

## Biosynthesis of iron oxide nanoparticles for potential agronomical application

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### Abstract

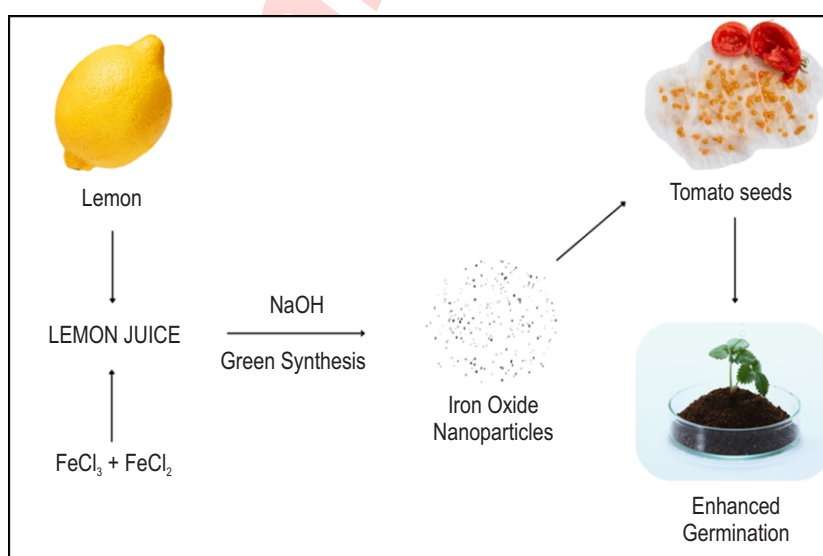
**Aim:** Green synthesis of iron nanoparticles (IONPs) is a sustainable approach to develop IONP based fertilizers for agronomical applications. Study of unexplored biomass and biological sources are vital to expand the domain of IONP synthesis. The aim of the current study was to explore lemon juice as an unexplored precursor for IONPs synthesis.

**Methodology:** Characteristics of IONPs synthesised by biological route from lemon juice as a precursor were compared with chemically synthesized IONPs. These IONPs were studied for their agricultural application by assessing their potential to affect tomato seed germination.

**Results:** Large sized IONPs (43.83 nm) were generated from lemon juice as a precursor as compared to chemically synthesized IONPs (27.86 nm). Biological IONP increased tomato seed germination by 59.1%. With reduced use of chemicals and at par performance, this study confirms biological IONPs (generated from lemon juice) as promising alternative to chemically synthesized IONPs.

**Interpretation:** This work established a new low cost organic solution, lemon juice, as a promising precursor for green synthesis of IONPs, with effective performance in enhanced tomato seeds germination, indicating their potential for agronomic applications.

**Key words:** Green synthesis, Iron oxide nanoparticle, Lemon juice, Nanofertilizer, Seed germination



## Introduction

Nanoparticles (NPs) are diverse types of materials with dimensions below 100 nm. They have gained considerable interest in recent years due to their unique properties and varied functionality. Reduction in size causes marked variations in the mechanical, thermal, optical and magnetic properties of any material (Hossain *et al.*, 2022). Due to these unique characteristics, nanoparticles are being increasingly used in various domestic and commercial applications, including catalysis, energy storage, agriculture and environment (Joudeh *et al.*, 2022). Metallic nanoparticles have harnessed increasing interest in recent times (Falke *et al.*, 2024). These materials can be synthesized by various routes and modified with various biomolecules such as antibodies, ligands, and various pharmaceutically active compounds of interest (Upadhyay *et al.*, 2023). Additionally, many nanoparticles, primarily gold, silver and zinc are being explored for their antimicrobial activities and other biomedical applications (Nishanthi *et al.*, 2019; Suthar *et al.*, 2024).

Biological method for nanoparticle synthesis, referred to as biological synthesis or green synthesis, is being actively advocated in recent times (Samuel *et al.*, 2022). Production of nanoparticles by green routes is considered safer, low in cost, and environment friendly, as compared to physical and chemical methods of synthesis (Makarov *et al.*, 2014; Dixit *et al.*, 2024). There are reports on the synthesis of metal nanoparticles via biological synthesis using precursors such as microorganisms (yeast, algae, diatoms, fungi, bacteria), plants (leaves, stem), agro-waste (fruit peel, fruit juice etc) and human cells (Salem *et al.*, 2021). These biological sources can transform inorganic metal ions into metal nanoparticles with unique desirable properties. Plant extract is one of the most widely explored and successful precursor for the biological or green synthesis of metal nanoparticles. Plants contain many phytochemicals, including many secondary metabolites such as carotenoids, alkaloids, flavonoids and terpenoids. Many of these metabolites possess a polyhydroxy group, which has been proposed to play a key role in metal reduction (Marslin *et al.*, 2018).

