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Influence of green synthesised iron and zinc nano-particles in enhancing yield and nutrient content of *rabi* sorghum (*Sorghum bicolor* L. Moench) through seed priming and foliar application

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Abstract
A field study conducted in MARS, Dharwad, Karnataka, during the r*abi* season of 2023-24 aimed to assess the efficacy of green-synthesized iron (Fe) and zinc (Zn) nanoparticles (NPs) compared to conventional fertilizers. The standard concentrations for seed priming (SP) and foliar spray (FS), determined in lab and controlled conditions were applied in the field. The standardized SP and FS concentration for Fe, Zn and (Fe + Zn) under lab and controlled conditions were (200, 500 and 400 ppm) and (400, 500 and 400 ppm), respectively. Foliar spraying occurred at the panicle initiation and flowering stages. The experiment was laid out in RCBD with three replications comprising fourteen treatments *viz.*, T_1-SP with Fe NPs @ 200 ppm, T_2-SP with Zn NPs @ 500 ppm, T_3-SP with (Fe + Zn) NPs @ 400 ppm, T_4-FS of Fe NPs @ 400 ppm, T_5-FS of Zn NPs @ 500 ppm, T_6-FS of (Fe + Zn) NPs @ 400 ppm, T_7-SP with Fe NPs @ 200 ppm + FS of Fe NPs @ 400 ppm, T_8-SP with Zn NPs @ 500 ppm + FS of Zn NPs @ 500 ppm, T_9-SP with (Fe + Zn) NPs @ 400 ppm + FS of (Fe + Zn) NPs @ 400 ppm, T_{10}-FS of $FeSO_4$ @ 0.5%, T_{11}-FS of $ZnSO_4$ @ 0.5%, T_{12}-FS of ($FeSO_4$ + $ZnSO_4$ @ 0.5%), T_{13}-RPP (RDF + 15 kg $ZnSO_4$ ha^{-1} and seed treatment with *Azospirillum* @ 500 g ha^{-1}) and T_{14}- Control (RDF-50:25 kg N and P_2O_5 ha^{-1}). RDF was applied to all the treatments. Results revealed that, SP with (Fe + Zn) NPs @ 400 ppm + FS with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher grain yield, stover yield, grain and stover Fe content (44.75 q ha^{-1}, 7.73 t ha^{-1}, 39.95 and 43.42 mg kg^{-1}) grain and stover Zn content (29.06 and 29.54 mg kg^{-1}) compared to control (T_{14})(36.17 q ha^{-1}, 6.26 t ha^{-1}, 34.36 and 36.18 mg kg^{-1}, 25.41 and 25.76 mg kg^{-1}, respectively).

Keywords: Nanoparticles, green-synthesis, seed priming and foliar spray

Introduction
Sorghum, commonly referred to as "Jowar" in India, holds the esteemed title of the "king of millets" and ranks as the third most crucial crop in the country, following rice and wheat. It serves as a significant source of food, feed, fodder, and ration for humans, cattle, and poultry, especially in the Deccan Plateau covering approximately 5.0 million hectares in Maharashtra, Karnataka, and Andhra Pradesh. Indian soils are being continuously supplied with the bulk of the major nutrients only through fertilizers over the decades. The deficiencies in both secondary and micronutrients do occur and in many cases are quite apparent requiring urgent attentions to break the limits of yield barrier and to maximize crop profitability. Keeping in view of its importance, seed priming and foliar supplementation of nutrients needs to be emphasized. In addition to that, foliar feeding has proved to be the fastest way of correction nutrient deficiencies and increasing yield and quality of crop products and it also minimizes environmental pollution and improves nutrient utilization by reducing the amount of fertilizers added to the soil whereas conventional fertilizers and its method of application showed reduced efficiency. Keeping the above facts in view, study has been conducted to assess the influence of green synthesised Fe and Zn nanoparticles in enhancing the yield of *rabi* sorghum through seed priming and foliar application.

Materials and Methods
The field study conducted in MARS, Dharwad, Karnataka, during the r*abi* season of 2023-24 aimed to assess the efficacy of green-synthesized iron (Fe) and zinc (Zn) nanoparticles (NPs)

compared to conventional fertilizers. Soil was clay loam in texture, slightly alkaline in reaction (7.20), normal in salt content (0.32 dSm^{-1}), low in available nitrogen (243.64 kg ha^{-1}), medium in available phosphorus (24.60 kg ha^{-1}) and high in available potassium (353.70 kg ha^{-1}) content. The experiment was laid out RCBD with three replications comprising fourteen treatments *viz.*, T_1-SP with Fe NPs @ 200 ppm, T_2-SP with Zn NPs @ 500 ppm, T_3-SP with (Fe + Zn) NPs @ 400 ppm, T_4-FS of Fe NPs @ 400 ppm, T_5-FS of Zn NPs @ 500 ppm, T_6-FS of (Fe + Zn) NPs @ 400 ppm, T_7-SP with Fe NPs @ 200 ppm + FS of Fe NPs @ 400 ppm, T_8-SP with Zn NPs @ 500 ppm + FS of Zn NPs @ 500 ppm, T_9-SP with (Fe + Zn) NPs @ 400 ppm + FS of (Fe + Zn) NPs @ 400 ppm, T_{10}-FS of $FeSO_4$ @ 0.5%, T_{11}-FS of $ZnSO_4$ @ 0.5%, T_{12}-FS of ($FeSO_4$ + $ZnSO_4$ @ 0.5%), T_{13}-RPP (RDF + 15 kg $ZnSO_4$ ha^{-1} and seed treatment with *Azospirillum* @ 500 g ha^{-1}) and T_{14}- Control (RDF-50:25 kg N and P_2O_5 ha^{-1}). RDF was applied to all the treatments. Foliar spraying occurred at the panicle initiation and flowering stages. The standard concentrations for seed priming (SP) and foliar spray (FS), determined in lab and controlled conditions were applied in the field. The standardized SP and FS concentration for Fe, Zn and (Fe + Zn) under lab and controlled conditions were (200, 500 and 400 ppm) and (400, 500 and 400 ppm), respectively. The procedure followed for green synthesis and characterization of Fe and NPs, recording observations for yield parameters and nutrient content of *rabi* sorghum are explained below.

