis. These findings indicate that optimal calcium availability promotes membrane stability, enzymatic regulation, and overall plant vigor, whereas excessive calcium may impair physiological processes. Therefore, appropriate management of calcium supplementation is essential for maximizing radish growth and yield under controlled cultivation conditions. Further studies focusing on physiological and biochemical mechanisms underlying calcium-mediated responses are recommended to optimize nutrient management strategies.

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