Iron oxide nanoparticles (IONPs) are one of the preeminent and biocompatible nanoparticles being explored for many promising applications. Iron oxide nanoparticles show low susceptibility to oxidation and exhibit flexible surface chemistry with wide range of environmental applications, such as pollutant adsorption and antimicrobial activity against various pathogens (Naveen *et al.*, 2021). They possess catalytic properties similar to various oxidases, such as peroxidases, catalase and superoxide dismutase, enabling them to function as potential enzyme mimicking analytes (Gao *et al.*, 2020). Iron plays an important role in plant metabolism: increasing growth rate, leaf length and chlorophyll content among other beneficial impacts (Palmqvist *et al.*, 2017). Iron in the form of iron nanoparticles can be a potential source of fertilizer for plants, causing enhanced metabolic functions and stress resilience (Naik *et al.*, 2025).

Nano-fertilizers have the potential to enhance crop growth and output, boost fertilizer application efficiency, reduce nutrient losses, and cause minimal adverse environmental effects as compared to chemical fertilizers (Raiesi Ardali *et al.*, 2022). Magnetic nanoparticles have been reported to positively impact the germination of cucumber and lettuce seeds as well as exhibit phyto-protective effects, thereby holding the potential to reduce the dosage load of persistent agrochemicals and pesticides on the environment (Shilova *et al.*, 2021).

A number of precursors are being explored for the green synthesis of IONPs (Yew *et al.*, 2016; Naveen *et al.*, 2021). In one of the earlier work, the extracts of *Hordeum vulgare* and *Rumex acetosa* plants were reported to yield amorphous IONPs. Organic acids like oxalic acid and citric acid in the extract play an important role in nanoparticles stabilization (Makarov *et al.*, 2014). Synthesis of iron oxide nanoparticles has been reported from the fruits of *Cynometra ramiflora* (Bishnoi *et al.*, 2018) and orange (Susithra *et al.*, 2024). Although lemon peel and leaves have been explored for nanoparticle synthesis, to the best of our knowledge, there are no previous reports on the use of lemon juice for green synthesis of IONPs. Additionally, no detailed comparative studies have been conducted on the potential of chemically and biologically synthesised IONPs for use in tomato cultivation. Increasing soil degradation and micronutrient deficiencies, call for additional biologically derived, sustainable eco-friendly interventions that can replace chemical fertilizers, provide additional nutrients to crops and promote soil fertility and plant growth, thereby strengthening environment and supporting sustainable development goal 13 (Climate Action).

The aim of the current study was to explore the biological (green) synthesis of iron oxide nanoparticles (IONPs) from lemon juice and assess the agricultural applications of synthesized IONPs. A comparative analysis of the properties and efficacy of IONPs synthesized from green and chemicals routes was also undertaken. IONPs will serve as an alternative to chemical fertilizers in iron deficient soils and function as a sustainable product for pre-sowing and foliar treatment of seeds and vegetative plants. Use of such environment friendly agri-inputs will strengthen climate smart agriculture, support resource efficiency, reduce environmental footprint caused by chemical additives, enhance soil quality, and provide opportunities for a safe agricultural management, thereby supporting Environmental Social Governance (ESG) framework.

## Materials and Methods

**Biological synthesis of iron oxide nanoparticles (IONPs):** Synthesis of IONPs was carried out using both chemical and biological (green) synthesis routes. Lemon juice extract was used for the green synthesis of Iron oxide nanoparticles. Fresh lemons procured from local market in Pune city, Maharashtra, India, were thoroughly washed to remove external dirt adhered to the surface. The lemon juice subsequently obtained was filtered using a normal tea strainer and centrifuged at 4000 rpm for 10

min. to obtain a clear solution. The filtered lemon juice was mixed with sterile milli Q water in 1:1 ratio and stored at 4 °C for further use. A modified method from a previous report was adopted for the IONP synthesis (Yekeen *et al.*, 2025). Solution of 0.2 M Iron (III) chloride Hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) and 0.1 M Iron II chloride Tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ) were prepared and stirred for 30 min for complete mixing. Lemon juice extract was introduced drop wise into the metal solution with constant mixing. The mixture was additionally stirred for an hour, followed by drop wise addition of NaOH (1 M) to the solution mixture with constant stirring for about 2 hr. The pH of the solution increased from around 1.5 to 2.0. The solution mixture was centrifuged at 5000 rpm for 10 min at 10 °C to obtain a pellet. The pellet obtained was washed thrice with milli Q water and dried for 5 hr at 60 °C in a hot air oven. The pellet once dried was crushed to powder form, weighed and stored for further analysis. The synthesis was carried out in triplicate and the mean weight of nanoparticles obtained was calculated.