Green synthesis of zinc nanoparticles by using neem leaf extract

Preparation of aqueous leaf extract of neem

Fresh leaves were collected from the campus of University of Agricultural Sciences, Dharwad and washed thoroughly with distilled water. About 50 g of leaves were chopped into small pieces. Finely chopped leaves were soaked in a beaker containing 200 ml distilled water. Later the mixture was boiled for 30 minutes. The extract was filtered using Whatmann No. 1 filter paper and filtrate was collected and refrigerated at 4°C for further use.

Stock solution preparation

Around 1 gram of iron sulphate ($FeSO_4$) was dissolved in 1 litre of distilled water to get 1000 ppm of iron sulphate ($FeSO_4$) solution. Around 1 gram of zinc acetate was dissolved in 1000 ml of distilled water to get 1000 ppm of zinc acetate stock solution.

Protocol for green synthesis of iron and zinc nanoparticles

Iron sulphate ($FeSO_4$) 1000 ppm stock solution was used as a precursor and neem leaf extract was used as reducing agent. For synthesis of FeNPs, the freshly prepared 50 ml $FeSO_4$ solution was added drop wise to the 50 ml leaf extract in a 1:1 ratio. The resultant mixture was heated at 80°C with continuous stirring using magnetic stirrer for 2 hours. The synthesized nanoparticles were characterized by particle size analyzer.

The freshly prepared 25 ml Zinc acetate solution was added drop wise to the 25ml leaf extract in a 1:1 ratio were kept on the magnetic stirrer at 70 °C along with stirring for 500 rpm for 60 minutes and change in colour of the solution were observed and particle size analyzer were observed.

Characterization of green iron and zinc nanoparticles

1. UV-Visible spectroscopy

UV-Visible spectroscopy was used to study surface plasmon resonance of metal nanoparticles. Reduction of Zn and Fe ions to Zn and FeNPs were confirmed by UV-Vis spectrophotometer (SP-UV 500 DB, Germany) at scanning wavelengths of 200-700 nm based on their optical absorbance peak. As the size of the nanoparticle decreases, the band gap increases and thus, the optical absorbance increases as compared to that of the bulk particles and therefore their colour changes.

The synthesized zinc and iron nanoparticles were first sonicated using solid probe Ultra sonicator (inkarp 20kHz ±50Hz. Sonics Vibracell) for uniform distribution of nanoparticles. An aliquot of extract was used as control and the absorbance maximum was recorded at room temperature.

2. Particle Size Analyzer (PSA)

Characterization of synthesized nanoparticles was carried out by particle size analyzer (PSA). The nanoparticles were further subjected to dynamic light scattering (DLS) to measure average particle size, in a high-performance particle-size analyzer (model 23000, Nicomp, USA). The sample were placed in disposable polystyrene cuvettes and the analysis was carried out at room temperature in triplicate with a scattering angle of 90°.

3. Scanning Electron Microscope (SEM)

Characterization of synthesized nanoparticles was done by scanning electron microscope (SEM). The zinc and iron nanoparticles were then subjected to surface morphology analysis by scanning electron microscopy (SEM; Carl Zeiss, Germany). A pinch of powder sample obtained from muffle furnace was uniformly placed on an aluminium stub with carbon adhesive tape and coated with gold in a sputter coater and SEM images were captured with an EVO 18 microscope operated with smart SEM software and examined at a working distance between 8-9 mm and different voltage of 5-10 kV.

4. X-ray diffractometer (XRD)

The zinc and iron nanoparticles were then subjected to X-ray diffraction analysis using X-ray diffractometer (XRD). Enough powder from the labelled sample was filled on an empty sample holder and gently pressed the powder flush with the sample holder using a glass slide. The excess powder from the sample holder edges was removed and carefully placed in the appropriate XRD slot. X-ray diffractometer equipped with Cu Kα radiation source using Cu as filter at a setting of 40 kV/30 mA. All data were collected under the experimental conditions at the angular range $50 \leq 2\theta \leq 800$.

Grain yield

The panicles from the net plot were harvested. After threshing, grains were separated, cleaned and weighed accordingly to different treatments. Grain weight or yield was computed to quintal per ha (q ha^{-1}).

Stover yield

Stover yield was recorded after the complete sun drying of stalks from each net plot and expressed in tonnes per ha (t ha^{-1}).

