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Potential effects of Metal Oxide Nanoparticles on Leguminous Plants: Practical Implications and Future Perspectives

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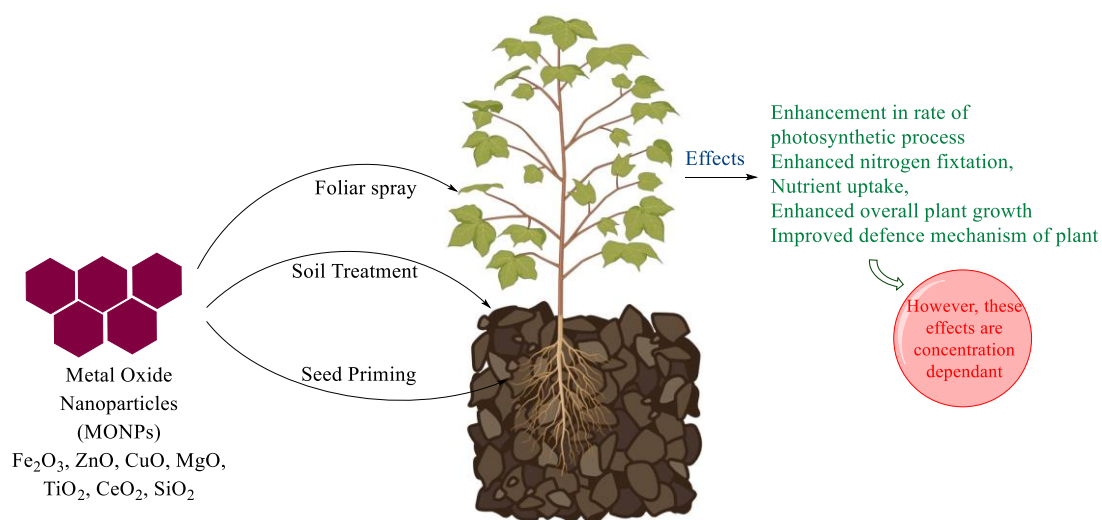
Abstract

The use of nanoparticles in agriculture is increasing due to their ability to readily enter plants. Among these nanoparticles, metal oxide nanoparticles (MONPs) have garnered significant attention owing to their unique attributes such as stability and catalytic activity. Extensive studies have demonstrated the positive effects of various MONPs, including iron oxide (Fe₂O₃ or Fe₃O₄), copper oxide (CuO), zinc oxide (ZnO), titanium dioxide (TiO₂), cerium oxide (CeO₂), silicon dioxide (SiO₂), and magnesium oxide (MgO) on the growth and productivity of legume plants in normal and abiotic stress conditions. These nanoparticles enhance symbiotic interactions with bacteria, photosynthesis, nutrient uptake, and nitrogen fixation. However, excessive utilization of MONPs can have detrimental consequences, including reduced photosynthesis, nitrogen fixation, and altered gene expression, leading to diminished crop yield. To ensure the safe and effective application of MONPs, it is imperative to consider factors such as MONP type, concentration, and exposure method. The main objective of this review is to provide a comprehensive overview of

30 the current research landscape concerning the role of MONPs and their effects on leguminous
31 plants. Furthermore, it aims to identify potential avenues for future research in this domain. By
32 addressing these aspects, this review endeavors to enhance our understanding of the implications
33 and potential risks associated with the agricultural use of MONPs, facilitating informed decision-
34 making and promoting sustainable practices.

35 **Keywords: MONPs, abiotic stress, legumes, crop yield**

36 Graphical Abstract



37

38 Introduction

39 Nanotechnology has gained significant attention in the field of agriculture due to its potential for
40 improving crop productivity. Researchers have extensively studied the ability of nanoparticles to
41 penetrate plants due to their nano size (Agrawal et al., 2022; Faizan et al., 2020). However, among
42 the nanoparticles armamentaria, metal oxide nanoparticles are commonly utilized due to their
43 unique features such as shape, size, concentration, composition, and physiochemical properties
44 that play a crucial role in influencing the effects of MONPs on different plant species (Palacio-
45 Márquez et al., 2021a; Xie et al., 2018). In agriculture, commonly employed in MONPs are ZnO,
46 Fe₂O₃ or Fe₃O₄, SiO₂, TiO₂, CuO, CeO₂, and MgO (Amde et al., 2017; Gebre and Sendeku, 2019a).
47 Traditional physical and chemical methods have been used for nanoparticle synthesis; however,
48 these methods have drawbacks such as high pressure, temperature risks, and environmental

49 pollution (Panakkal et al., 2021). In contrast, biological methods offer advantages such as
50 biocompatibility and eco-friendliness (Vithanage et al., 2023).

51 MONPs can be applied directly to soil or plants, affecting plant metabolism through symplastic
52 and apoplastic pathways. However, the interaction between plants and MONPs is not yet fully
53 understood and requires further investigation at the molecular level. MONPs have the potential to
54 be used as pesticides and fertilizers to enhance agricultural productivity while reducing inputs and
55 nutrient losses (Gilbertson et al., 2020). Different studies have shown the positive effects of
56 MONPs on plant growth and development. For instance, the application of Fe₂O₃ NPs to broad
57 beans (*Vicia faba* L.) increased root node numbers, and ZnO nanoparticles improved yield in lentil
58 (*Lens culinaris* Medik.) crops (S. M. Alwan et al., 2022; Kolenčik et al., 2022a). Additionally, these
59 MONPs impart tolerance from various abiotic stress such as drought, salt, cold, temperature, and
60 heavy metal by improving photosynthetic activity, antioxidant enzymes, and ultimately plant
61 growth (Sarraf et al., 2022) such as TiO₂ nanoparticles enhanced germination and photosynthesis
62 in the same plant species (Khan et al., 2019). However, increasing the dosage of nanoparticles can
63 have detrimental effects, for instance, SiO₂ and TiO₂ nanoparticles reduced germination, plant
64 length, and chromosomal structure in broad beans (*Vicia faba* L.) (Thabet et al., 2019) and CeO₂
65 nanoparticles decreased nitrogen fixation and photosynthetic pigments in mungbeans (*Vigna*
66 *radiata* L.) (Kamali-Andani et al., 2022). Similarly, ZnO nanoparticles were found to decrease
67 nodulation in alfalfa (*Medicago sativa* L.) plants (Sun et al., 2022). Leguminous plants, known for
68 their high protein content and soil fertility enhancement through nitrogen fixation, are particularly
69 affected by MONPs. The impact of MONPs such as Fe₃O₄, ZnO, and CeO₂, on the symbiotic
70 relationships between legumes and bacteria is crucial to understand. These nanoparticles can also
71 have adverse effects on soil microorganisms, reducing their growth and enzymatic activity, which
72 can vary depending on the morphology, composition, and concentration of the nanoparticles and
73 the plant species (Burke et al., 2015; Prakash et al., 2021a; Sun et al., 2022). Moreover, MONPs
74 like CeO₂, ZnO, and TiO₂ have been found to reduce the count of soil microorganisms, including
75 *Azotobacter*, fungal and actinomycetes colonies, and inhibit their enzymatic activity, as well as
76 negatively impacting phosphorus and potassium-solubilizing bacterial growth (Chai et al., 2015;
77 Verma et al., 2020).

78 The increasing use of MONPs in various industries raises concerns about their impact on the
79 environment and human health. These nanoparticles can enter the food chain through plants,

80 potentially causing adverse effects(Panakkal et al., 2021). Their release into water bodies can
81 further affect aquatic ecosystems and the food chain. Therefore, it is imperative to identify the
82 detailed impact of MONPs on symbiotic bacteria and plant nodulation. Soil polluted with MONPs
83 may pose a threat to agricultural output and sustainability by disrupting the symbiotic association
84 between *Rhizobium* and legume plants. To address these issues, a potential strategy is the
85 controlled release of nutrients through metal oxide nano-fertilizers. This approach aims to optimize
86 the benefits of MONPs while minimizing potential risks. This review aims to provide an evaluation
87 of the current research concerning Metal Oxide Nanoparticles (MONPs) and their effects on
88 leguminous plants. It will address the existing research gaps to shed light on the consequences and
89 potential dangers of using MONPs in agriculture.

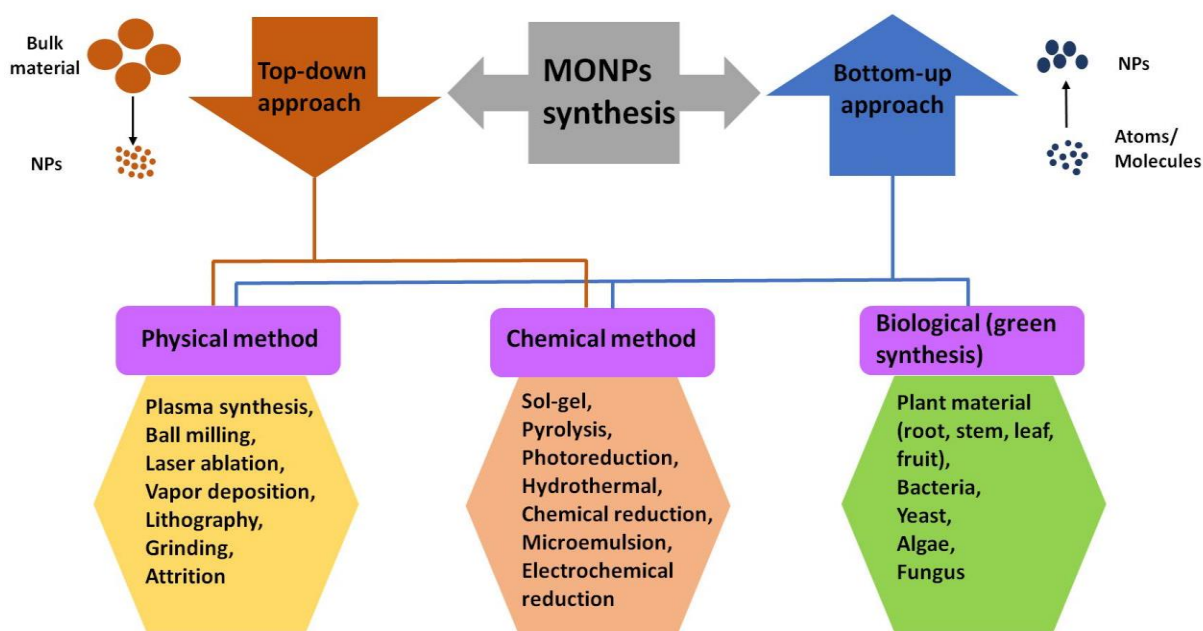
90 **Synthesis methods for metal oxide nanoparticles**

91 Recent advancements in nanotechnology have led to the development of new techniques for
92 synthesizing nanoparticles with specific characteristics such as shape, size, and properties.
93 Generally, two approaches (top-down and bottom-up) were followed for synthesizing
94 nanoparticles. The top-down approach involves reducing the size of bulk materials to the nanoscale
95 using mechanical forces. In contrast, the bottom-up approach involves synthesizing nanoparticles
96 from smaller molecules using physical, chemical, or biological techniques (**Figure 1**). In the top-
97 down approach, mechanical methods such as plasma synthesis, ball milling, laser ablation, and
98 physical vapor deposition are used to compress bulk materials down to the nanoscale(Chavali and
99 Nikolova, 2019; Koul et al., 2021; Saebnoori et al., 2022). A number of MONPs created through
100 these techniques such as Fe₃O₄ by plasma synthesis, CuO by electrospinning, ZnO by laser
101 ablation, and TiO₂ by ball milling(Jaber et al., 2021; Lee et al., 2018; Lyu et al., 2019; Sun et al.,
102 2023). On the other hand, the bottom-up approach involves chemical reactions to synthesize
103 MONPs using techniques such as sol-gel, wet-chemical reduction, pyrolysis, photoreduction, and
104 hydrothermal methods (Dadkhah and Tulliani, 2022). Chemical processes such as sol gel,
105 pyrolysis and wet chemical methods have been utilized to produce MONPs like TiO₂, ZnO, and
106 Fe₃O₄ (Malek et al., 2018; Mathumba et al., 2017; Taziwa et al., 2017).
107 While physical and chemical approaches have shown potential in various applications, there are
108 challenges associated with them including stability, toxicity, low yield, high cost, and the need
109 for skilled operators (Purohit et al., 2019; J. Singh et al., 2018). These techniques often involve the