**Chemical synthesis of IONPs:** Solutions of Iron III chloride Hexahydrate (0.2 M) and Iron II chloride tetrahydrate (0.1 M) were prepared in distilled water. Total volume of the solution was 90 ml. The solution was mixed with the help of a magnetic stirrer for 30 min., followed by drop wise addition of 1 M NaOH to the solution with constant stirring for 3 hr. The change in pH was recorded. The final solution obtained was centrifuged at 5000 rpm for 10 min. at 10 °C to obtain a pellet. The pellet obtained was washed thrice with milli Q water to remove impurities and dried for 5 hrs at 60 °C. The pellet once dried was crushed into powder form, weighed and stored for further analysis. The synthesis was carried out in triplicate and the mean weight of the nanoparticles obtained was calculated (Inestrosa-Izurrieta *et al.*, 2023).

**Characterization of IONPs:** IONPs synthesized by chemical and biological methods were analyzed using Dynamic Light Scattering (Brookhaven Instruments, model: Nano Brook 90Plus Zeta) for size estimation and Fourier Transform Infrared Spectroscopy (FT-IR) (Bruker Alpha II) for assessment of surface functional groups (Salim *et al.*, 2025). Atomic Absorption Spectroscopy (AAS) (Agilent 4200) was done for the confirmation of metal content.

**Effect of IONPs on seed germination:** Seeds of *Solanum lycopersicum* (Tomato), variety PKM-1 (Indian tomato), were selected to study the effect of chemically and biologically synthesized IONPs on seed germination carried out on blotting paper (Almutairi *et al.*, 2015). Tomato seeds purchased from the local market were washed with sterile distilled water to remove dirt, treated with 4% sodium hypochlorite solution for 2 min. to ensure sterility and subsequently washed thoroughly in sterile distilled water. The sterile seeds were soaked in IONPs solution ( $0.3 \text{ mg l}^{-1}$ ) for 12-14 hrs. Seeds were then placed at a distance of 2 cm from each other in sterile glass Petri plates lined with double layer of blotting paper at the base. A total of 48 seeds were used for each treatment. The seeds were then watered with IONP solution every three to four days. Sterile distilled water served as a negative control. Seeds were incubated at room temperature

(25-30 °C) under normal light conditions for 15 days. Parallel set up was done under dark conditions with incubation temperature of 20 °C. Seed germination was monitored each day. Seed germination (%) was calculated as per Singh *et al.* (2024).

**Statistical analyses:** Software GraphPad PRISM was used for the analysis of differences in the effect of chemically synthesized IONPs (IONP-CH) and biologically synthesized IONPs (IONP-LE).

## Results and Discussion

Green nanomaterials are known to enhance crop growth, resilience against pests, and improve nutrient delivery by means of enhanced uptake and solubility of metals (Khan *et al.*, 2024). Magnetic  $\text{Fe}_3\text{O}_4$  nanoparticles have gained much interest in nanoscience and nanotechnology, primarily owing to their distinct physico-chemical characteristics such as super-paramagnetism, particle size, morphology, and low toxicity (Elizondo-Villarreal *et al.*, 2022). Additionally, iron in nanoform is reported to be more effective in enhancing iron bio-availability in plants, especially under iron deficient conditions. Finding effective biological sources as precursors thereby assumes importance to ensure production of environment friendly and biocompatible iron oxide nanoparticles. This work explores lemon juice as a raw material for IONPs synthesis and its effectiveness for seed germination, as compared to chemically synthesised IONPs.