Nutrient content analysis

Plant samples of sorghum crop collected at harvest were used for estimation of nutrient content. The dried sample were ground and passed through 40 mesh sieve. The ground material was collected in butter paper bag and used for chemical analysis.

Iron and zinc content

Iron and zinc determined by using Atomic Absorption Spectrophotometer and expressed the concentration in milligram per kilogram (mg ka^{-1}) (Tandon, 1998) [17].

Statistical analysis and interpretation of data

The data recorded from the experiment at different growth stages were subjected to statistical analysis as given by Gomez and Gomez (1984) [6]. The level of significance used in 'F' test was P = 0.01 (1%) and P = 0.05 (5%). Critical difference (CD) at 1% and 5% values were calculated whenever the 'F' test was found significant.

Results and Discussion

Characterization of biosynthesized iron and zinc nanoparticles

1. UV-Visible spectroscopy

The formation of FeNPs and ZnNPs were preliminarily confirmed by the change in colour of the reactant mixture, later the synthesized samples were subjected to the UV-Visible spectrometer where FeNPs green synthesized through neem leaf extract produced a strong absorption peak at 365 nm (Fig 3A) and for FeNPs, the peak due to surface resonance was observed at 294 nm (Fig 3B).

2. Particle size analyser

The Particle Size Analyser showed the mean diameter of nanoparticles in relation to volume-weight, intensity-weight, and number weight. The neem leaf extract was used to synthesize FeNPs with mean diameter of 51.7 nm (Fig 4A). The PSA analysis revealed that average diameter of the synthesized Zn NPs using neem leaf extract was 48.7 nm (Fig 4B).

2. Scanning electron microscope

Characterization through SEM of FeNPs green synthesized through neem leaf extract revealed the shape of the nanoparticles to be spherical in green synthesized Fe samples (Fig 5A).

Characterization of ZnNPs through SEM biosynthesized through neem leaf extract showed the shape of the nanoparticles to be spherical in green synthesized Zn samples (Fig 5B).

3. X-ray diffraction

X-Ray Diffraction (XRD) characterization is a powerful non-destructive technique for characterizing crystalline materials. It provides information on crystal structure, phase, preferred crystal orientation, and other structural parameters, such as average particle size, crystallinity, strain, and crystal defects. The sharp peaks obtained in the Zn and FeNPs synthesized using neem leaf extract resulted in crystalline and amorphous nature, ZnNPs synthesized through neem leaf extract, the intense peaks were showed at 31.76°, 34.42 36.26°, 47.50°, 56.57°, 62.84%, 66.37°, 67.88° and 68.99° which corresponds to (100), (002). (101), (102), (110), 103), (200), (112) and (201) planes (Fig 6B), respectively. FeNPs synthesized through neem leaf extract the peaks were observed at 30.10°, 35.52°, 48.45°, 57.17° and 62.85° which corresponds to (220), (311), (422), (511) and (440) (Fig 6A), respectively.

Grain and stover yield

The results presented in Table 1 showed that seed priming with (Fe + Zn) NPs @ 400 ppm + Foliar spray with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher grain yield of 44.75 q ha^{-1} which was 23.72 percent higher than the control (36.17 q ha^{-1}). The highest was on par with SP with Zn NPs @ 500 ppm + FS with Zn NPs @ 500 ppm (T_8) (43.59 q ha^{-1}). Seed priming with (Fe + Zn) NPs @ 400 ppm + Foliar spray with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher stover yield of 7.73 t ha^{-1} which was 23.48 percent higher than the control (6.26 t ha^{-1}). The highest was on par with SP with Zn NPs @ 500 ppm + FS with Zn NPs @ 500 ppm (T_8) (7.55 t ha^{-1}) as presented in Table 1. Increase in yield is due to sufficient supply of iron and zinc has enhanced the supply of other nutrients and regulated the overall plant growth and development and resulted in an increase number of grains per panicle due to stimulation of physiological processes such as photosynthesis, translocation and assimilation of photosynthates, as well as the formation of more number of panicle during panicle initiation (Talib *et al.*, 2016 and Janmohammadi *et al.*, 2016) [8]. Improvement in biochemical and physiological processes that is due to Zn which acted as a cofactor for a number of enzymes, finally impacted on better crop growth and yield. Zinc nanoparticles have the capacity to pass through the surface of leaves and release Zn ions across the cuticle due to their extremely smaller size. In addition to that, the highest thousand grain weight suggested that the cytokinin hormone's enhanced activity has resulted in larger individual grain sink size (Bihmidine *et al.*, 2013) [3]. Application of zinc nanoparticles resulted in the highest grain yield, and this might be because of the smaller size and greater surface area of nano fertilisers, which improved the absorption and translocation of Zn in plant tissue (Subbaiah *et al.*, 2016) [15]. The increase in overall yield is the result of zinc, which helps in increasing the fertilisation percentage during the blooming stage. This facilitated the transport of photosynthetic by-products to the pollen grains, enhancing their vitality (Al-Tameemi *et al.*, 2019) [2]. According to Harsini *et al.* (2014) [7], using nano chelated iron fertiliser can increase nitrogen uptake by considerably affecting 1000-grain weight, grain yield, biological yield, and harvest index when compared to the control. Due to the ultra-small size and diffusible nature of nano nutrients, which boosted their effectiveness in penetrating through the leaf surface and releasing ions across the cuticle, the nano composite increased the absorption of Fe in plants. The foliar application of iron nanoparticles increased the photosynthetic and metabolic activity of the plant, considerably raised the nutritional content, growth, and yield (Sekaran and Singaravel, 2022 and Prerna *et al.* 2021) [12, 11].