110 use of hazardous substances, which can generate toxic fumes, contribute to environmental
111 pollution, and pose risks to living organisms. Additionally, the nanostructures produced by these
112 methods may not be suitable for agricultural use due to potential health hazards for humans (Gebre
113 and Sendeku, 2019b). To overcome these limitations, researchers have turned to green synthesis
114 techniques as a solution (**Figure 1**). Green synthesis, a bottom-up approach, involves the use of
115 plant extracts and microorganisms to create MONPs. These methods are biocompatible and
116 environmentally sustainable due to the lower toxicity of the compounds used and mild conditions.
117 Plant extracts from various sources such as Indian mulberry (*Morinda citrifolia* L.), cassava
118 (*Manihot esculenta* Crantz), yam (*Dioscorea* spp. L.), lemon peel (*Citrus limon* (L.) Osbeck),
119 pomegranate peel (*Punica granatum* L.), and cumin (*Cuminum cyminum* L.) seeds have been
120 employed for the fabrication of MONPs (Dubey and Singh, 2019; Herrera-Barros et al., 2018;
121 Mathew et al., 2021; Sundrarajan et al., 2017). Aqueous solutions of cabbage (*Brassica oleracea*
122 L.) and leaf extracts from guava (*Psidium guajava* L.), bay laurel (*Laurus nobilis* L.), mesquite
123 (*Prosopis juliflora* L.), Jwarancusa lemongrass (*Cymbopogon jwarancusa* L.) and common olive
124 (*Olea europaea* L.) have also been used (Fakhari et al., 2019; Irum et al., 2020; Issam et al., 2021;
125 Osuntokun et al., 2019; Saha et al., 2018). Besides plants, yeast, algae, and fungi, and bacteria
126 have been investigated as biological sources for synthesizing MONPs (Hassan et al., 2021; Majeed
127 et al., 2021; Peiris et al., 2018; Salem et al., 2019). Plant materials are often preferred over
128 microorganisms due to their ease of use, cost-effectiveness, and scalability (Gebre and Sendeku,
129 2019a). These green synthesis techniques provide stable and non-toxic plant metabolites that can
130 act as stabilizing and reducing agents. Additionally, advanced methods like microwave irradiation,
131 which utilizes dielectric heating, have been proposed for plant extract preparation (Ganguly and
132 Sengupta, 2023). Consequently, there is a growing demand for environmentally friendly materials
133 and methods as an alternative for the production of nanostructures. To characterize and study these
134 nanostructures, various spectroscopic methods such as UV-Visible absorption spectroscopy, FTIR,
135 XRD, DLS, TEM, SEM, and EDAX are commonly used. These techniques provide valuable
136 information about their size, shape, structure, elemental composition, concentration, and other
137 physical and chemical properties. Each method offers unique insights that cannot be obtained
138 through a single technique alone (Nair et al., 2022). The distinctive physical and chemical
139 properties of these nanoparticles such as their unique edges and corners and increased density,

140 make them highly useful in various industries, including agriculture, medicine, and environmental
141 preservation.

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152 **Figure 1. Different methods for synthesizing metal oxide nanoparticles (MONPs).**

153 **Uptake and accumulation of metal oxide nanoparticles in leguminous plants**

154 MONPs can be applied to plants through foliar application, which involves spraying them on the
155 leaves, or soil fertilization, which involves adding them to the soil. Understanding the pathways
156 through which plants absorb MONPs is crucial, with the cuticle and stomatal pathways being the
157 two main routes (Avellan et al., 2019). The cuticle, which is the outermost layer of leaves, acts as

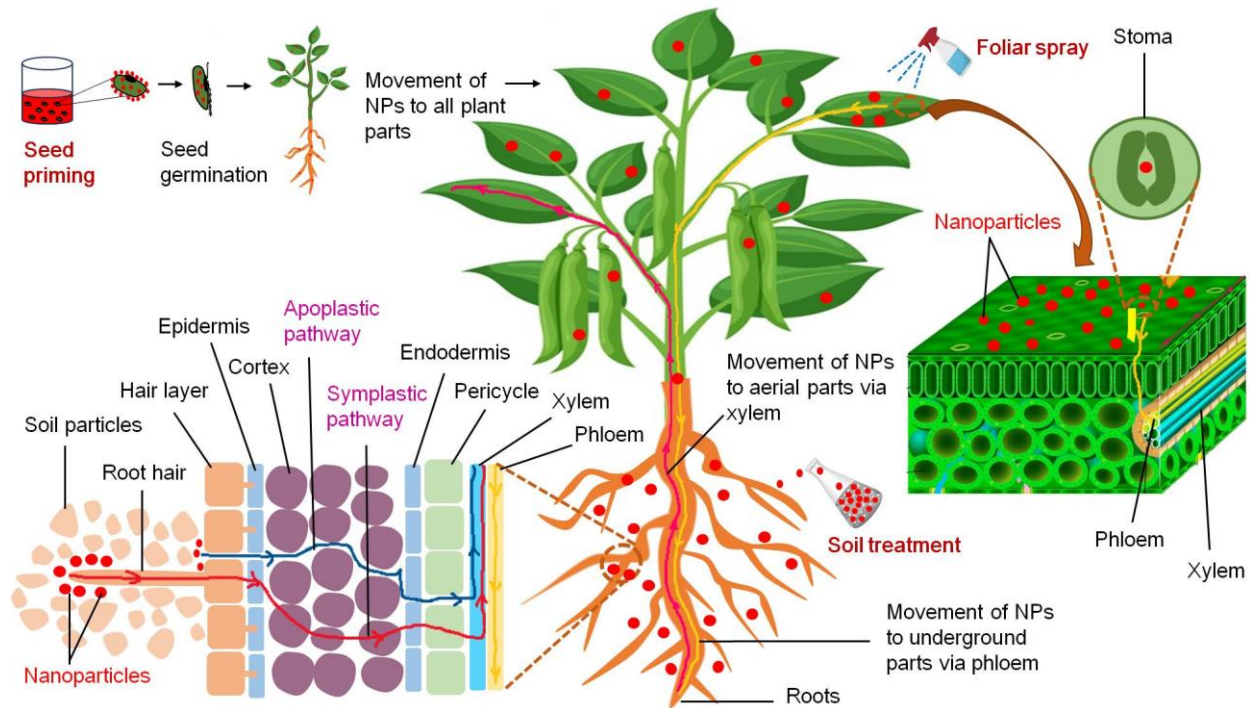
158 a barrier against nanoparticle penetration (**Figure 2**). However, nanoparticles with diameters
159 ranging from 0.6 to 4.8 nm can pass through the cuticle efficiently via the stomatal and cuticular
160 pathways (Lv et al., 2019). Some studies even suggest that nanoparticles as small as 5 nm can
161 penetrate the cuticle (Rastogi et al., 2017). Nevertheless, the exact route taken by these
162 nanoparticles is still not fully understood, as it can vary depending on factors such as the plant
163 species, nanoparticle characteristics, experimental conditions, and the plant's growth stage,
164 environmental conditions, and presence of pests or diseases (Huang et al., 2021; Rani et al., 2022).
165 In addition to the cuticular pathway, nanoparticles can also enter and migrate through stomatal
166 pores in plants (Khan et al., 2022). The entry of nanoparticles through stomata is influenced by
167 factors such as stomatal density, size, aperture opening, leaf structure, and the activity of specific
168 pumps within plant cells (Wang et al., 2023). Numerous studies have reported the penetration of
169 nanoparticles, including CeO₂, TiO₂, Fe₂O₃, and ZnO through stomata. Furthermore, these
170 nanoparticles can interact with various cellular components, affecting their toxicity, genotoxicity,
171 and metabolic processes (A Ebrahimi et al., 2016; Khan et al., 2021; Mahdieh et al., 2018; Salehi
172 et al., 2018a). Techniques such as fluorescence, confocal microscopy, SEM, and TEM have been
173 employed to investigate the entry of different MONPs through stomata and their accumulation in
174 deeper plant tissues, particularly in leguminous species like common beans (*Phaseolus vulgaris*
175 L.) (Dina M Salama et al., 2019; Salehi et al., 2021a; Zhang et al., 2019; Zhu et al., 2020). Even
176 plant species can influence the absorption of nanoparticles through leaves (Su et al., 2019).
177 Once MONPs enter the substomatal cavity, they can travel through plant tissues via apoplastic and
178 symplastic pathways (Liu et al., 2020) (**Figure 2**). When applied to leaves, MONPs are typically
179 transported to the roots through the phloem, whereas seed or soil treatments lead to their movement
180 from roots to shoots. It is worth noting that chemicals transported downward through the phloem
181 are not circulated upward through the xylem (Lv et al., 2019). Several studies have shown that
182 MONPs can enter plants through stomata and eventually reach the root system. For instance, Xiong
183 et al. found that foliarly sprayed CuO NPs with a size of about 40-200 nm reached the roots through
184 the phloem in lettuce (*Lactuca sativa* L.) plants (Xiong et al., 2021). TEM analysis revealed the
185 presence of Fe₃O₄ NPs ranging from 10 to 40 nm in pumpkin (*Cucurbita pepo* L.) phloem sap,
186 indicating their entry into the plant through the apoplastic pathway. However, no transfer from
187 roots to shoots was observed (Tombuloglu et al., 2020). Similarly, TEM examination of wheat
188 (*Triticum aestivum* L.) plant root sections detected Fe₃O₄ NPs, suggesting their entry via the

189 apoplastic pathway. Fe₃O₄ NPs caused an increase in iron levels in the root tissues, as evidenced
190 by a hysteresis loop in the M-H curve, demonstrating their presence in different regions of the
191 wheat plant. However, the aerial samples exhibited weak ferromagnetic behavior, suggesting that
192 Fe₃O₄ NPs did not move from the roots to the above ground parts (Iannone et al., 2016).

193 The movement of nanoparticles in the root cells of wheat plants was investigated using techniques
194 such as TEM. The results demonstrated the transport of nanoparticles from the leaves to roots via
195 phloem pathway (“Annual report 2013-2014,” 2014). Similarly, X-ray fluorescence microscopy
196 (XRF), Laser confocal scanning microscopy, and SEM was used to observe the movement of Zn
197 NPs and polystyrene NPs from roots to shoots in common bean (*Phaseolus vulgaris* L.) and wheat
198 (*Triticum aestivum* L.) plants (da Cruz et al., 2019; Lian et al., 2020).

199 Seed priming was also identified as an effective technique for facilitating the uptake and
200 accumulation of MONPs in plants. During seed priming, seed pretreatment with metal oxide
201 nanoparticles (MONPs) was observed to enhance essential aspects of plant growth, including
202 germination, seedling growth, and overall plant performance (Mazhar et al., 2023b, 2023a). The
203 uptake and accumulation of nanoparticles through seed priming occur primarily through the seed
204 coat and embryo (**Figure 2**). The inserted nanoparticles could influence various physiological and
205 biochemical processes, enhancing plant growth and development. Multiple studies demonstrated
206 that seed priming with MONPs such as ZnO (Mazhar et al., 2023a), Fe₂O₃ (V. Pawar et al., 2019),
207 CuO (Sarkar et al., 2021), TiO₂ (Mathew et al., 2021), MgO (P Sharma et al., 2022) could improve
208 seed germination rates, seedling vigor, and plant biomass.

209 Additionally, nanoparticles absorbed through seed priming could affect plant morphology, nutrient
210 uptake, and stress tolerance (Ghorbani et al., 2021). The uptake of nanoparticles by seeds and their
211 subsequent distribution within plant tissues were displayed with advanced microscopic techniques
212 such as SEM and TEM (Kashyap and Siddiqui, 2021). These techniques have revealed the
213 presence of nanoparticles in various plant organs, including roots, stems, leaves, and fruits,
214 indicating their systemic movement within the plant.



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217 **Figure 2. Uptake mechanism of MONPs in plants through the soil and foliar application.**

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219 **Role of metal oxide NPs in leguminous plant**

220 The agricultural sector faces numerous challenges such as food security, seed germination, plant
 221 growth improvement, targeted pesticide delivery, diagnostic devices, food quality enhancement,
 222 storage, and pest management, but nanotechnology holds great potential for addressing various
 223 challenges in agriculture (Vijayakumar et al., 2022). Nanomaterials, at the nanoscale, exhibit
 224 exceptional properties such as quantum size effects, increased surface area, and unique magnetic
 225 properties, which often result in higher activity compared to their bulk counterparts (Singh et al.,
 226 2022). MONPs have shown promising effects on plant growth, particularly in leguminous crops.
 227 Their application as fertilizers can reduce reliance on chemical inputs, enhance nutrient
 228 bioavailability, optimize water-nutrient ratios, and ultimately increase plant production (Salehi et
 229 al., 2020). MONPs have demonstrated positive effects on plant morphology, including
 230 improvements in germination percentage, shoot and root length, and total biomass of seedlings.
 231 Numerous studies have reported the beneficial effects of MONPs on different leguminous plants

232 such as soybeans (*Glycine max* (L.) Merr.) (Stowers et al., 2018), yellow medick (*Medicago*
233 *falcata* L.) (Kokina et al., 2020), and peas (*Pisum sativum* L.) (Kashyap and Siddiqui, 2021) in
234 enhancing various physiological parameters related to plant growth. However, the widespread use
235 of MONPs in industry and agriculture has raised concerns about their potential toxicity and
236 environmental impact. Upon entry into the environment, these nanoparticles interact with plants,
237 affecting their physiological, biochemical, and genetic makeup, and potentially leading to negative
238 effects on human health and the ecosystem (Kamali et al., 2019; Rastogi et al., 2017). This
239 discussion highlights both the positive and negative effects of various MONPs on different crops.