In the current study, iron oxide nanoparticles were synthesised by two different routes: chemical and biological. Lemon juice was selected as a precursor for green synthesis of IONPs. During the synthesis of nanoparticles, factors such as temperature, pH, and molar ratios play a critical role (Iravani, 2011). Hence, the method for the synthesis of IONP by lemon juice extract was optimized for all these variables. Different combinations of parameters such as pH, temperature and molar ratios were tried for the synthesis of nanoparticles (data not shown). Combination of 0.2 M Iron III chloride hexahydrate and 0.1 M Iron II chloride tetrahydrate, with metal: biological precursor ratio of 9:1, 25-35 °C temperature and incubation time of 3 hr gave the best results in terms of yield (239.5 mg from 100 ml solution). The pH of metal-lemon juice solution varied from 1.2-2.0. Similar variation in pH has been reported earlier to promote reduction of  $\text{Fe}^{3+}$  ions (Timoshnikov *et al.*, 2020).

The synthesized IONPs were attracted by a strong magnet and hence easily separated from the solution. Successful chemical synthesis of IONPs was confirmed by a marked change in color concomitantly with change in the pH of metal solution. Change in color from yellowish to reddish black indicated the formation of IONPs in the solution. Although the synthesis parameters remained the same, except the absence of lemon juice, yield was significantly high (427.6 mg from 100 ml solution). However, NaOH was consumed in large quantity to enable increase in pH to around 11.8-12.0 for effective IONPs precipitation. This is the reason, biological synthesis of IONPs is a

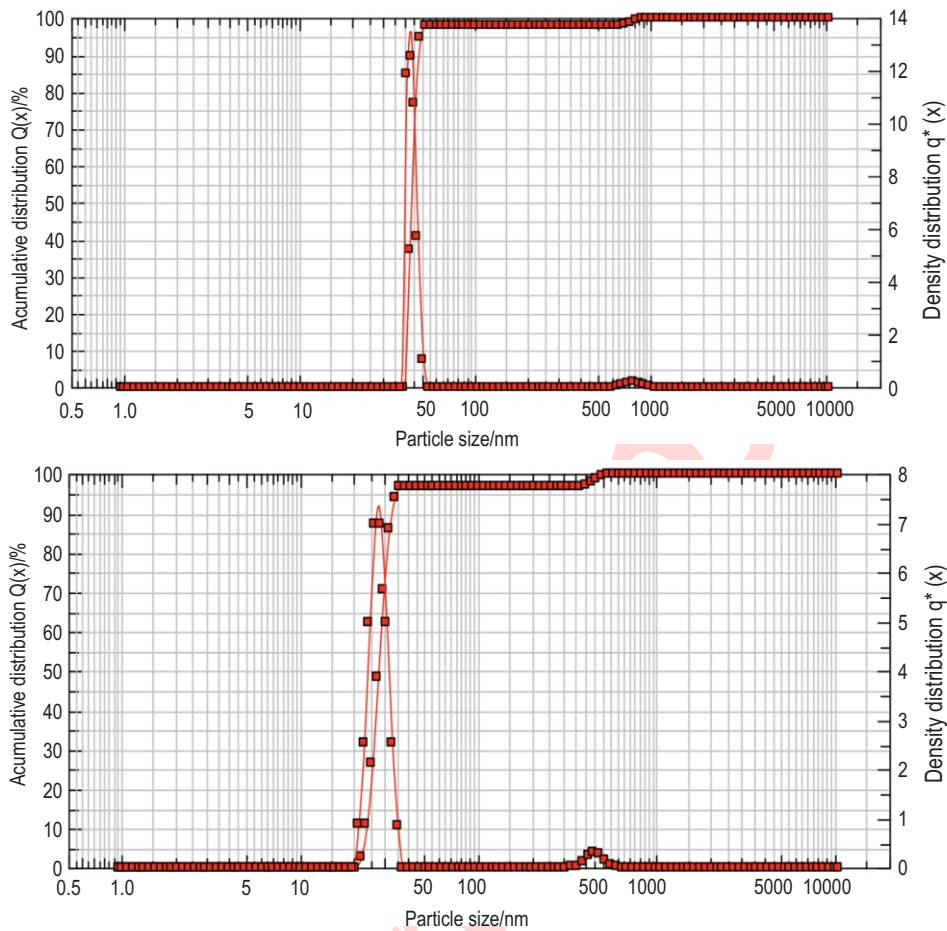


Fig. 1: Dynamic Light Scattering of Iron oxide nanoparticles synthesized by (a) biological route (b) chemical route.

more environment friendly and sustainable route as compared to chemical synthesis.