Grain and stover iron content (mg kg^{-1})

Seed priming with (Fe + Zn) NPs @ 400 ppm + Foliar spray with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher grain iron content of 39.95 mg kg^{-1} which was 16.26 percent higher than the control (34.36 mg kg^{-1}). The highest followed by SP with Fe NPs @ 200 ppm + FS with Fe NPs @ 400 ppm (T_7) (39.54 mg kg^{-1}), FS of (Fe + Zn) NPs @ 400 ppm (T_6) (39.26 mg kg^{-1}) and FS of Fe NPs @ 400 ppm (T_4) (38.87 mg kg^{-1}) as depicted in Fig 1. Seed priming with (Fe + Zn) NPs @ 400 ppm + Foliar spray with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher grain iron content of 43.42 mg kg^{-1} which was 20.01 percent higher than the control (36.18 mg kg^{-1}). The highest followed by FS of (Fe + Zn) NPs @ 400 ppm (T_6) (42.73 mg kg^{-1}) and SP with Fe NPs @ 200 ppm + FS with Fe NPs @ 400 ppm (T_7) (42.73 mg kg^{-1}). Similar results were reported by Mahmoud *et al.* (2019) [10] who revealed the increased uptake of iron and zinc at harvest stage was attributed to improved absorption because nanoparticles can enter the plant system through stomata, lenticels, cuticle, and even through the physically damaged part of the plant leaves more effectively as well as greater leaf penetration. For nutrient uptake and translocation, it must pass through numerous physical and chemical barriers, such as plant cell walls. These barriers are extremely selective for tiny particles during xylem and phloem loading and uploading. The interaction of NPs and inorganic fertiliser molecules with cellulose allows the tiny particles but prevents the entry of larger particles. As a result, smaller particles may readily overcome the barriers and contribute to nutrient uptake (Sundaria *et al.*, 2019) [16]. Nano fertilisers have high surface areas and particles that are smaller than the pore size of plant leaves, which can boost penetration into plant tissues from the applied surface and improve nutrient absorption and usage efficiency (Al-Juthery *et al.*, 2018) [1].

Grain and stover zinc content (mg kg^{-1})

Seed priming with (Fe + Zn) NPs @ 400 ppm + Foliar spray with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher grain zinc content of 29.06 mg kg^{-1} which was 14.36 percent higher than the control (25.41 mg kg^{-1}). The highest was on par with SP with Zn NPs @ 500 ppm + FS with Zn NPs @ 500 ppm (T_8) (28.72 mg kg^{-1}) and followed by FS of (Fe + Zn) NPs @ 400 ppm (T6) (28.56 mg kg^{-1})and FS of Zn NPs @ 500 ppm (T5) (28.41 mg kg^{-1}) (Fig 2). Seed priming with (Fe + Zn) NPs @ 400 ppm + Foliar spray with (Fe + Zn) NPs @ 400 ppm (T_9) recorded significantly higher stover zinc content of 29.54 mg kg^{-1} which was 14.67 percent higher than the control (25.76 mg kg^{-1}). The highest was followed by SP with Zn NPs @ 500 ppm + FS with Zn NPs @ 500 ppm (T_8) (29.44 mg kg^{-1}), (T6) (29.27 mg kg^{-1}) and (T5) (29.08 mg kg^{-1}) as depicted in Fig 2. Singh *et al.*, 2019 [14] revealed that due to their small size, nanoparticles are easily passes through the membrane pores inside cells, further increasing their bioavailability. Nanoparticles' increased capabilities are due to quick absorption and bioavailability and due to their huge surface area. Increased the zinc content dramatically in wheat grain and root and shoot. This might be due to increased Zn translocation into grain due to improved source-sink channels and increased usage efficiency of ZnNPs in comparison to zinc sulphate (Lateef *et al.*, 2016 and Yadav *et al.*, 2023) [9, 18]. Foliar spraying of Zn nanoparticles increased grain Zn content due to its enhanced effectiveness of ZnONPs compared to zinc sulphate is attributed to the smaller size of the nanoparticles, which promotes adherence to the leaf surface, with the slow dissolution of the ZnONPs (Yu *et al.*, 2017 and Du *et al.*, 2019) [19, 5]. Zinc application improves plant physiology as evidenced by increased photosynthesis, nitrogen absorption, auxin activity, thermo-tolerance, and water usage efficiency stated by Sher *et al.* (2022) [13].