240 **IRON OXIDE**

241 Iron oxide nanoparticles have gained significant attention for their positive impact in plants (Yang
242 et al., 2020). Iron is an essential nutrient element for plants and plays a crucial role in various
243 metabolic processes, including photosynthesis, nitrogen metabolism, respiration, stomatal closure,
244 and nucleic acid synthesis. Iron acts as a cofactor for several enzymes involved in redox reactions,
245 electron transport chain, peroxide activation, and decomposition (Rai et al., 2021). While iron is
246 traditionally used in its ionic form, the use of iron oxide nanoparticles has shown promising effects
247 due to their efficient absorption and ability to form complexes with other molecules, facilitating
248 the delivery of iron to different plant parts (Poddar et al., 2020). The higher surface area of Fe₂O₃
249 or Fe₃O₄ NPs allows them to bind with carrier proteins or ion transporters, facilitating their
250 absorption through the plasma membrane of plant cells (Deng et al., 2022). Various studies have
251 found that Fe₂O₃ NPs are transported via xylem vessels, and the transfer and accumulation depend
252 on particle size, concentration, exposure time, and plant species (Saleem and Khan, 2023; Yang et
253 al., 2023). Upon interacting with the soil, Fe₂O₃ NPs actively boost the presence of soluble iron.
254 This is facilitated by the release of soluble iron ions from the nanoparticles, utilizing their
255 significant surface area and small size. As a result, plants can absorb these ions, providing an
256 essential nutrient for their growth (Zia-ur-Rehman et al., 2018).

257 Plants respond to iron oxide nanoparticles by altering the various physiological processes for
258 instance, increase in the photosynthetic pigments and upregulation of gene expression. Iron oxide
259 nanoparticles (Fe₂O₃) have roles in water purification and can be used as catalysts, adsorbents, and
260 iron fertilizers (Algarni and Al-Mohaimed, 2022; Gillispie et al., 2019). Iron oxide nanoparticles
261 interact with plants when the pH is mildly acidic and help convert to Fe⁺³ ions into Fe⁺² ions that

262 can be utilized by plants The introduction of iron oxide nanoparticles improves the growth of plants
263 by enhancing their biomass and length. The roots absorb these nanoparticles which are then
264 transported to parts of the plant through shoots (Khan et al., 2023). However, the reactivity of iron
265 poses a dual nature, as low concentrations of iron have beneficial effects including increased
266 height, root length, biomass, and chlorophyll levels, while higher concentrations caused a
267 phototoxic effect on plants due to their involvement in the Fenton (Fe) reaction, generating
268 hydroxyl radicals (OH) within the plant. The availability of iron in plants is regulated by various
269 enzymes, transporters, scavengers, and chaperones (Distéfano et al., 2021). Researchers
270 investigated the role of iron oxide nanoparticles, specifically Fe₂O₃, in improving seed
271 germination, root growth, pod number, root nodules, and chlorophyll content in legume crops such
272 as chickpeas (*Cicer arietinum* L.), green peas (Palchoudhury et al., 2018) (*Pisum sativum* L.), broad
273 beans (Abdel-Salam, 2018; S. Alwan et al., 2022; Palchoudhury et al., 2018; Rani et al., 2022)
274 (*Vigna radiata* L.), and yellow medick (Kokina et al., 2020) (*Medicago falcata* L.) (**Table 1**). In
275 peanut (*Arachis hypogea* L.) seeds, Fe₂O₃ NPs boosted glycoprotein production and enhanced root
276 length, plant height, chlorophyll content, plant biomass, root development, and SPAD (soil plant
277 analysis development) values (Rui et al., 2016; S Suresh et al., 2016a). Nevertheless, the research
278 is only focused on hydroponic conditions, raising concerns about potential nanomaterial toxicity.
279 Comparative analyses with traditional iron fertilizers are needed for a thorough understanding.
280 Legume seeds such as green gram (*Vigna radiata* L.), green peas (*Pisum sativum* L.), chickpeas
281 (*Cicer arietinum* L.) when soaked with Fe-enriching hematite (α -Fe₂O₃ NPs) resulted in improved
282 root length, and seedling growth in chickpea plants (Boutchuen et al., 2019; Palchoudhury et al.,
283 2018; V. A. Pawar et al., 2019). These studies suggested that using lower nanoparticle
284 concentrations in seed pre-soaking solutions can reduce fertilizer usage, minimizing
285 environmental impact. The comparative analysis and investigation of varied pH conditions provide
286 valuable insights for the sustainable and efficient use of nano fertilizers in promoting food
287 production. Furthermore, foliar application of Fe₂O₃ NPs and fulvic acid-coated Fe₂O₃ NPs in
288 soybean (*Glycine max* (L.) Merr.) plants has demonstrated increased chlorophyll content, biomass,
289 root developmental indices, and biological nitrogen fixation. The mildly acidic pH in the
290 hydroponic environment facilitates the conversion of Fe⁺³ ions to Fe⁺² ions, readily absorbed by
291 plants. Consequently, the exposure to iron nanoparticles has led to enhanced biomass and root
292 length, fostering positive contributions to soybean (*Glycine max* (L.) Merr.) plant growth (Yang et

293 al., 2020). In meantime, Cao and colleagues conducted a pivotal study focusing on the foliar
294 application of differently sized γ -Fe₂O₃ nanomaterials (NPs) to assess their impact on soybean
295 (*Glycine max* (L.) Merr.) growth and physiology (Cao et al., 2022). The investigation revealed
296 size- and concentration-dependent effects, with small-sized NPs (S-Fe₂O₃, 4-15 nm) at 30 mg/L
297 exhibiting superior growth promotion compared to a commercial iron fertilizer (EDTA-Fe). Other
298 studies have shown the positive effects of Fe₂O₃ NPs on chlorophyll fluorescence, root length, and
299 miRNA expression in yellow medick (*Medicago falcata* L.) as well as the role of Fe₃O₄ NPs and
300 *Rhizobium* in enhancing iron content, nodule leghemoglobin, nitrogenase activity, nodule number,
301 plant length, and dry weight in common beans (*Phaseolus vulgaris* L. cv. Red Guama) (De Souza-
302 Torres et al., 2021a; Kokina et al., 2020). However, these reported studies did not provide an
303 explanation of the underlying mechanisms, through which Fe₃O₄ nanoparticles affected root
304 length, chlorophyll fluorescence nitrogen fixation and miRNA expression in yellow medick
305 (*Medicago falcata* L.) and common beans (*Phaseolus vulgaris* L.). and common beans. The seed
306 soaking with polyethylene glycol-coated Fe₃O₄ nanoparticles (Fe₃O₄-PEG; at a concentration of
307 1,000 mg Fe/L) had a beneficial effect on the germination and seedling growth of bean plants
308 (*Phaseolus vulgaris* L.) (Duran et al., 2017). Moreover, recent research has indicated that foliar
309 application of Fe₂O₃ NPs improved nitrate reductase activity, chlorophyll content, iron level,
310 biomass, and plant yield in green beans (*Phaseolus vulgaris* L. cv. 'Strike') (GUTIÉRREZ-
311 RUELAS et al., 2021) (**Table 1**). This study highlighted the enhanced assimilation of nitrogen,
312 emphasizing the potential of nano-fertilizers in improving crop yield and nutrient absorption. Irum
313 and his co-workers found that iron oxide NPs made from *Cymbopogon jwarancusa* leaf extract
314 effectively stimulate in-vitro callus, support shoot regeneration, and improve root induction in
315 chickpea (*Cicer arietinum* L.) plants (Irum et al., 2020). The Punjab-Noor 09 chickpea variety
316 stands out with high callogenesis (96%) and shoot regeneration (88%), along with increased iron
317 content (4.88 mg/g) in the regenerated plants. This environmentally friendly method highlights the
318 promise of iron oxide NPs in advancing tissue culture for vital legume crops like chickpeas.
319 Further, Saleem and Khan found that the application of Fe₂O₃ NPs, either alone or in combination
320 with *Rhizobium pusense*, enhances seed germination, protein and pigment content, plant length,
321 and dry biomass in green grams, making them a potential nano-fertilizer for legume plants (Saleem
322 and Khan, 2023). Conversely, Pawar et al. revealed adverse effects of iron oxide nanoparticles
323 (Fe₂O₃ NPs) on chickpea (*Cicer arietinum* L.) seedling growth, particularly at higher

324 concentrations (16mg/L). These negative impacts include reduced seedling length, decreased fresh
325 and dry weights, altered root-to-shoot ratio, and indications of phytotoxicity through stress indices
326 (V. A. Pawar et al., 2019) (**Table 1**). This study showed the significance of choosing nanoparticle
327 concentrations carefully to avoid adverse effects on plant growth. So, further research is required
328 to determine the optimal concentration and application method for different plant species and
329 growth stages. Moreover, it is necessary to assess the long-term effects of repeated exposure to
330 iron oxide nanoparticles, their potential accumulation in soil and plant tissues, their impact on soil
331 microorganisms and non-target organisms in the ecosystem, considering their corrosive nature,
332 larger surface area, and small size. Such investigations would provide insights into the safety and
333 sustainability of using these nanoparticles in agricultural practice.

334 **Table 1: Effects of various concentration of iron oxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
Fe ₂ O ₃	2, 10, 50, 250, and 1000 mg/kg	<i>Arachis hypogea</i> L.	Pot experiment	Soil application	Improved root length, plant biomass, plant height, antioxidant enzyme activity, and the SPAD value	(Rui et al., 2016)
	500 and 4000 mg/L	<i>Arachis hypogea</i> L.	Pot experiment	Seed priming	Increased glycoprotein content	(S. Suresh et al., 2016)
	0.00554 mg/L	<i>Pisum sativum</i> L., <i>Vigna radiata</i> L., <i>Cicer arietinum</i> L., and <i>Phaseolus vulgaris</i> L.	Growth vials solution	Seed priming	Increased root length in chick peas (88%), green peas (160%), and green grams (366%)	(Palchoudhury et al., 2018)

22 and 1100 mg/L	<i>Cicer arietinum</i> L., <i>Phaseolus vulgaris</i> L., <i>Vigna radiata</i> L., and <i>Phaseolus vulgaris</i> L.	Petriplate	Seed priming	Growth boosts (230-830%) across four legume species, enhanced survival, accelerated fruit production, and yields healthier second-generation plant	(Boutchuen et al., 2019)
4, 8, and 12 mg/L	<i>Cicer arietinum</i> L. variety Digvijay	Pot experiment	Seed priming	Enhanced seedling growth	(V. Pawar et al., 2019)
1, 5, 10, 15, 20 mg/L	<i>Cicer arietinum</i> L.	Growth vials solution	In growth medium	Enhanced chickpea growth, induce callus, promoted shoot regeneration, and improved root development	(Irum et al., 2020)
15, 30, and 60 mg/pot	<i>Glycine max</i> (L.) Merr.	Pot experiment	Direct soil application and foliar spray	Increased nodulation number, root surface area, root volume, shoot	(Yang et al., 2020)

					and root fresh weight and dry weight	
25, 50, and 100 mg/L	<i>Phaseolus vulgaris</i> L. cv. 'Strike'	Pot experiment	Foliar spray	Biomass, nitrate reductase activity, Fe content, and yield were increased	(GUTIÉRREZ-RUELAS et al., 2021)	
30 mg/L	<i>Glycine max</i> (L.) Merr.	Pot experiment	Foliar spray	Increased the shoot and nodule biomass, improved soybean yield (13.7%) and promoted the nutritional quality (e.g., free amino acid content) of the seeds.	(Cao et al., 2022)	
100, 250, and 500 mg/kg	<i>Vigna radiata</i> L.	Pot experiment	Soil application	Plant length, biomass, seeds per plant, and protein	(Saleem and Khan, 2023)	

					content were enhanced	
Fe ₃ O ₄	1, 2, and 4 mg/L	<i>Medicago falcata</i> L.	Hydroponic experiment	In hydroponic solution	Improvement in root length, chlorophyll fluorescence, and miRNA expression	(Kokina et al., 2020)
	2000 mg/L	<i>Phaseolus vulgaris</i> L. cv. Red Guama	Polythene culture	Soil application	Increased iron level, nitrogenase activity, leghaemoglobin content, nodule number, plant length, and dry weight were observed	(De Souza-Torres et al., 2021b)
	1,000 mg/L	<i>Phaseolus vulgaris</i> L. cv. Sintonia	Petriplate	Seed priming	Increased radicle elongation	(Duran et al., 2018)
Negative effects						
Fe ₂ O ₃	0.016 mg/L	<i>Cicer arietinum</i> L. variety Digvijay	Pot experiment	Seed priming	Decreased seedling growth	(V. Pawar et al., 2019)

336 ZINC OXIDE

337 Zinc (Zn) is an essential micronutrient that acts as a co-factor in the biosynthesis of proteins and
338 enzymes in plants such as alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, RNA
339 polymerase, phospholipase, and Cu-Zn SOD. It plays a crucial role in lipid metabolism, nitrogen
340 fixation, and gene expression by participating in DNA replication and transcription processes
341 (Kaur and Garg, 2021; A. Singh et al., 2018).