Studies on lemon derived precursors for nanoparticles synthesis is limited. Silver nanoparticles, with antimicrobial activity, have been synthesized using lemon peels (Nisha *et al.*, 2014). There are reports on silver nanoparticles synthesis using citrus fruit peels including lemon and orange (Niluxsshum *et al.*, 2021). However, reports on IONPs synthesis from citrus fruits juices is scarce. Lemon peel extract was used to synthesize iron oxide nanoparticles with anticorrosive properties (Elizondo-Villarreal *et al.*, 2022). In a previous report,  $\text{CoFe}_2\text{O}_4$  nanoparticles with potential drug delivery applications, were synthesized from lemon juice (Cheraghi *et al.*, 2021). Susithra *et al.* (2024) reported IONP synthesis from orange. However, high temperature of 350-450°C was reported for the complete synthesis of nanoparticles as compared to 25-30°C used in the current work. There are multiple ways that have been proposed by which lemon juice facilitates nanoparticle formation and stabilization. Active phytochemicals in the lemon juice can act as reducing agents to convert metal chlorides to nanoparticles (Dao *et al.*, 2021).

Addition of new functional groups on nanoparticle surface is expected to enhance the colloidal stability of the material. Hence, citric acid can act as capping and stabilizing agent. It has been reported that citric acid molecules bind to nanoparticles preventing them from agglomerating. The citric acid coating also enhances the nanoparticles' biocompatibility and dispersibility in aqueous solutions, making them suitable for various applications (Portilla *et al.*, 2024). Additionally biological synthesis results in reduced use of chemicals, such as NaOH, making the process less harsh and more environment friendly.

Chemically and biologically synthesized IONPs were compared for their physical and chemical characteristics, using spectroscopic and microscopic techniques, to understand their size, functional groups, concentration of metal present in the sample and their purity. The hydrodynamic diameter of the synthesized IONPs was assessed by dynamic light scattering (DLS) technique (Fig. 1a,b). The average hydrodynamic size of the biologically and chemically synthesized IONPs was 43.83 nm and 27.86 nm, respectively. As expected, the nanoparticles synthesized through chemical methods were smaller in size

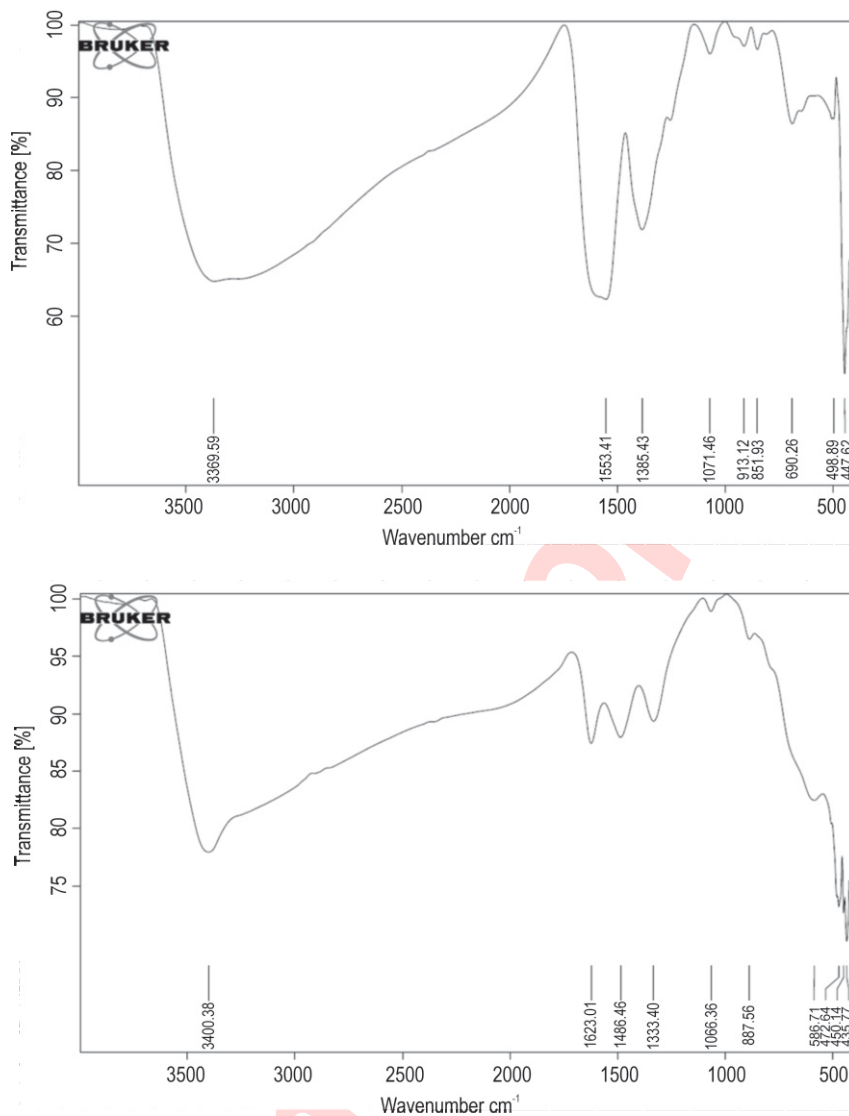
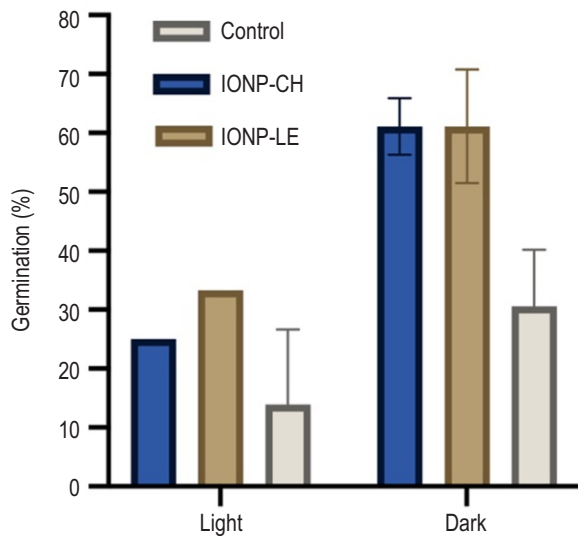