Table 1: Grain yield and fodder yield and harvest index of *rabi* sorghum as influenced by seed priming and foliar spraying with iron and zinc nanoparticles under field condition

Treatments	Grain yield (q ha^{-1})	Stover yield (t ha^{-1})
T_1	36.41	6.31
T_2	36.48	6.44
T_3	36.53	6.40
T_4	39.05	7.29
T_5	40.36	7.41
T_6	41.43	7.45
T_7	39.23	7.39
T_8	43.59	7.55
T_9	44.75	7.73
T^{10}	36.98	6.45
T_{11}	37.41	6.57
T_{12}	37.68	6.81
T_{13}	38.46	7.07
T_{14}	36.17	6.26
S.Em±	0.44	0.07
CD at 0.05	1.34	0.22

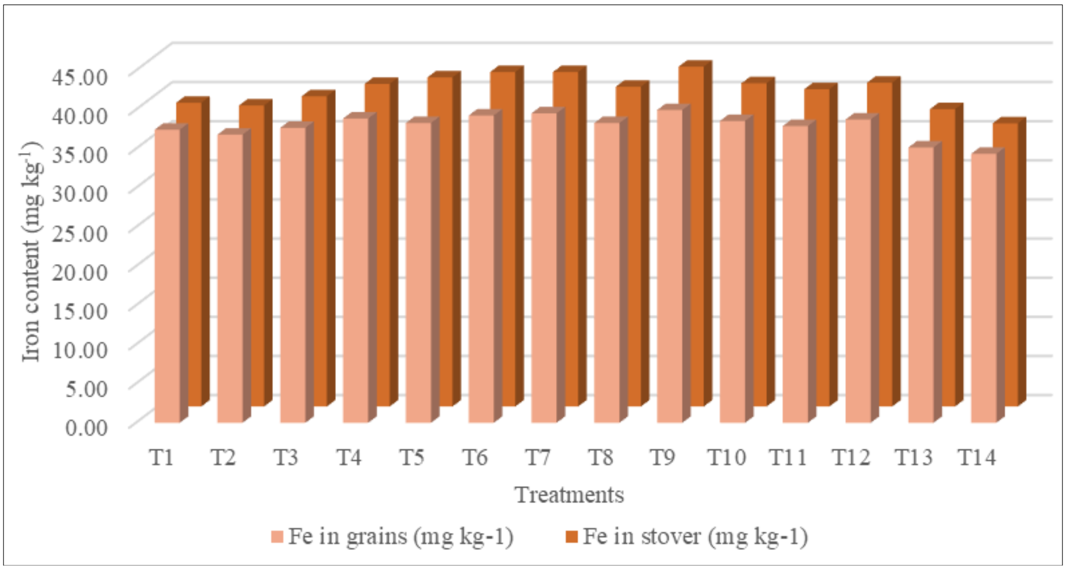

Fig 1: Iron content in *rabi* sorghum as influenced by seed priming and foliar spraying with iron and zinc nanoparticles under field condition

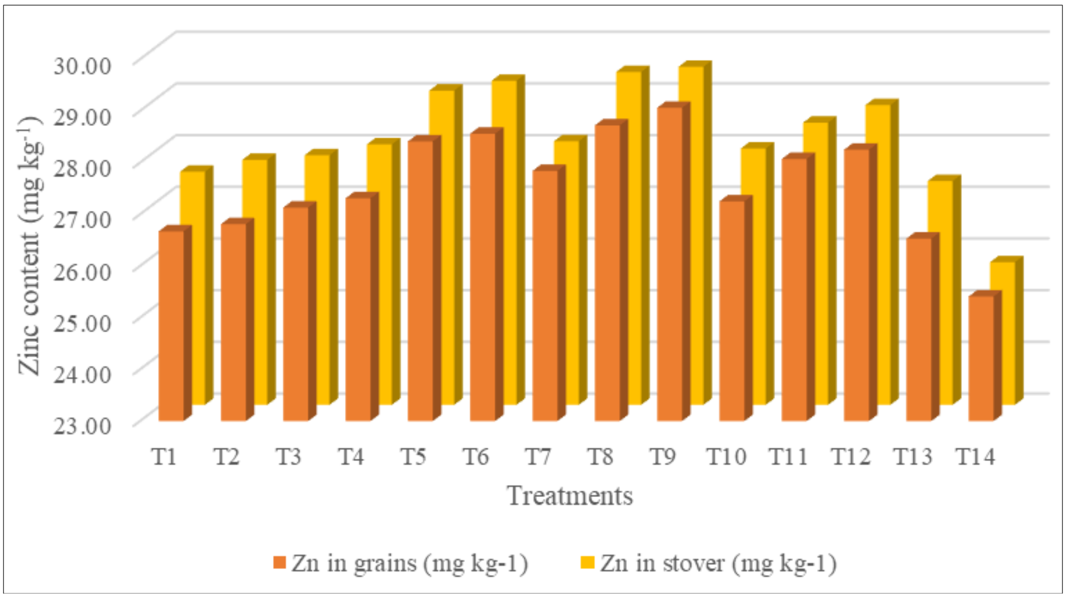

Fig 2: Zinc content in *rabi* sorghum as influenced by seed priming and foliar spraying with iron and zinc nanoparticles under field condition