342 Zinc oxide nanoparticles (ZnO NPs) are receiving attention in plant applications due to their high
343 bioavailability, smaller size, and enhanced absorption properties (Siddiqui et al., 2018). However,
344 high concentrations of zinc can have detrimental effects on plants, including seed germination
345 inhibition, chlorosis, nutrient imbalances, and growth inhibition. These negative effects are
346 attributed to increased lipid peroxidation, protein degradation, and other underlying mechanisms.
347 It is important to note that the response to zinc varies among different plant species. Several studies
348 have investigated the positive as well as negative effects of ZnO NPs on legume plants (**Table 2**).
349 For instance, Mukherjee et al. studied the effects ZnO NPs on peas (*Pisum sativum* L.) at low and
350 high concentrations (250 and 1000 mg/kg). The soil application of ZnO NPs (1000 mg/kg) led to
351 an increase in chlorophyll and carotenoid content, as well as higher zinc content in various plant
352 tissues compared to untreated pea plants (Mukherjee et al., 2016). In kidney beans (*Phaseolus*
353 *vulgaris* L.), the effects of soil treatment with ZnO NPs (125 mg/kg) was investigated by Medina-
354 Velo et al. (Medina-Velo et al., 2017). They found the increased levels of zinc in leaves (136%),
355 stems (139%), and nodules (203%) of kidney beans, along with elevated manganese (74%) and
356 boron (122%) levels in the stem. These findings demonstrated the potential of ZnO NPs to
357 influence nutrient uptake and accumulation in legume plants. Further the effects of seed priming
358 and foliar application of different zinc forms was compared in pinto beans (*Phaseolus vulgaris* L.)
359 (Mahdieh et al., 2018). They reported that foliar treatment with ZnO NPs had a more significant
360 impact on the growth and yield of pinto bean plants compared to seed priming. Additionally, zinc
361 oxide nanoparticles (ZnO NPs) positively impacted *Phaseolus vulgaris* L. growth, with significant
362 improvements in yield at a low concentration of 300 mg/kg. Higher doses (600 and 1000 mg/kg)
363 showed phytotoxic effects, while ZnO NPs enhanced zinc uptake, bioaccumulation, and
364 antioxidant enzyme activities in plant tissues (Akanbi-gada et al., 2019).

365 Foliar treatments of zinc oxide nanoparticles (ZnO NPs) have shown positive effects in various
366 plant species. Kolenčik et al. reported increased pod number, seed weight, and overall plant yield
367 in field-grown lentil (*Lens culinaris* Medik.) plants with ZnO NPs foliar treatment (1 mg/L)
368 (Kolenčik et al., 2022b). Similarly, Salama et al. observed enhanced branch, leaf, and overall
369 production in common bean (*Phaseolus vulgaris* L.) plants with foliar treatment of ZnO NPs (30
370 mg/L) (Dina M Salama et al., 2019). This positive impact was linked to the upregulation of specific
371 genes encoding enzymes and proteins, although higher concentrations induced genotoxic effects.
372 Palacio-Marquez et al. found that foliar application of ZnO NPs at 25 mg/L significantly improved
373 biomass accumulation and production in green beans (*Phaseolus vulgaris* L.) (Palacio-Márquez et
374 al., 2021b).

375 Various studies have investigated the impact of zinc oxide nanoparticles (ZnO NPs) on different
376 plant species depending on factors such as particle size and concentration. Yusefi-Tanha et al.
377 investigated the size and concentration-dependent effects of ZnO NPs on soybean (*Glycine max*
378 (L.) Merr.) plants (Yusefi-Tanha et al., 2022a). ZnO NPs with a size of 38 nm, at concentrations
379 up to 160 mg Zn/kg, enhanced seed yield. In contrast, a higher concentration of 400 mg/kg resulted
380 in increased oxidative stress indicators and altered antioxidant enzymatic activity in soybeans.
381 Skiba et al. reported that ZnO NPs influenced the uptake and translocation of manganese, iron,
382 and copper in green peas (*Pisum sativum* L.) grown under hydroponic conditions (Skiba et al.,
383 2020). This study revealed how ZnO NPs impact the uptake and translocation of essential elements
384 in plants, providing insights into their influence in overall plant growth. Salehi et al. found that
385 lower concentrations of ZnO NPs positively affected zinc content, plant height, and peroxidase
386 and catalase activity in bean (*Phaseolus vulgaris* L.) plants. However, higher concentrations
387 resulted in damaging effects on photosynthetic activity, protein levels, increased lipid
388 peroxidation, and decreased antioxidant activity (Salehi et al., 2021b). Yusefi-Tanha et al. and
389 Salehi et al. reported their toxic effects at higher concentrations were evident in soybean (*Glycine*
390 *max* (L.) Merr.) and common bean (*Phaseolus vulgaris* L.) crops (Salehi et al., 2021c; Yusefi-
391 Tanha et al., 2022b). Conversely, Sun et al. (2022) and Verma et al. (2023) highlighted both
392 detrimental and beneficial impacts on alfalfa (*Medicago sativa* L.) and field pea (*Pisum sativum*
393 L.) crops, respectively (Sun et al., 2022; Verma et al., 2023). In a subsequent investigation, the
394 exposure of peas (*Pisum sativum* L.) to zinc oxide nanoparticles (ZnO NPs) resulted in a growth
395 impact that depended on the size of the nanoparticles. The more toxic ZnO NPs (50 nm) at a

396 concentration of 1000 mg/L resulted in a 20–30% slowdown in root elongation. Metabolically,
397 there was a significant increase in sucrose and GABA levels in both roots and shoots. Additionally,
398 pea (*Pisum sativum* L.) seedlings exhibited enhanced content of metabolites related to the
399 aspartate–glutamate pathway and the TCA cycle, such as citrate and malate. This suggests that
400 ZnO NPs influence various metabolic processes in peas, indicating a potential role in altering plant
401 growth and metabolic pathways (Stałańska et al., 2023)(Stałańska et al., 2023). Despite the
402 positive and detrimental effects of ZnO NPs on plants at different concentrations, the underlying
403 mechanisms explaining these effects remain largely unknown. Further studies are needed to
404 explore how ZnO NPs behave, interact, move, and get absorbed by plants and soil. Understanding
405 their physical and chemical characteristics over time is crucial for a more detailed assessment.

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416 **Table 2: Effects of various concentration of zinc oxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
ZnO	1000 mg/kg	<i>Pisum sativum</i> L.	Pot culture	Mixed in soil	Improved Chl a and carotenoid content as well as increased Zn accumulation, were observed in the root, shoot, and leaf tissues	(Mukherjee et al., 2016)
	125 mg/kg	<i>Phaseolus vulgaris</i> L. var. red hawk kidney	Pot culture	Mixed in soil	The Zn level was enhanced in nodules, stems, and leaves, while concentrations of Mn and B also showed improvement	(Medina-Velo et al., 2017)
	50, 100 and 150 mg/L	<i>Phaseolus vulgaris</i> L.	Pot culture	Seed priming and foliar spray	Foliar application resulted in enhanced vegetative characteristics, yield, and crop quality	(Mahdieh et al., 2018)

	1 mg/L	<i>Lens culinaris</i> Medik.	Field experiment	Foliar spray	Seed weight, number of pods, and overall plant yield was increased	(Kolenčík et al., 2022b)
	10, 20, 30, and 40 mg/L	<i>Phaseolus vulgaris</i> L.	Field experiment	Foliar spray (spray after 20 days)	The number, fresh weight, and dry weight of leaves and branches were also enhanced along with improved seed production	(Dina M. Salama et al., 2019)
	40, 80, and 160 mg/kg	<i>Glycine max</i> (L.) Merr. cv. Kowsar	Polythene bags	Soil application	Improved seed yield	(Yusefi-Tanha et al., 2020a)
	250 mg/L	<i>Phaseolus vulgaris</i> L.	Pot culture	Foliar and soil application	Foliar application showed positive effects on peroxidase and catalase activity as well as increased Zn content in leaves, and plant height	(Salehi et al., 2021b)
	200 mg/kg	<i>Glycine max</i> (L.) Merr.	Pot culture	Soil application	Root architecture and Zn accumulation were improved leading to better nutrient and water uptake	(Yusefi-Tanha et al., 2022b)

1.5 mg/L	<i>Phaseolus vulgaris</i> L., <i>Vigna radiata</i> L., and <i>Vigna unguiculata</i> L.	Hogland solution	Roots dipped in Hogland solution for 24 hours	Increased nitrogenase activity	(Kumar et al., 2015)
25 mg/L	<i>Phaseolus Vulgaris</i> L. cv. Strike	Plastic pot culture	Foliar application	Improved biomass accumulation and production	(Palacio-Márquez et al., 2021a)
300, 600, and 1000 mg/kg	<i>Phaseolus Vulgaris</i> L.	Pot culture	Mixed in soil	Increased plant productivity at lower concentration, but Zn level, SOD, APX, and CAT activity	(Akanbi-gada et al., 2019)
20 mg/L	<i>Vigna radiata</i> L.	Pot culture	Foliar application	Improved Zn and protein content, leaf area index, 1000-seed weight, seed yield, root biomass, and active nodule content	(Sahoo et al., 2021)
10, 20, and 50 mg/L	<i>Pisum sativum</i> L.	Pot culture	Foliar application	Improved plant height and yield	(Verma et al., 2023)

Negative effects						
ZnO	400 mg/kg	<i>Glycine max</i> (L.) Merr. cv. Kowsar	Polythene bags	Soil application	Increased oxidative stress	(Yusefi-Tanha et al., 2020b)
	2000 mg/L	<i>Phaseolus vulgaris</i> L.	Pot culture	Foliar and soil application	Decreased photosynthetic efficiency and compromised antioxidant defense system as well as increased protein degradation and lipid peroxidation	(Salehi et al., 2021c)
	2000 mg/L	<i>Phaseolus vulgaris</i> L.	Pot culture	Foliar application	Tapetum abnormalities, cell apoptosis, and reduced seed development, seed dry weight, and yield were observed	(Salehi et al., 2022)
	250 and 750 mg/L	<i>Medicago sativa</i> L.	Pot culture	Soil application	Decreased nodule number and root nodule area, and nitrogen fixation	(Sun et al., 2022)

500 mg/kg	<i>Glycine max</i> (L.) Merr.	Pot culture	Soil application	Reduction in root architecture and Zn accumulation, nutrient, and water uptake	(Yusefi-Tanha et al., 2022a)
1000 mg/L	<i>Pisum sativum</i> L.	Petriplate	Added in petriplate	Decreased root elongation by 20-30%	(Stalanowska et al., 2023)
600 and 1000 mg/kg	<i>Phaseolus Vulgaris</i> L.	Pot culture	Mixed in soil	Decreased yield parameters and increased the duration of flowering and fruit emergence	(Akanbi-gada et al., 2019)
1.5 and 10 mg/L	<i>Cyamopsis tetragonoloba</i> , <i>Vigna aconitifolia</i> Jacq., <i>Vigna radiata</i> L., and <i>Vigna unguiculata</i> L.	Hogland solution	Roots dipped in Hogland solution containing ZnO NPs for 24 hours	Decreased nitrogenase activity in cluster bean, green gram, and cowpea at 1.5 µg/mL but in moth bean nitrogenase activity reduced at both concentration	(Kumar et al., 2015)

	100 (priming), 250, 500, and 750 mg/L (chronic effects)	<i>Pisum sativum</i> L.	1% water agar in large Petri dishes for priming and 150 mL of FP medium with ZnO NPs for chronic effects	Seed priming	Reducing the count of first and second-order lateral roots, stem length, leaf surface area, and transpiration, affecting bacterial surface, root nodulation, nitrogen fixation, and triggered early senescence of nodules	(Huang et al., 2014)
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427 COPPER OXIDE

428 Copper (Cu) is a vital redox metal that serves as a cofactor for various proteins, including Cu/Zn-
429 superoxide dismutase, cytochrome C oxidase, plastocyanin, and ethylene receptors. It's role in
430 vital biological processes such as iron oxidation, carbohydrate metabolism, and chlorophyll
431 synthesis are well established in plants (Mir et al., 2021). Insufficient copper levels can disrupt
432 critical physiological functions such as nitrogen metabolism and respiration, resulting in oxidative
433 stress and the accumulation of detrimental free radicals. While the effects of bulk CuO on plants
434 have been extensively studied, the implications of CuONPs on legume crops are still under
435 investigation.