Fig. 2: FTIR spectrum of Iron oxide nanoparticles synthesized by (a) biological routes (b) chemical route.

compared to those synthesized by biological routes. This difference in size was primarily due to coating of large plant derived biomolecules, such as carotenoids, alkaloids, flavonoids, terpenoids, etc., from lemon extract on the surface of IONPs. These biomolecules can be decorated over the surface of nanoparticles, thus stabilizing its structure resulting in larger sized IONPs. Aydogen *et al.* (2022) synthesized Fe-FeO nanoparticles of size ranging from 66.68 to 156 nm from lemon peel extract. Similar sized IONPs were reported in previous studies wherein humic acid and citric acid coated IONPs were synthesized for increased growth of tomato plants (Raiesi Ardali *et al.*, 2022).

FTIR spectroscopic analyses was carried out to determine the nature of functional groups in biological and

chemically synthesized IONPs (Fig. 2a, b). The FTIR spectrum of biological IONPs revealed the presence of prominent peaks at 3369, 1553, 1365, 1071, 447  $\text{cm}^{-1}$ . The peak at 3369  $\text{cm}^{-1}$  and 1553  $\text{cm}^{-1}$  can be due to stretching of O-H bond of carboxylic acids and C=C bond, respectively, while the peak at 1385  $\text{cm}^{-1}$  can be attributed to C-N bond stretching, as well as OH bending vibration of phenols (Faruruwa *et al.*, 2024). The peak at 1071  $\text{cm}^{-1}$  may be related to stretching of O-H bond of alcohols. Presence of these peaks indicate the presence of various biomolecules derived from lemon extract, including phenol containing flavonoids and phenolic acids, that can reduce and coat the nanoparticle surface. Phenols and alcohols can act as reducing agents, donating electrons to iron ( $\text{Fe}^{3+}$ ), reducing it to form  $\text{Fe}_2\text{O}_3$  or  $\text{Fe}_3\text{O}_4$ . Although the exact mechanism of metal ions bio-reduction by biomolecules remains unclear, studies indicate that functional