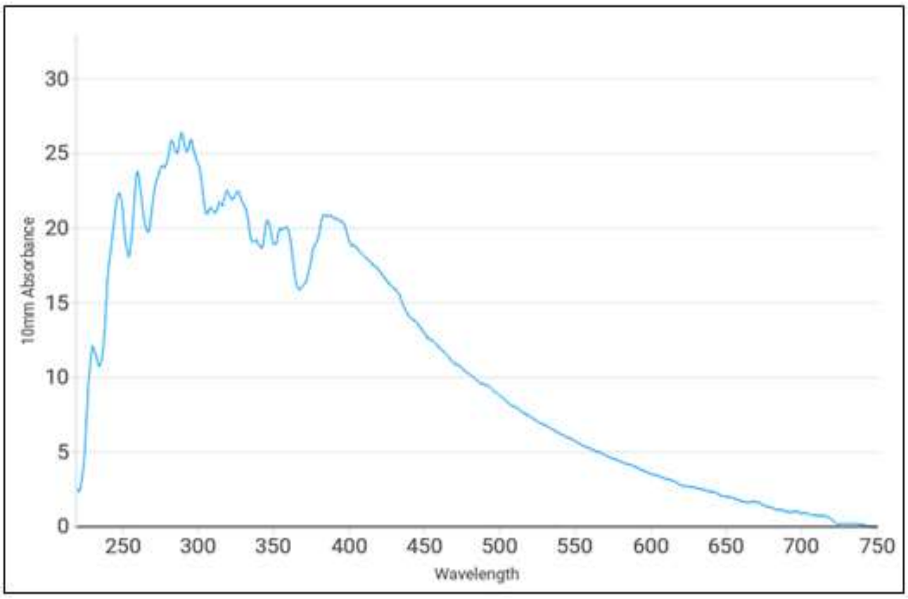

Fig 3A: Characterization of green synthesized FeNPs by UV- Visible spectrophotometer

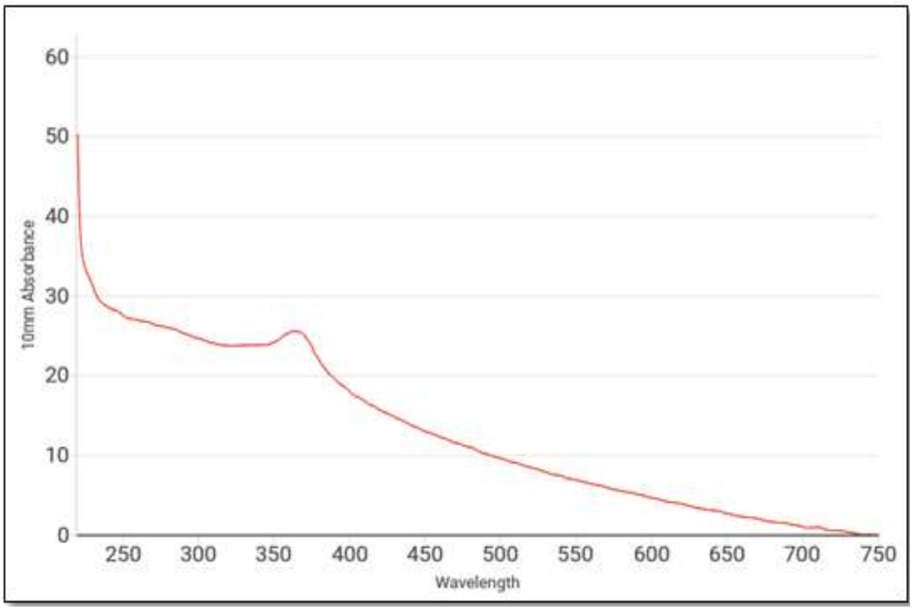

Fig 3B: Characterization of green synthesized ZnNPs by UV- Visible spectrophotometer