436 Recent investigations provided insights into the influence of CuONPs on legumes (**Table 3**). For
437 instance, priming peanuts (*Arachis hypogea* L.) with CuONPs resulted in enhanced protein content
438 without compromising overall plant growth (S Suresh et al., 2016b). Similar positive effects on
439 shoot length, root length, leaf number, and elemental composition were observed in peas (*Pisum*
440 *sativum* L.) treated with CuONPs. In comparison to untreated plants, the presence of nickel in the
441 pods decreased when conventional copper oxide was replaced with CuONPs (Ochoa et al., 2017a).
442 Another study demonstrated that the addition of CuONPs (100 mg/kg) with indole-3-acetic acid
443 (IAA; 100 μ M) led to increased iron and aluminum content in pea seeds, thereby augmenting their
444 nutritional value (Ochoa et al., 2018). This enhanced iron level could be attributed to the activity
445 of iron and copper-linked reductases in cell membranes. Copper oxide nanoparticles (CuONPs)
446 revealed concentration-dependent effects on plant physiology. Gautam et al. reported significant
447 changes in soybean at 200 mg/L, while toxicity was observed at higher concentrations (Gautam et
448 al., 2016). Singh et al. highlighted toxic effects on *Vigna radiata* L. germination and seedling
449 growth, with both adverse and potentially favorable outcomes at different concentrations (Singh
450 et al., 2017). Moreover, low concentrations of CuONPs (1 and 10 mg/L) positively influenced
451 germination and root growth in common bean (*Phaseolus vulgaris* L.) plants, whereas high
452 concentrations (100 and 1000 mg/L) exhibited adverse effects (Duran et al., 2017). Liu et al.
453 demonstrated concentration-dependent effects of copper oxide nanoparticles resulting in increased
454 copper content, oxidative stress and gene expression in soybean (*Glycine max* (L.) Merr.) roots
455 (Liu et al., 2021). Karmous et al. found positive impacts at lower concentrations (10 mg/L) in
456 mungbeans (*Vigna radiata* L.) but detrimental effects at higher levels (100–2000 mg/L) of copper
457 oxide nanoparticles (Karmous et al., 2022). CuONPs and CuO coated with 3-aminopropyl

458 triethoxysilane nanoparticles applied to mungbean seeds were found to enhance water absorption
459 and germination rates, indicating improved metabolic processes (Sarkar et al., 2021).
460 However, the utilization of smaller CuONPs (25 nm) resulted in increased copper uptake and
461 nutritional value but led to reduced seed yield and antioxidant activity in soybean (*Glycine max*
462 (L.) Merr.) seeds, yielding contradictory outcomes (Yusefi-Tanha et al., 2020c). This disparity was
463 attributed to the higher accumulation of nano-Cu ions within the soybean plant system (Yusefi-
464 Tanha et al., 2020d). Adverse effects of CuONPs on green pea (*Pisum sativum* L.) (1 mg/L) and
465 slender sesbania plants (*Sesbania grandiflora* L.) (100, 200, 300, and 400 mg/L) were observed,
466 including decreased seed germination, stunted seedlings, and slowed root development. These
467 consequences were associated with diminished photosynthetic activity and metabolic processes
468 (Ghosh et al., 2017; Santos et al., 2021). Similarly, Kavitha et al. demonstrated that higher
469 concentrations of CuONPs (500 mg/L) adversely affected seed germination and cyto-physiological
470 activities in mungbean (*Vigna radiata* L.) plants by inducing alterations in chromosomal structure
471 and mitotic index (Kavitha et al., 2022). More recently, a study on common beans (*Phaseolus*
472 *vulgaris* L.) revealed detrimental effects of CuONPs, such as chromosomal breakage, genetic
473 material damage, and cell distortion at various stages of cell division (metaphase, anaphase, and
474 telophase) in root cells (Tasar, 2022). It is crucial to prioritize research into the molecular and
475 physiological mechanisms governing the impact of CuO NPs on plant growth, development, and
476 metabolic processes. These insights will guide the development of customized strategies, ensuring
477 the safe and effective use of CuO NPs in agriculture.

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479 **Table 3: Effects of various concentration of copper oxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
CuO	500 mg/L	<i>Arachis hypogea</i> L.	Pot culture	Seed priming	Influenced protein content positively in leaves	(S Suresh et al., 2016b)
	50 mg/kg	<i>Pisum sativum</i> L.	Pot culture	Mixed in soil	Improved plant growth and increased Fe and Ni content in pods	(Ochoa et al., 2017b)
	100 mg/kg	<i>Pisum sativum</i> L.	Pot culture	Mixed in soil	Increased Fe, B, and Al content	(Ochoa et al., 2018)
	1 and 10 mg/L	<i>Phaseolus vulgaris</i> L.	Petriplate	Seed priming	Stimulated root growth and seed germination	(Duran et al., 2017)
	16, 48, and 80 mg/L	<i>Vigna radiata</i> L.	Petriplate	Seed priming	Enhanced seed imbibition and germination rate	(Sarkar et al., 2021)
	50, 100, 200, and 500 mg/kg	<i>Glycine max</i> (L.) Merr.	Polythene pot culture	Mixed in soil	Increased Cu ²⁺ uptake in leaves, shoots, roots, and seeds	(Yusefi-Tanha et al., 2020c)
	10 mg/L	<i>Vigna radiata</i> L.	Petriplate	Impregnated in filter paper	Improved essential minerals, free amino acids, total soluble sugars,	(Karmous et al., 2022)

					antioxidant polyphenols and flavonoids content	
	200 mg/L	<i>Glycine max</i> (L.) Merr.	Murashige and Skoog	Mixed in solution	Increased plant germination, shoot and root length, vigor index and total chlorophyll content	(Gautam et al., 2016)
Negative effects						
CuO	100 and 1000 mg/L	<i>Phaseolus vulgaris</i> L.	Petriplate	Seed priming	Decreased seed germination rate and inhibited root growth	(Duran et al., 2017)
	0.25, 0.50, and 1.00 mg/L	<i>Lathyrus sativus</i> L.	Petriplate	Impregnated in filter paper	Inhibited seed germination and resulted in reduced seedling growth	(Ghosh et al., 2017)
	50, 100, 200, and 500 mg/kg	<i>Glycine max</i> (L.) Merr. cv. Kowsar	Polythene pot culture	Mixed in soil	Led to decreased seed yield and reduced antioxidant enzyme activity	(Yusefi-Tanha et al., 2020d)
	100, 200, 300, and 400 mg/L	<i>Sesbania virgata</i> (Cav.) Poir.	Petriplate	Seed priming	Resulted in reduced root length	(Santos et al., 2021)
	500 mg/L	<i>Vigna radiata</i> L.	Petriplate	Impregnated in filter paper	Decreased seed germination and negatively affected cytophysiological activity	(Kavitha et al., 2022)
	50, 150, and 300 mg/L	<i>Phaseolus vulgaris</i> L.	Petriplate	Impregnated in filter paper	Induced increased chromosomal damage and cell distortion at	(Tasar, 2022)

					different phases of cell division in the roots	
2, 5 and 10 mg/L	<i>Glycine max</i> (L.) Merr.	Plastic pot culture	Root treatment		Increased ROS, MDA, relative electrical conductivity, inhibit root growth, destruction in pro-meristem, primary meristem, meristematic zone, and decreased mitotic index	(Liu et al., 2021)
400, 600, and 800 mg/L	<i>Glycine max</i> (L.) Merr.	Murashige and Skoog medium	Mixed in solution		Reduced plant germination, shoot and root length, vigor index and total chlorophyll content	(Gautam et al., 2016)
1, 10, 100, 500, and 1000 mg/L	<i>Vigna radiata</i> L.	Petriplate	Seed priming		Inhibited germination, seedling growth, and decreased sugar and protein content	(Singh et al., 2017)
100, 1000, and 2000 mg/L	<i>Vigna radiata</i> L.	Petriplate	Impregnated in filter paper		Altered quality of phenolic compounds, macronutrients (Na, Mg, and Ca) and micronutrients (Cu, Fe, Mn, Zn, and K).	(Karmous et al., 2022)

481 TITANIUM DIOXIDE

482 Titanium is a beneficial element that plays a significant role in nitrogen fixation, nutrient
483 absorption, and stimulation of enzymatic activities, and exhibits a hormesis effect in legume roots
484 (Lyu et al., 2019). Recently, there has been growing interest in utilizing manufactured TiO₂ NPs
485 in agriculture to enhance crop yield. Current studies have generally reported more beneficial
486 effects of TiO₂ NPs on leguminous plants compared to detrimental outcomes (**Table 4**). For
487 example, AL-oubaidi and Kasid observed enhanced fresh and dry weight and the generation of
488 secondary metabolites in chickpea (*Cicer arietinum* L.) callus embryos treated with TiO₂ NPs (AL-
489 oubaidi and Kasid, 2015). Foliar application of TiO₂ NPs to common beans (*Phaseolus vulgaris*
490 L.) at concentrations ranging from 10, 20, 30, and 50 mg/L increased chlorophyll content,
491 antioxidant enzyme activity, 8-deoxy-2-hydroxyguanosine, seed protein, grain weight, and crop
492 yield (A Ebrahimi et al., 2016; Ahmad Ebrahimi et al., 2016). Similarly, Khan et al. found that the
493 response of lentil (*Lens culinaris* Medik.) plants to TiO₂ NPs was concentration-dependent, with
494 lower concentrations positively influencing parameters such as vigor index, seed germination,
495 photosynthetic pigments, and biomass, while higher concentrations had adverse impacts, including
496 increased lipid peroxidation, DNA damage, and reactive oxygen species generation (Khan et al.,
497 2019). Priya et al. demonstrated that applying TiO₂ NPs at a concentration of 10 mg/L improved
498 various seedling traits in mungbean (*Vigna radiata* L.) plants, including germination rate, root and
499 shoot length, fresh and dry weight, and vigor index (Priya et al., 2020).

500 Furthermore, Verma et al. showed that common bean plants responded favorably to lower doses
501 of TiO₂ NPs (30 and 60 mg/L), which increased shoot and root growth, leaf number, chlorophyll
502 content, and seed weight (Verma et al., 2020). However, higher doses (240 mg/L) negatively
503 impacted these parameters. In kidney bean (*Phaseolus vulgaris* L.) seedlings, seed priming with
504 TiO₂ NPs at various doses (ranging from 0.10% to 2.50%) increased root and shoot length and
505 antioxidant activity (Paul et al., 2020a). Similarly, Mathew et al. found that applying TiO₂ NPs to
506 mungbean (*Vigna radiata* L.) plants at various concentrations (25 to 250 mg/L) enhanced seed
507 germination and growth rate compared to the control (Mathew et al., 2021). Further, it was found
508 that pea (*Pisum sativum* L.) defense mechanisms restricting water uptake were overcome by TiO₂
509 photocatalytic activity, stimulating photosynthesis. The impact of TiO₂ nanoparticles on
510 photosynthesis process in green pea was investigated through hydroponic cultivations at three Ti
511 levels (10, 50, and 100 mg/L). The nanoparticles penetrated plant tissues, altering CO₂ assimilation

512 and gas exchange parameters. The most significant effects were observed at 50 mg/L TiO₂
513 nanoparticles, enhancing photosynthesis efficiency and related parameters. Changes in element
514 concentrations, particularly Cu, Zn, Mn, and Fe, were noted, emphasizing their involvement in
515 photosynthesis (Skiba et al., 2024). Evaluating nanosized titanium dioxide (nTiO₂) interaction with
516 *Vigna radiata* L. demonstrated improved plant performance, increased seed germination, root and
517 shoot length, and elevated chlorophyll, flavonoid, and phenolic contents. Despite mild oxidative
518 stress, antioxidant enzyme activity countered lipid peroxidation (Thakur et al., 2021).

519 Kushwah et al. discovered that concentrations of TiO₂ NPs between 15 and 240 mg/L enhanced
520 root growth in green pea (*Pisum sativum* L.) plants but decreased the number and weight of
521 germinated seeds (Kushwah et al., 2022a). Higher concentrations of nanoparticles further reduced
522 seed germination, seed weight, survival rate, and overall plant growth compared to the control.

523 Kushwah and Patel reported that faba beans (*Vicia faba* L.) exposed to higher concentrations of
524 TiO₂ NPs exhibited aberrant chromosomal structure and meiotic activity (Kushwah and Patel,
525 2020). TiO₂ NPs exposure during seed germination in pea seeds (50 mg/L) was assessed over five
526 days. There were adverse effects on germination rate, mean daily germination, tissue dry weights,
527 water supply, solute leakage, oxidative stress, and antioxidant enzyme activities. Oxidative and
528 metabolic disturbances were identified as significant factors influencing pea (*Pisum sativum* L.)
529 seed germination. These studies suggested TiO₂ nanoparticles could affect plant growth and
530 physiological processes, but the outcomes depend on concentration, exposure duration, and plant
531 species (Basahi, 2021). So, it is essential to identify the appropriate concentration range of TiO₂
532 NPs that maximizes benefits while minimizing potential risks for different leguminous crops.
533 Further research is necessary to fully understand the precise mechanisms by which TiO₂ NPs affect
534 plant growth, development, and biochemical activity. This knowledge will be crucial for
535 maximizing the potential use of TiO₂ NPs in agriculture. Additionally, long-term studies are
536 needed to assess the ecological risks and feasibility of utilizing TiO₂ NPs in agricultural practices.