**Fig. 3:** Effect of iron oxide nanoparticles (IONPs) on tomato seed germination in the presence and absence of light (IONP-CH: Chemically synthesized Iron oxide nanoparticles; IONP-LE: Biologically synthesized iron oxide nanoparticles)

groups such as -COOH, O-H, C-O-C, C=C, -CHO etc. are likely involved in the reduction process (Ishak et al., 2019). Presence of peaks at 498 and 447  $\text{cm}^{-1}$  is indication of the presence of metal oxygen bonds, as reported in an earlier study on iron oxide nanoparticles synthesized from *Avicennia marina* flowers. The formation of Fe-O was confirmed by bands at 618 and 467  $\text{cm}^{-1}$  (Karpagavinayagam and Vedhi, 2019). Adsorption band at 586 and 447  $\text{cm}^{-1}$  are also similar to peaks reported by Miri et al. (2021) and can correspond to vibrational band of O-Fe-O and Fe-O, respectively. FTIR spectra of chemically synthesized IONPs revealed a prominent peak at 3400  $\text{cm}^{-1}$ , and less prominent peak at 1623, 1465 and 450  $\text{cm}^{-1}$  corresponding to O-H bond vibrations of hydroxyl group, C=O bond, C-H bending, and metal oxygen bonds, respectively.

Considering the relevance of iron as a micronutrient essential for plant growth, the effect of IONPs on seed germination was assessed. A significant difference was observed in the effect of light on germination ( $p < 0.001$ ), with higher germination of tomato seeds recorded in the absence of light

source (Fig. 3). Effect of biological IONP (IONP-LE) and chemical IONP (IONP-CH) on tomato seed germination was significant as compared to the control ( $p=0.0012$  and  $p=0.0047$ ). Additionally, germination of IONP treated seeds was also faster as compared to the control (data not shown). However, the final comparative effect of chemically and biologically synthesized IONPs was not significant ( $p=0.4645$ ) indicating that both IONPs were equally effective in promoting germination of tomato seeds. In one of the closest studies, similar results were obtained on the use of chemically synthesized humic acid coated-iron oxide nanoparticles that caused increase in growth and yield of tomato plants (Raiesi Ardali et al., 2022). Increased bioavailability of Fe to plants, increase in chlorophyll content and alleviation in oxidative stress were the probable reasons for the increased plant growth. IONPs in the range of 20-30  $\text{mg l}^{-1}$  were found to be effective for optimum growth of lemon balm plant (Farnoosh et al., 2024). Several reports are available on the use of silver and gold nanoparticles for medical and agronomical applications (Castillo-Henriquez et al., 2020). However, studies on the use of IONPs for agriculture application are scarce.

A vital nutrient for plants, iron is involved in photosynthesis, in nitrogen fixation, nitrate synthesis, hormone production, respiration, and various other physiological and metabolic processes as an enzyme co-factor (Raiesi Ardali et al., 2022). Therefore, IONPs can be a viable substitute to address the shortcomings of conventional agrochemicals (Yoon et al., 2020). Table 1 gives a comparative overview of IONPs synthesized through two different routes, biological using lemon extract (IOLE) and chemical synthesis (IOCH). With effects comparable to that of synthetic IONPs, biologically synthesized IONPs offer a promising alternative as an agricultural intervention to cause enhanced seed germination and hence, a climate resilient crop. This work established the efficacy of lemon extract to yield effective nanoparticles, with better bioavailability and uptake, for potential application in the field of agriculture. Using nanoparticles to enhance seed germination can support Environmental Sustainability and Governance (ESG) framework, by means of reduced use of chemical fertilizers, improved food security, sustainable agri-supply chains, thereby contributing to sustainable and climate smart agriculture.

Nanofertilizers have emerged as an alternative to chemical fertilizers and are vital to restore soil health in nutrient deficient soil. In the present study IONPs were successfully synthesized using lemon juice extract. This work offers a detailed

**Table 1:** Comparison of iron oxide nanoparticles developed from biological route using lemon extract (IOLE) and by chemical synthesis (IOCH)

	Size (nm)	Yield (mg per 100ml)	Average germination after 10 days(%)		Metal content ( $\text{mg l}^{-1}$ )
			Dark	Light	
IOCH	27.86	427.5	61.11	25.00	67.45
IOLE	43.93	239.0	61.11	33.33	91.81

process on the use of a new precursor for the green synthesis of IONPs and opens up new avenues in the research on the synthesis and application of organic iron nanoparticles using citrus fruits. Nanoparticles synthesized through biological methods differed in their size and morphology from their chemical counterparts. However, both chemically and biologically synthesized nanoparticles were equally effective in causing enhanced tomato seed germination. The synthesized nanoparticles, with property to enhance seed germination, hence, showed the potential application in the agricultural sector, and a promising intervention for sustainable agriculture in the era of soil degradation and climate change, in line with the ESG framework.

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