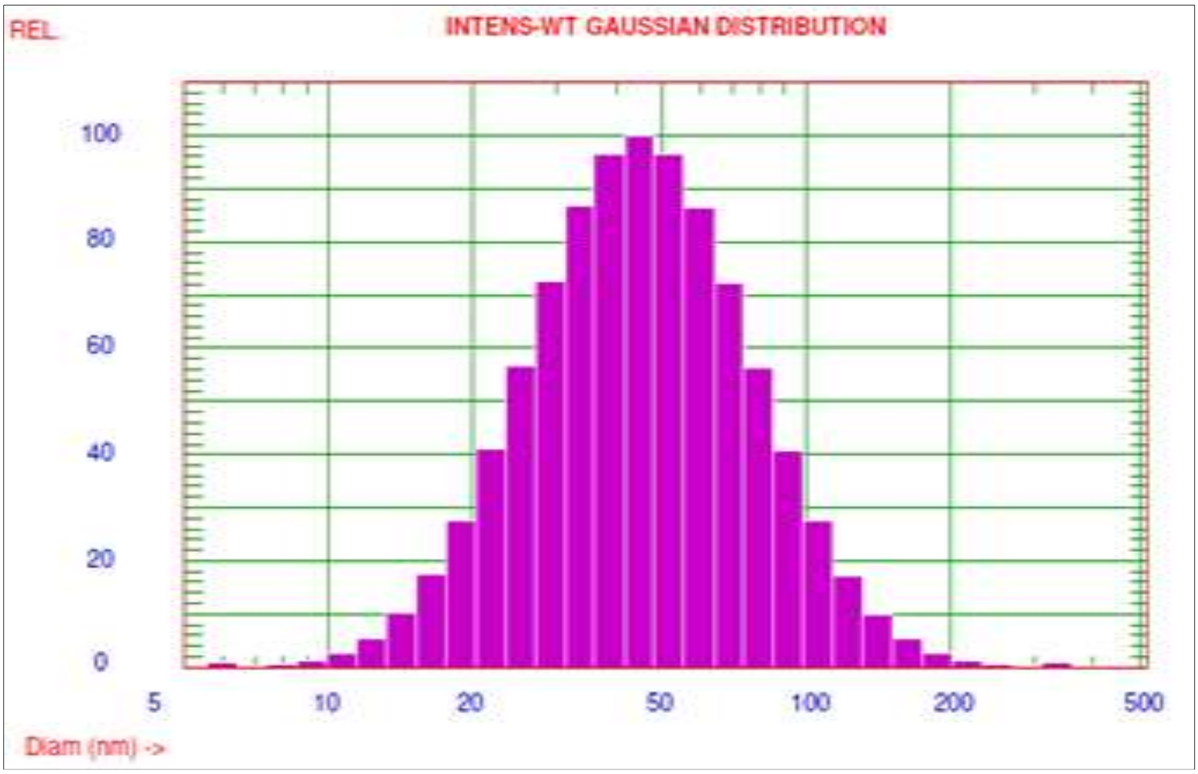

Fig 4A: Characterization of green synthesized Fe NPs by Particle size analyzer (PSA)

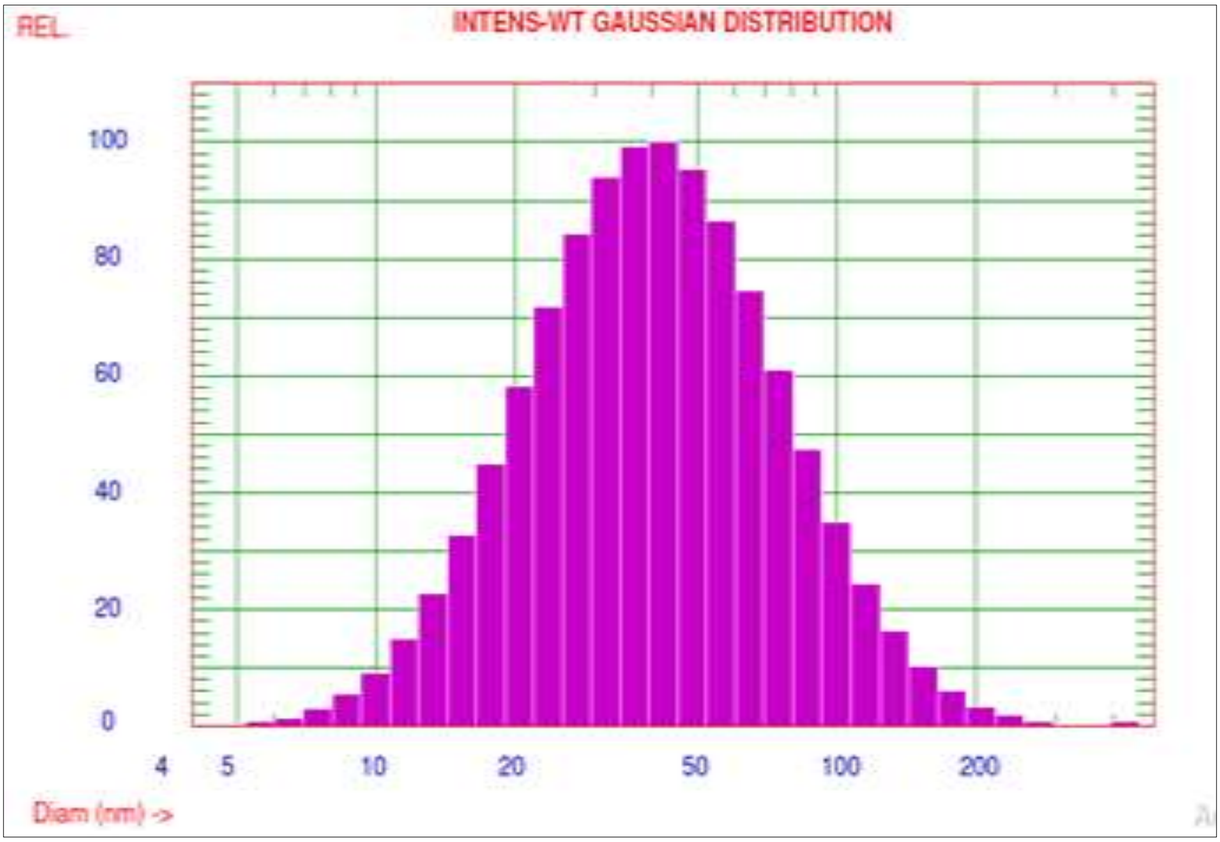

Fig 4B: Characterization of green synthesized Zn NPs by Particle size analyzer (PSA)

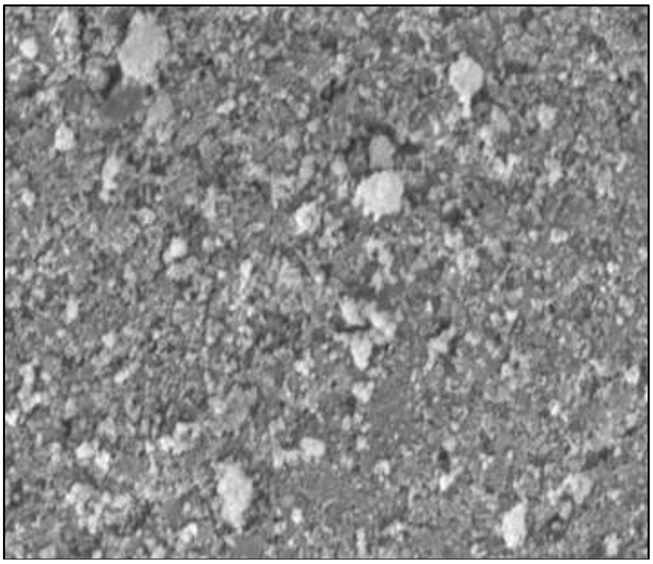

Fig 5A: Characterization of biosynthesized Fe NPs by Scanning electron microscope (SEM)