537 **Table 4: Effects of various concentration of titanium dioxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
TiO ₂	4.5 and 6 mg/L	<i>Cicer arietinum</i> L.	Culture vessels	Added in culture medium	Increased callus fresh weight, dry weight, and secondary metabolite production	(AL-oubaidi and Kasid, 2015)
	10, 20, 30, and 50 mg/L	<i>Phaseolus vulgaris</i> L.	Factorial experiment	Spraying at different plant growth stages	Enhanced chlorophyll a content, seed protein levels, grain weight, crop yield, antioxidant activity, and the 8-deoxy-2-hydroxyguanosine content	(A Ebrahimi et al., 2016; Ahmad Ebrahimi et al., 2016)
	10 mg/L	<i>Vigna radiata</i> L.	Glass plate	Seed priming	Improved seed germination rates, root-shoot length, total fresh and dry weight, and vigor index	(Priya et al., 2020)
	100, 250, 500, 750, 1000, 1500, 2000, and 2500 mg/L	<i>Phaseolus vulgaris</i> L.	Germination paper	Seed priming	Increased shoot-root length, lateral root formation, and antioxidant enzyme activities	(Paul et al., 2020b)
	25, 50, 200, 150, 200, and 250 mg/L	<i>Vigna radiata</i> L.	Petriplate experiment	Seed priming	Increased germination indices	(Mathew et al., 2021)

	15, 30, 60, 120, and 240 mg/L	<i>Pisum sativum</i> L.	Pot experiment	Seed priming	Increased root growth but decreased shoot growth, seed germination, and weight	(Kushwah et al., 2022b)
	10, 50, and 100 mg/L	<i>Pisum sativum</i> L.	Hydroponic culture	Mixed in solution	Increased photosynthesis efficiency, transpiration, stomatal conductance, rubisco and ETC activity, micro and macronutrients	(Skiba et al., 2024)
	10 and 100 mg/L	<i>Vigna radiata</i> L.	Hydroponic culture	Mixed in solution	Increased seed germination, root and shoot length, fresh and dry weight, chlorophyll, flavonoid, phenolic content	(Thakur et al., 2021)
Negative Effects						
TiO ₂	25, 50, 75, 100, and 200 mg/L	<i>Lens culinaris</i> Medik.	Petriplate and pot culture	Seed priming	Increased DNA damage, lipid peroxidation, and reactive oxygen species (ROS) accompanied by a decrease in the vigor index, seed germination, fresh weight, dry weight, and photosynthetic pigments	(Khan et al., 2019)
	15, 30, 60, 120, and 240 mg/L	<i>Vicia faba</i> L.	Pot experiment	Seed priming	Reduction in seed germination, seed weight, rate of survival, plant growth, and an increase in chromosomal abnormalities	(Kushwah and Patel, 2020)
	15, 30, 60, 120, and 240 mg/L	<i>Phaseolus vulgaris</i> L.	Plastic pot experiment	Exogenous treatment	Decreased the root and shoot growth rate of seedlings, plant height, leaf number, seed weight, and chlorophyll content	(Verma et al., 2020)

	50 mg/L	<i>Pisum sativum</i> L. cv. Merveille de Kelvedon	Petriplate	Moistened filter paper	Decreased germination rate, mean daily germination, embryo, coat, and cotyledon dry weights, water supply, solute leakage, and elevated oxidative stress and antioxidant enzyme activities	(Basahi, 2021)
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538

539 CERIUM OXIDE

540 CeO₂ NPs have attracted interest recently due to their potential to increase crop yield (Prakash et
541 al., 2021b). Applying cerium to soil positively impacts overall plant growth by enhancing nitrogen
542 assimilation and photosystem II activity (Awad et al., 2023). However, the effects of cerium on
543 plants depend on several factors, like concentration, exposure time, and plant species (**Table 5**).
544 Higher concentrations of CeO₂ NPs were observed to reduce Mg, K, Ca, Cu, Mn, and Fe in roots
545 and shoots in peas (*Pisum sativum* L.) (Skiba et al., 2021). According to Cao et al., CeO₂ NPs in
546 soybeans at 100 mg/kg increased the activity of the enzyme rubisco carboxylase, the rate of
547 photosynthetic reaction, and plant development (Cao et al., 2017). In comparison, higher
548 concentrations had the opposite effects. With 100 and 500 mg/kg concentrations, CeO₂ NPs
549 accelerated photosynthetic rate and nodule formation in soybean (*Glycine max* (L.) Merr.) plants
550 (Stowers et al., 2018). Similar improvements in shoot elongation, biomass, stress-related enzyme
551 activity, and antioxidants were seen in common beans (*Phaseolus vulgaris* L.) when CeO₂ NPs
552 were applied at 50 and 100 mg/L (Salehi et al., 2020). When applied foliarly to mungbeans, CeO₂
553 NPs at dosages of 250 and 500 mg/L increased dry matter and antioxidant activity, but at 1000
554 mg/L, they had the opposite effect (Kamali-Andani et al., 2022).

555 In contrast, a study on common bean plants found that foliar treatment with CeO₂ NPs (250, 500,
556 1000, and 2000 mg/L) resulted in altered proteomics and metabolomics, electrolyte leakage,
557 stomatal length, increased osmolytes and phytosiderophores concentration, lipid degradation, and
558 changes in the electron transport chain machinery and photosynthesis rate (Salehi et al., 2018b).
559 Common beans were subjected to a foliar application of CeO₂ NPs (250–2000 mg/L), resulting in
560 modifications to the proteome, metabolomics, electrolyte leakage, stomatal length, osmolyte
561 concentration, lipid breakdown, and photosynthesis rate (Salehi et al., 2018b). Moreover, the
562 application of CeO₂ NPs at 2000 mg/L resulted in adverse effects such as chromosomal
563 abnormalities, pollen abortion, and lower yield (Salehi et al., 2021a). The levels of Cu, Fe, Zn, and
564 Mn in pea (*Pisum sativum* L.) plants were also decreased by CeO₂ NPs (Skiba and Wolf, 2019).
565 Further, Viezcas found CeO₂ NPs adversely affected soybean (*Glycine max* (L.) Merr.) plant
566 growth and nitrogen fixation. However, in Mesquite (*Prosopis juliflora* (Sw.) DC.) plants, the
567 exposure of CeO₂ NPs showed increased cerium uptake in roots. His study pointed out the
568 necessity of assessing nanoparticle transfer into the food chain, particularly in plants'
569 reproductive/edible parts, highlighting potential concerns for plant health and food safety

570 (Hernandez Viezcas, 2013). Research on nutrient uptake, photosynthesis, and plant metabolism
571 must be done in-depth to understand how CeO₂ NPs interact with plants. Standardized techniques
572 and comparative studies under controlled conditions will improve the validity and comparability
573 of findings across legume species, helping establish guidelines for the safe and efficient use of
574 CeO₂ NPs in agriculture.

575 **Table 5: Effects of various concentration of cerium oxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
CeO ₂	100 mg/kg	<i>Glycine max</i> (L.) Merr. var. 'Tohya'	Pot culture	Mixed in soil	Improved photosynthetic rate, rubisco activity, and plant growth were observed	(Cao et al., 2017)
	100 and 500 mg/kg	<i>Glycine max</i> (L.) Merr.	Pot culture	Added in soil	Increased net photosynthetic rate and the number of nodules	(Stowers et al., 2018)
	50 and 100 mg/L	<i>Phaseolus vulgaris</i> L. var. khomain	Agar medium	Mixed with agar medium	Proline content, shoot elongation, root, and shoot fresh weight, JAs, and antioxidant content were enhanced	(Salehi et al., 2020)
	250 and 500 mg/L	<i>Vigna radiata</i> L.	Pot culture	Foliar application	Dry matter and antioxidant enzymatic activity showed improvement	(Kamali-Andani et al., 2022)
Negative Effects						
CeO ₂	500 mg/kg	<i>Glycine max</i> (L.) Merr. var. 'Tohya'	Pot culture	Mixed in soil	Decreased in the photosynthetic rate, rubisco activity, and plant growth	(Cao et al., 2017)

250, 500, 1000, and 2000 mg/L	<i>Phaseolus vulgaris</i> L.	Pot culture	Foliar and soil application	Increased electrolyte leakage, stomatal length, osmolytes, phytosiderophores concentration, and lipid degradation	(Salehi et al., 2018b)
200 mg/L	<i>Pisum sativum</i> L.	Hydroponic	Mixed with Hoagland nutrient solution	Zinc concentration, root length, metal concentration, and solution-root transfer were restricted	(Skiba and Wolf, 2019)
50, 100, and 500 mg/kg	<i>Glycine max</i> (L.) Merr.	Pot culture	Added in soil	Diminished plant growth and nitrogen fixation	(Hernandez Viezcas, 2013)
2000 mg/L	<i>Phaseolus vulgaris</i> L.	Pot culture	Foliar spray	Pollen showed increased structural and chromosomal damage, resulting in decreased seed yield	(Salehi et al., 2021a)
1000 mg/L	<i>Vigna radiata</i> L.	Pot culture	Foliar application	Starch granules, ROS, and chloroplast swelling increased, while photosynthetic pigments, nitrogen fixation, and plant growth were reduced	(Kamali-Andani et al., 2022)

577 **SILICON DIOXIDE**

578 Silicon (Si) positively affects crop productivity and stress tolerance when applied in bulk form
579 (Shivaraj et al., 2022). In the case of rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) plants
580 grown in soils contaminated with As, Cd, and Cr, using SiO₂ NPs has shown promising results
581 (Manzoor et al., 2022; Wang et al., 2021). Based on existing research conducted on leguminous
582 plants, the effects of SiO₂ NPs have been observed to be predominantly detrimental rather than
583 advantageous (**Table 6**). Ross et al. observed reduced growth in mungbean (*Vigna radiata* L.)
584 plants treated with SiO₂ NPs compared to the control group (Ross et al., 2016). This could be
585 attributed to the fact that silicon nanoparticles possess the potential to influence nutrient
586 absorption, cellular activities, and hormonal balance in mungbean physiology, consequently
587 hindering growth processes.

588 Similarly, Thabet et al. reported detrimental effects on germination, shoot length, and
589 chromosomal structure in broad bean (*Vicia faba* L.) seeds treated with SiO₂ NPs at 25, 50, and
590 75 mg/L 14 concentrations. On the other hand, Kashyap and Siddiqui discovered that pea (*Pisum*
591 *sativum* L.) plants exhibited enhanced growth, chlorophyll and carotenoid content, proline content,
592 plant length, total fresh weight, dry weight, and nodule number when treated topically with SiO₂
593 NPs at low concentration (100 mg/L) (Kashyap and Siddiqui, 2021). They suggested that pea
594 plants' interaction with SiO₂ NPs at a lower concentration could promote nutrient absorption,
595 physiological activities, and symbiotic interactions with nitrogen-fixing bacteria. Given the
596 distinctive biological characteristics of leguminous plants and their symbiotic relationships with
597 nitrogen-fixing bacteria, it is imperative to conduct further investigation to fully understand the
598 precise interactions and potential differential effects of SiO₂ NPs in these plants.

599

600 **Table 6: Effects of various concentration of silicon dioxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
SiO ₂	100 mg/L	<i>Pisum sativum</i> L.	Pot culture	Foliar and soil application	Increased levels of proline, chlorophyll, and carotenoids, along with higher fresh and dry weight, increased plant length, and number of nodules	(Kashyap and Siddiqui, 2021)
Negative Effects						
SiO ₂	20 mg/L	<i>Vigna radiata</i> L.	Petriplate	Nanopore solution	Reduced growth of seedlings	(Ross et al., 2016)
	25, 50, and 75 mg/L	<i>Vicia faba</i> L.	Petriplate	Seed priming	Decreased germination percentage, shorter shoot length, and increased occurrence of chromosomal aberrations	(Thabet et al., 2019)

601

602 **MAGNESIUM OXIDE**

603 Magnesium (Mg), an essential macronutrient and a pivotal component of the chlorophyll molecule,
604 is critical in regulating photosynthetic enzymes, plant development, and physiological processes.
605 It is also indispensable for synthesizing and functioning nucleic acids and adenosine triphosphate
606 (ATP) (Leher, 2022). Several studies have explored the impacts of MgO NPs on legume plants
607 **(Table 7)**. For instance, Elakkiya et al. conducted a study on long beans (*Vigna unguiculata* L.
608 ssp. *sesquipedalis*), elucidating that the application of MgO NPs at a concentration of 5 mg/L led
609 to an increase in chlorophyll content (Elakkiya V. et al., 2020). Improved chlorophyll content
610 could enhance photosynthetic efficiency, increasing plant growth energy production. Similarly,
611 Kocak et al. observed that MgO NPs, at varying concentrations (185, 370, and 555 mg/L),
612 enhanced shoot formation, with the highest efficacy observed at 555 mg/L. Additionally, applying
613 MgO NPs at 370 mg/L resulted in an augmentation of shoot number, shoot length, root number,
614 root formation, and root length in cowpeas (*Vigna unguiculata* L.) (Koçak et al., 2021). Thus, it
615 could be inferred that MgO nanoparticles positively impacted metabolic processes, enhancing
616 nutrient uptake and utilization, ultimately resulting in improved plant growth, shoot formation, and
617 root development. In the case of mungbean plants (*Vigna radiata* L.), Abdallah et al. reported that
618 MgO NPs, at a concentration of 100 mg/L, positively influenced shoot-root fresh weight, dry
619 weight, as well as the number and length of root nodules (Abdallah et al., 2022). This may be
620 attributed to improved nutrient availability, creating a conducive environment for symbiotic
621 interactions with nitrogen-fixing bacteria. Similarly, Sharma et al. documented that treatment with
622 MgO NPs enhanced chlorophyll, carbohydrate, protein, phenol, and flavonoid levels (Priya
623 Sharma et al., 2022a, 2022b, 2022c). Furthermore, it stimulated root and shoot elongation, along
624 with augmenting the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase
625 (SOD), and ascorbate peroxidase (APX) in horse gram and black chickpea (*Cicer arietinum* L.)
626 plants. Additionally, MgO NPs, sized 35–40 nm, positively impacted cowpea plantlets *in vitro*.
627 Higher concentrations (555 mg/L) increased callus formation by 25%, while 370 mg/L enhanced
628 shoot multiplication, indicating potential efficiency improvements in cowpea (*Vigna unguiculata*
629 L.) tissue culture (Koçak et al., 2023). Recently, Abdelfattah et al. innovatively synthesized MgO
630 NPs using *Saccharomyces cerevisiae* extract, with notable impacts on cowpea *in vitro* parameters
631 (Abdelfattah et al., 2023). At 200 mg/L concentration, the MgO NPs demonstrated significant
632 enhancements in shoot length, shoot dry-weight, and root dry-weight by 27.35%, 45.09%, and

633 31.91%, respectively. Moreover, MgO NPs increased photosynthetic pigments and biochemical
634 constituents, showcasing insecticidal efficacy against cowpea pests and dose-dependent effects on
635 rats' biochemical and histopathological profiles. Nevertheless, it is noteworthy that certain studies
636 have reported adverse effects of MgO NPs (**Table 7**). Sharma et al. found that exposure to MgO
637 NPs resulted in increased toxicity, leading to alterations in root length, chlorophyll content, plant
638 biomass, antioxidant activity, non-enzymatic antioxidants, carbohydrate, and protein accumulation
639 in black gram (*Cicer arietinum* L.), mungbean (*Vigna radiata* L.), and lentil (*Lens culinaris*
640 Medik.) plants. Consequently, further research endeavors are necessary to elucidate the factors
641 contributing to the variability in the response of legume plants to MgO NPs, including disparities
642 in nanoparticle characteristics, plant species, experimental conditions, and exposure duration (P
643 Sharma et al., 2022; Priya Sharma et al., 2022a, 2022c; Sharma et al., 2021).

644 **Table 7: Effects of various concentration of magnesium oxide nanoparticles on legume plants**

Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Positive Effects						
MgO	5000 mg/L	<i>Vigna unguiculata</i> L. ssp. <i>sesquipedalis</i>	Petriplate	MS medium	Increased chlorophyll concentration	(Elakkiya V. et al., 2020)
	370 and 555 mg/L	<i>Vigna Unguiculata</i> L.	Petriplate	MS medium	Improved number of shoots, roots, root length, shoot length, and callus formation	(Koçak et al., 2021)
	100 mg/L	<i>Vigna radiata</i> L.	Plastic cups	Added in soil	Enhanced shoot and root fresh weight, dry weight, root and shoot length, and root nodule number	(Abdallah et al., 2022)
	10, 50, 100, and 150 mg/L	<i>Macrotyloma uniflorum</i> (Lam.) Verdc. and <i>Cicer arietinum</i> L.	Petriplate	Seed priming	Increased shoot length, biomass, chlorophyll content, accumulation of carbohydrates, proteins, phenols, flavonoids, and antioxidants	(Priya Sharma et al., 2022b, 2022a)
	370 and 555 mg/L	<i>Vigna unguiculata</i> L.	Petriplate	MS medium	Improved shoot, root, and callus formation, shoot and root length, and plantlets' growth	(Koçak et al., 2023)

	150 and 200 mg/L	<i>Vigna unguiculata</i> L.	Pot culture	Foliar spray	Enhanced shoot length, shoot and root dry-weight, photosynthetic pigments, soluble proteins, and carbohydrates	(Abdelfattah et al., 2023)
Negative Effects						
MgO	10, 50, 100, and 150 mg/L	<i>Vigna mungo</i> L.	Petriplate	Seed priming	Decreased shoot and root length, chlorophyll content, biomass, phenol and flavonoid content, and antioxidant levels	(Sharma et al., 2021)
	10, 50, 100, and 150 mg/L	<i>Vigna radiata</i> L.	Petriplate	Seed priming	Reduced plant growth, fresh biomass, chlorophyll and protein content, enzymatic and non-enzymatic levels	(P Sharma et al., 2022)
	10, 50, 100, and 150 mg/L	<i>Lens culinaris</i> Medik.	Petriplate	Seed priming	Decreased shoot and root length, carbohydrate, chlorophyll, flavonoid, and protein content, as well as antioxidant levels	(Priya Sharma et al., 2022a)

646 **Effect on the nitrogen fixation**

647 Nitrogen fixation is a vital metabolic process that enhances the productivity of leguminous crops
648 by converting atmospheric nitrogen into ammonium with the help of diazotrophic microorganisms,
649 such as *Rhizobium*. These microorganisms form a symbiotic relationship with legume plants within
650 root nodules and utilize the nitrogenase enzyme for this conversion (Rapson et al., 2020).
651 Improving the symbiotic interaction between plants and bacteria is crucial for enhancing legume
652 production, and various nanoparticles, including metal oxide nanoparticles (MONPs), have been
653 investigated for this purpose. For instance, Muñoz-Márquez et al. (Muñoz-Márquez et al., 2022)
654 applied nano-Mo in green beans (*Phaseolus vulgaris* L.), Osman et al. (Osman et al., 2020) used
655 MoO₃ NPs in dry beans (*Phaseolus vulgaris* L.), and Alwan et al. (S. Alwan et al., 2022) employed
656 Fe₂O₃ NPs in broad beans (*Vicia faba* L.) for improving and understanding the symbiotic
657 interaction between plants and bacteria. However, the effects of MONPs on plants depend on their
658 type and concentration, exhibiting beneficial effects at lower levels but detrimental effects at
659 higher levels. For instance, Kumar et al. (**Table 2**) demonstrated that ZnO NPs (1.5 mg/L)
660 improved the activity of the nitrogenase enzyme in nodulated roots of green gram (*Vigna radiata*
661 L.), cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.), and cowpea (*Vigna unguiculata* L.) plants,
662 while higher concentrations (10mg/L) of ZnO NPs reduced the activity (Kumar et al., 2015).
663 Similarly, applying Fe₂O₃ or Fe₃O₄ NPs enhanced nitrogen fixation and nitrogenase activity in
664 soybeans (*Glycine max* (L.) Merr.) and green beans (*Phaseolus vulgaris* L.) (De Souza-Torres et
665 al., 2021b; GUTIÉRREZ-RUELAS et al., 2021; Huang et al., 2014; Yang et al., 2020).
666 Nevertheless, conducting a comparative study evaluating different MONPs and their varying
667 concentrations under controlled conditions would be beneficial for identifying the optimal and
668 safest formulations to enhance the symbiotic interaction between bean plants and diazotrophic
669 bacteria.

670 On the contrary, several studies have observed undesirable effects when exposing garden pea
671 plants (*Rhizobium leguminosarum* bv. *viciae* 3841) to TiO₂ and ZnO NPs. These effects include a
672 decrease in the number of secondary lateral roots, impairment of the nodulation process and nodule
673 development, and inhibition of nitrogen fixation. Furthermore, the application of ZnO NPs was
674 found to disrupt the symbiotic relationship between *Rhizobium* and legume plants, resulting in
675 reduced nodulation and impaired nitrogen fixation in lentil (*Lens culinaris* Medik.) and alfalfa
676 (*Medicago sativa* L.) plants. These findings indicate the potential toxic effects of nanoparticles on

677 the nitrogen fixation process (Kamali-Andani et al., 2022; Siddiqui et al., 2018; Sun et al., 2022).
678 To optimize the use of MONPs and minimize any potential negative effects, a comprehensive
679 understanding of how MONPs interact with legume plants and diazotrophic bacteria, affecting
680 nodulation, nodule formation, nitrogenase activity, and overall nitrogen fixation efficiency, is
681 essential. Additionally, it is critical to investigate the persistence, mobility, and accumulation of
682 MONPs in soil and their impact on microbial populations, soil health, and ecosystem dynamics.

683 **Effect of metal oxide nanoparticles under abiotic stress conditions**

684 Reducing agricultural land area due to abiotic stresses such as salinity, drought, cold, water
685 scarcity, temperature fluctuations, and heavy metal contamination poses a significant challenge,
686 especially given the increasing global population. Nowadays, scientists are investigating the use
687 of metal and metal oxide nanoparticles to address the impact of these abiotic stresses on
688 leguminous plants (Sarraf et al., 2022; Ulhassan et al., 2022). As a result, various scientific studies
689 have been conducted to determine the possible benefits of MONPs on leguminous crops (**Table**
690 **8**). In a study conducted by Gaafar et al., found that the application of zinc oxide nanoparticles
691 (ZnO NPs) mitigated salt stress in soybean (*Glycine max* (L.) Merr.) plants (Gaafar et al., 2020).
692 The most favorable outcomes were observed with a 50 mg/L concentration of ZnO NPs, resulting
693 in enhanced growth and decreased oxidative stress.

694 Similarly, the application of nano-ZnO demonstrated positive effects in alleviating salinity stress
695 in cowpeas (*Vigna unguiculata* L.), suggesting a potential role in increasing crop productivity
696 (Mohammad Alabdallah and Saeed Alzahrani, 2020). Biosynthesized ZnO nanoparticles
697 positively impacted faba bean (*Vicia faba* L.), indicating a potential role in improving tolerance to
698 salinity (Mogazy and Hanafy, 2022). Furthermore, foliar-applied Zn nanoparticles and nano-
699 loaded *Moringa* extract showcased positive effects on *Vicia faba* L. under saline conditions (Ragab
700 et al., 2022). Meanwhile, titanium dioxide nanoparticles (nTiO₂) also displayed concentration-
701 dependent effects, with lower concentrations positively influencing *Vicia faba* L. growth under
702 salty conditions (Abdel Latef et al., 2017). Lately, nTiO₂ has demonstrated a protective effect
703 against salinity-induced stress in *Vicia faba* L., resulting in improved growth, reduced DNA
704 damage, and upregulation of antioxidant genes (Omar et al., 2023). These findings demonstrate
705 the efficacy of MONPs, especially ZnO NPs and nTiO₂, alleviating salt stress in leguminous crops
706 and offering valuable perspectives for sustainable agriculture.

707 Further studies discussed the role of nanoparticle, particularly zinc oxide (ZnO), iron oxide (FeO),
708 titanium dioxide (TiO₂), and silicon dioxide (SiO₂), in mitigating the adverse effects of drought
709 stress on various crops, including *Glycine max* (L.) Merr. (soybean), *Vigna radiata* L. (mungbean),
710 *Pisum sativum* L. (pea), *Cicer arietinum* L. (chickpea), *Lathyrus sativus* L. (grass pea), and *Vigna*
711 *unguiculata* L. (cowpea) (**Table 8**). These nanoparticles positively impacted seed germination,
712 plant growth, physiological processes, antioxidant defense mechanisms, and yield parameters
713 under water-deficient conditions. Notably, ZnO nanoparticles improved *Glycine max* (L.) Merr.
714 (soybean) growth and countered oxidative stress induced by salt stress (Gaafar et al., 2020). The
715 underlying mechanism likely involves ZnO NPs influencing the plant's biochemical processes,
716 such as enhancing antioxidant activity, thus mitigating the negative impact of salt-induced stress.
717 Similarly, seed priming with ZnO nanoparticles enhanced *Vigna radiata* L. (mungbean)
718 performance, including growth, antioxidant defense, and yield, under water stress (Mazhar et al.,
719 2023a). It is attributed to the nanoparticles' ability to enhance osmolyte accumulation, such as
720 proline and soluble sugars, thereby enhancing the plant's resilience to drought. Iron oxide
721 nanoparticles also proved effective in improving the activity of *Pisum sativum* L. (pea) plants
722 under drought, as seen in increased root length, antioxidant enzyme activity, and yield (Mazhar et
723 al., 2023b). TiO₂ nanoparticles positively affected *Cicer arietinum* L. (chickpea), improving
724 chlorophyll index and osmotic potential, and reducing cellular inflammation under drought stress
725 (Ghorbani et al., 2021). The mechanisms potentially involve the nanoparticles' influence on plant
726 physiological processes, resulting in enhanced water use efficiency and diminished cellular
727 inflammation during water scarcity. Furthermore, nano-iron foliar spray enhanced *Glycine max*
728 (L.) Merr. (soybean)'s drought tolerance, yield, and seed quality, offering the potential for reducing
729 losses caused by drought stress (Dola et al., 2022). Silicon dioxide nanoparticles also showed
730 promising results in mitigating drought stress in *Vigna unguiculata* L. (cowpea) by improving
731 plant growth, biochemical attributes, and fatty acid profiles (Zadegan et al., 2023).