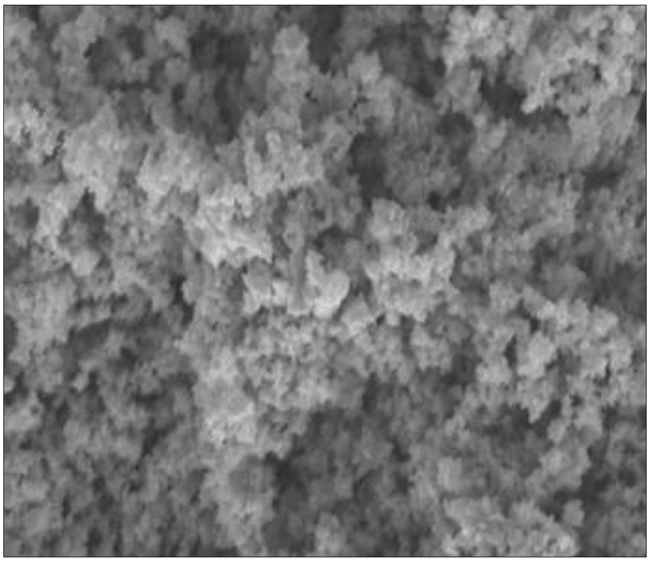

Fig 5B: Characterization of biosynthesized Zn NPs by Scanning electron microscope (SEM)

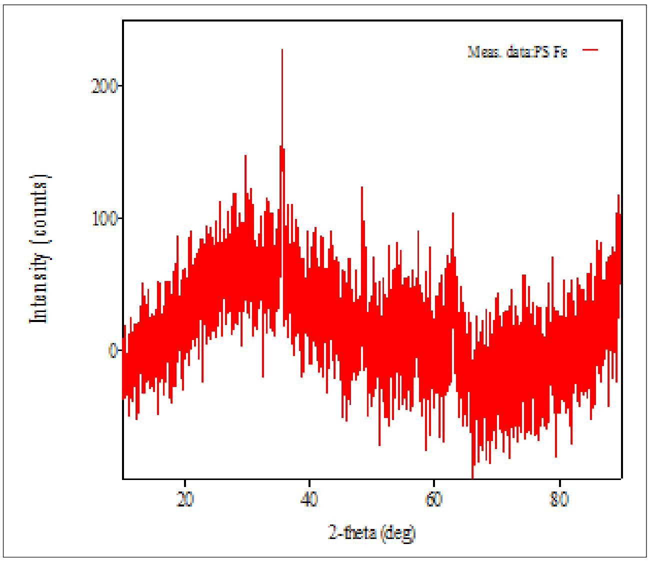

Fig 6A: Characterization of biosynthesized Fe NPs by X-ray diffraction (XRD)

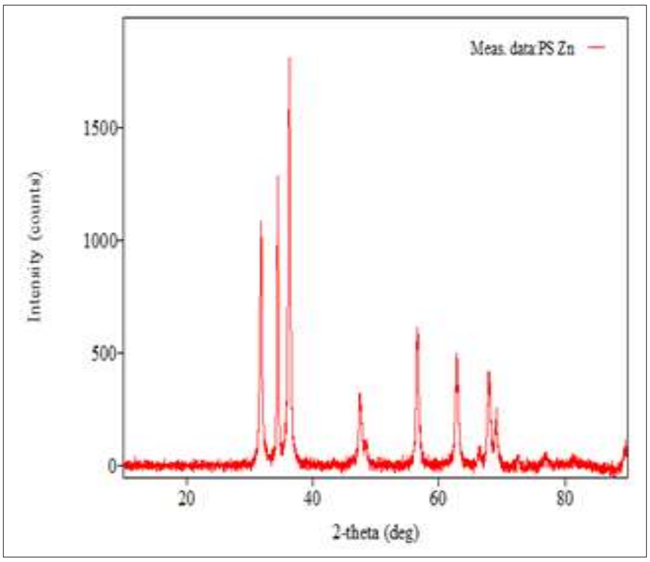

Fig 6B: Characterization of biosynthesized Zn NPs by X-ray diffraction (XRD)

Conclusion

- The organic compounds present in the neem leaf extract act as both reducing and stabilizing agents for synthesis of iron and zinc nanoparticles.
- Seed priming and foliar application of green synthesized iron and zinc nanoparticles at panicle initiation and flowering stage recorded significantly higher yield and grain nutrient content in *rabi* sorghum.
- Green synthesis of nanoparticles using neem leaf extract is considered to be an environmentally friendly and cost effective approach.

Based on the results, it may be concluded that seed priming of (Fe + Zn) NPs @ 400 plus two foliar spray of (Fe + Zn) NPs @ 400 at panicle initiation and flowering stages along with recommended dose of fertilizers to *rabi* sorghum found optimum for getting higher grain and stover yield, having higher iron and zinc content compared to recommended dose of fertilizers alone and recommended package of practice.

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