732 Aghdam et al. demonstrated that applying TiO₂ NPs to plants, such as lentils (*Lens culinaris*
733 Medik.), could reduce oxidative stress caused by drought (Aghdam et al., 2016). TiO₂ NPs
734 increased chlorophyll and carotenoid levels while decreasing the accumulation of hydrogen
735 peroxide (H₂O₂) and malondialdehyde (MDA), indicators of oxidative damage. Similarly, Singh
736 and Lee discovered that a different type of metal oxide nanoparticle (MONP) reduced the negative
737 impacts of cadmium on soybeans (*Glycine max* (L.) Merr.) by increasing photosynthetic efficiency

738 and overall growth (Singh and Lee, 2016). Hasanpour et al. revealed that TiO₂ NPs alleviate cold
739 stress in chickpeas (*Cicer arietinum* L.) by restoring rubisco activity and decreasing H₂O₂ levels
740 (Hasanpour et al., 2015). TiO₂ NPs possibly act as mediators in enhancing the plant's antioxidant
741 defense system, thus reducing oxidative stress induced by cold conditions. This restoration of
742 rubisco activity contributes to efficiently utilizing carbon dioxide during photosynthesis,
743 mitigating the negative impacts of cold stress on chickpea plants. Mustafa et al. demonstrated that
744 aluminum oxide (Al₂O₃) nanoparticles could improve soybean (*Glycine max* (L.) Merr.) growth
745 under flood conditions by controlling plant metabolism and reducing root cell death (Mustafa et
746 al., 2015). Reducing root cell death indicates improved oxygen uptake and nutrient absorption,
747 contributing to enhanced growth under flood stress. Kareem et al. showed that exogenous
748 application of ZnO NPs dramatically increases the production of osmolytes and antioxidants in
749 late-sown mungbean plants (*Vigna radiata* L.), suggesting a way to resist heat stress (Kareem et
750 al., 2022).

751 Furthermore, a further study found that foliar application of Fe₃O₄ NPs reduces the impacts of
752 drought stress in soybeans (*Glycine max* (L.) Merr.), resulting in higher plant yield and seed quality
753 (Dola et al., 2022). According to Ahmad et al. (2020), ZnO NPs treatment effectively mitigates
754 the harmful effects of arsenic (As) by limiting As uptake and modulating various biochemical
755 attributes within soybean plants, including the ascorbate-glutathione cycle, glyoxalase system, and
756 antioxidant enzymatic system (Ul Hassan et al., 2022). The formation of complexes between
757 nanoparticles and arsenic could be a probable mechanism resulting in a reduction in its uptake by
758 plant roots. These studies suggest that using nanoparticles could offer benefits in addressing abiotic
759 stresses in agriculture. Future research should aim to understand the long-term environmental
760 impacts and optimize nanoparticle formulations for sustainable and effective agricultural practices.

761 **Table 8: Positive effects of various metal oxide nanoparticles on legume plants under abiotic stress conditions**

Stress	Stress conc.	Type of MONPs	Effective MONPs Concentrations	Plant used	Experimental Type	Mode of Treatment	Effects	Reference
Salt Stress	250 mM	ZnO	50 mg/L	<i>Glycine max</i> (L.) Merr. cv. Giza 111	Plastic pot culture	Seed priming	Improved growth parameters and photosynthetic performance and reduced proline and MDA level	(Gaafar et al., 2020)
	10, 25, 50, 75 and 100% of seawater	ZnO	10 mg/L	<i>Vigna unguiculata</i> L. var. californica blackeye NO.46	Pot experiment	Foliar spray	Improved shoot and root length, fresh and dry weight, leaf area, and relative growth rate	(Mohammad Alabdallah and Saeed Alzahran i, 2020)
	150 mM	ZnO	50 mg/L	<i>Vicia faba</i> L. cv. Nubaria, 1	Plastic pot culture	Foliar application	Improved plant growth, gathering of antioxidants, secondary metabolites, and osmolytes	(Mogazy and Hanafy, 2022)

50 and 100 mM	ZnO	50 mg/L	<i>Vicia faba</i> L.	Field experiment	Foliar spray	Improved shoot length, numbers of leaves, RWC, shoot and roots fresh and dry weight, photosynthetic pigments, proline content and mineral elements	(Ragab et al., 2022)
50 and 100 mM	ZnO	50 mg/L	<i>Vicia faba</i> L.	Plastic pot culture	Foliar spray	Increased shoot length, number of leaves, RWC, shoot and root fresh and dry weight, photosynthetic pigments, proline, mineral elements, total phenol, and antioxidant enzymes	(Sherif et al., 2022)
50 and 100 mg/kg	ZnO	50 mg/L	<i>Medicago sativa</i> L.	Plastic pot culture	Foliar application	Increased proline, total soluble sugar, and total soluble protein content	(Hassan et al., 2023)
180 mM	TiO ₂	10 mg/L	<i>Vicia faba</i> L. cv. Misr-1	Plastic pot culture	Foliar spray	Increased activity of enzymatic antioxidants level, soluble sugars, amino acids, and proline	(Abdel Latef et al., 2017)
100 and 200 mM	TiO ₂	10 or 20 mg/L	<i>Vicia faba</i> L. (Sakha 101)	Pot culture	Mixed with irrigated water	Increased cell division, antioxidant genes, heat shock protein genes, and reduced chromosomal aberrations, and DNA damage	(Omar et al., 2023)

	4, 8 and 12 dSm ⁻¹	TiO ₂	20, 40, and 60 mg/L	<i>Lathyrus sativus</i> L.	Petriplate	Seed priming and soaked filter paper	Increased germination and seedling length	(Hojjat et al., 2020)
Drought Stress	Cutting of irrigation in 50% flowering and 50% pod setting stages	ZnO	5000 and 10000 mg/L	<i>Vigna radiata</i> L.	Field experiment	Foliar application	Improved height, proline content, grain, and biological yield	(Makarina et al., 2017)
	-0.5 and -1 MPa	ZnO	0.5 and 1 mg/L	<i>Glycine max</i> (L.) Merr.	Petriplate	Mixed with culture	Improved germination, non-enzymatic and enzymatic antioxidant enzymatic activity	(Sedghi et al., 2021)
	300 mm	ZnO	75 mg/L	<i>Vigna radiata</i> L.	Field experiment	Seed priming	Enhanced shoot and root length, number of leaves, vegetative and reproductive branches, antioxidant defense, osmolytes, yield and reduced MDA and H ₂ O ₂	(Mazhar et al., 2023a)

Required amount	Fe ₂ O ₃	20 and 50 mg/L	<i>Pisum sativum</i> L.	Pot culture	Mixed with irrigated water	Improved root length, tendrils Length, RWC, grain weight and count of grains per pod	(Ashraf et al., 2019)
40% field capacity	Fe ₃ O ₄	200 mg/L	<i>Glycine max</i> (L.) Merr.	Pot culture	Foliar application	Increased growth, physiology, yield, and quality of seeds	(Dola et al., 2022)
300 mm	FeO	75 mg/L	<i>Pisum sativum</i> L.	Field experiment	Seed priming	Increased root length, number of leaves, antioxidant enzymes, and yield and reduced MDA and H ₂ O ₂	(Mazhar et al., 2023b)
40, 60 and 90% field capacity	TiO ₂	5, 20, 10 and 40 mg/L	<i>Cicer arietinum</i> L.	Field experiment	Foliar spray	Improved total dry weight, fresh weight, and growth	(Ghorbani et al., 2021)
50% field capacity	TiO ₂	10, 100, and 500 mg/L	<i>Linum usitatissimum</i> L.	Pot culture	Foliar spray	Improved chlorophyll and carotenoid levels and decreased H ₂ O ₂ and MDA	(Aghdam et al., 2016)

	Polyethylene glycol (PEG), -0.27, -0.53, and -0.80 MPa	TiO ₂	20 mg/L	<i>Lathyrus sativus</i> L.	Petriplate	Seed priming	Elevate Germination parameter, root length, shoot length, root, and shoot fresh weight and dry weight	(Hojjat, 2020)
	40%, 60%, and 90% field capacity	TiO ₂	20 and 40 mg/L	<i>Cicer arietinum</i> L.	Pot culture	Poured in soil at seedling stage	Enhanced chlorophyll and carotenoid level, APX, POX, SOD, proline level, total phenols, and 2,2-diphenyl-1-picrylhydrazyl-hydrate	(Ghorbani et al., 2023)
	60, 100, and 140 mm evaporation	SiO ₂	50 and 100 mg/L	<i>Vigna unguiculata</i> L.	Field experiment	Foliar spray	Enhanced biological, seed yield, RWC, and decreased TSS, MDA, CAT, and SOD	(Zadegan et al., 2023)
Flood	-	Al ₂ O ₃	50 mg/L	<i>Glycine max</i> (L.) Merr.	Plastic case	2-day old plant transferred to solution	Improved plant growth and decreased cell death	(Mustafa et al., 2015)

Heat	<40/25° C for S1, >40/25° C for S2 and S3	ZnO	15, 30, 45, and 60 mg/L	<i>Vigna radiata</i> L. var. NM- 2016	Field (Plot) culture	Foliar application	Improved production of antioxidants and osmolytes and chlorophyll contents and gas exchange attributes, seeds per pod and pods per plant	(Kareem et al., 2022)
Cold	4°C	TiO ₂	5 mg/L	<i>Cicer arietinum</i> L.	Pot culture	Foliar spray	Decreased H ₂ O ₂ content and improved levels of CaLRubisco, CaSRubisco and Cachlorophyll a/bbinding protein genes	(Hasanp our et al., 2015)
Cadm ium	50, 100, and 150 mg/kg	TiO ₂	100, 200, and 300 mg/kg	<i>Glycine max</i> (L.) Merr.	Pot culture	Added in soil	Improved photosynthetic pigments and growth	(Singh and Lee, 2016)
Arsen ic	10 and 20 µM	ZnO	50 and 100 mg/L	<i>Glycine max</i> (L.) Merr.	Pot culture	Foliar spray	Improved shoot and root length, net photosynthetic rate, transpiration, stomatal conductance, and photochemical yield	(Ahmad et al., 2020)

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763

764 **Conclusion**

765 In conclusion, this article comprehensively summarizes recent research on MONPs uptake
766 mechanisms and their impact on leguminous crops. The uptake and accumulation of MONPs in
767 leguminous plants depend on factors such as particle size, application method, and plant species.
768 The role of specific MONPs, such as iron oxide, zinc oxide, copper oxide, titanium dioxide, cerium
769 oxide, silicon dioxide, and magnesium oxide, in legume growth has shown promise but also
770 presents challenges, particularly in understanding optimal concentrations and potential long-term
771 effects. Iron oxide nanoparticles have demonstrated positive impacts on plant growth, but their
772 reactivity raises concerns, necessitating further research to determine optimal conditions and
773 assess potential ecological impacts. Zinc oxide nanoparticles exhibit beneficial and detrimental
774 effects on different leguminous crops, emphasizing the need for careful consideration of
775 concentration and nanoparticle size. Copper oxide nanoparticles' concentration-dependent effects
776 on soybean and mungbean underscore the importance of understanding their influence on plant
777 physiology. Titanium dioxide nanoparticles generally show beneficial effects, but comprehensive
778 research is required to determine optimal concentrations and assess long-term ecological impacts.
779 Cerium oxide nanoparticles exhibit potential benefits in enhancing crop yield, but their effects vary
780 with concentration and plant species. Silicon dioxide nanoparticles have contrasting effects on
781 different leguminous plants, indicating the need for species-specific research to understand
782 interactions fully. Magnesium oxide nanoparticles positively impact chlorophyll content and
783 overall plant growth, necessitating further investigation into influencing factors. The effects of
784 MONPs on nitrogen fixation in leguminous plants are concentration-dependent, with potential
785 benefits at lower levels and adverse effects at higher concentrations. Optimizing MONP use
786 requires a comprehensive understanding of their interactions with diazotrophic bacteria,
787 nodulation processes, and nitrogenase activity. Under abiotic stress conditions, MONPs have
788 shown potential in mitigating the negative impacts of salt and drought stress on leguminous crops.
789 However, further research is needed to determine optimal concentrations, application methods,
790 and long-term environmental impacts.

791 Practical implications of this research involve using MONPs to enhance crop productivity, nutrient
792 uptake, and stress tolerance in leguminous plants. However, carefully considering concentration,
793 size, and application methods is crucial to avoid potential adverse effects. Future research should
794 focus on standardized techniques, comparative studies, and controlled conditions to establish

795 guidelines for the safe and efficient use of MONPs in agriculture. Additionally, long-term studies
796 are essential to assess ecological risks, soil accumulation, and impacts on non-target organisms.
797 Addressing these knowledge gaps will contribute to developing sustainable agricultural practices
798 incorporating nanotechnology.

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