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## Revitalizing Germination and Starch Quality of Aged Rice Seeds Through Innovative Nano Priming with Advanced Nanoparticle

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### Abstract

Aging or dormant rice seeds' ability to germinate is crucial to agricultural productivity. To address this, seed nano preparing has arisen as an expected system. In any case, regular strategies for orchestrating nanoparticles (NPs) include poisonous synthetic compounds as decreasing specialists, raising natural worries. In this review, we present a green blend approach for Fe<sub>2</sub>O<sub>3</sub> NPs utilizing Citrus hystrix plant remove as a plant substitute for poisonous reductants. This study focused on germination tests and starch metabolism analysis to evaluate the performance of aged rice seeds treated with Fe<sub>2</sub>O<sub>3</sub> NPs. Furthermore, we meant to streamline seed development through tests, for example, starch agar plate  $\alpha$ -amylase examine, cell reinforcement chemical measure and histochemical limitation. The trial configuration followed a randomized block plan with three replications. The germination tests assessed seed quality and suitability, giving insights into the field execution. Factual examination affirmed the meaning of the information, utilizing one-way investigation of difference (ANOVA). The germination performance of aged rice seeds is positively impacted by nano priming with Fe<sub>2</sub>O<sub>3</sub> NPs, as shown by our findings. Additionally, the antioxidant enzyme assay and analysis of starch metabolism revealed improved physiological processes in treated seeds. This study demonstrates that green-synthesized NPs have the potential to be an environmentally friendly and cost-effective alternative for agricultural seed enhancement.

## INTRODUCTION

Lately, there was an enormous improvement in the volume of environmental change and its effects on agrarian efficiency and cultivators' business in Southeast Asia<sup>[1]</sup>. For the reason to increment further efficiency in agrarian items, speed as well as delicacy is the early on interest for long haul productive development, seriousness and supportability. About portion of the total populace depends on rice as their essential wellspring of nourishment<sup>[2]</sup>. Rice seed item stages enjoy various upper hands over microorganism frameworks concerning cost-viability, versatility, wellbeing, item steadiness and efficiency<sup>[3]</sup>. Rice seeds can develop under complete submergence, though seed germination of other grain crops is hindered under lowered conditions as a result of hypoxic stress<sup>[4]</sup>. These rice seeds become old or matured due to the warm tacky circumstances; According to Wang *et al.*, rice seed germination rates will eventually fall to zero. 2022). As seeds age, their germination rate will decline. In rice seeds, the bounce declination process is the abecedarian metabolic event that ensures energy force and respiration during germination. It is another significant process in seed development. It likewise impacts the grain yield and quality<sup>[5]</sup>. Quick fire seed germination and seedling development are required for fruitful foundation in the attractive cultivation area in the current script of population growth. Seed preparing is a framework where seeds are soaked not entirely until they can begin germination and other metabolic cycles<sup>[6]</sup>.

Nano-preparing is a clever seed-preparing innovation that further develops seed germination, development and yield by giving protection from vivid burdens in stores<sup>[7]</sup>. By setting off skip declination through the sensation of amylase, nano-planning animates seed germination<sup>[8]</sup>. Nanopores help the shoot absorb water and stimulate reactive oxygen species (ROS)/malignant growth counteraction specialist structures in seeds as a result of this development. As a debaser for the quick hydrolysis of starch, it likewise frames hydroxyl progressives to release cell walls<sup>[9]</sup>. For the purpose to make the exploration a more engaging one, ferric oxide ( $\text{Fe}_2\text{O}_3$ ) NPs had been incorporated in an eco-friendly way and *Oryza sativa* was chosen as the model. Since it has various seeds with heaps of *Oryza sativa* type are normally matured seeds because of improper storage facility conditions under encompassing temperatures by most extreme cultivators<sup>[10]</sup>. The general idea and end of the survey are to convey a common place structure for the embodiment of  $\text{Fe}_2\text{O}_3$  and encourage a system for the conflation of  $\text{Fe}_2\text{O}_3$  NPs and present another planning style using  $\text{Fe}_2\text{O}_3$  NPs. The germination and starch metabolic course of rice made seeds is crucial for standard needing to support<sup>[11]</sup>.  $\text{Fe}_2\text{O}_3$  nanoparticles have been incorporated in a green

manner with the help of a kaffir lime splint extract as a nano priming agent. Utilizing a tool to explore statistical analysis is crucial after all of the analysis has been completed.

**Problem Definition:** Enhancing germination as well as starch metabolism is pivotal for attaining improved performance and efficiency. Many other materials have been used in incorporation for the analysis of germination and starch metabolism, but still, there is a need to add investigation of aged rice seeds with respect to the chosen NPs ( $\text{Fe}_2\text{O}_3$ ) of nano priming technologies and also focus on its advantages and challenges.

The following is the structure of the presented research study: in segment 2, the current exploration concentrates on connected with the improvement of germination and starch digestion of matured rice seeds utilizing various NPs of nano preparing advances are given., The research study materials are discussed in section 3., in segment 4, the trial plan system is given., in segment 5, the subsequent result of the trial configuration is made sense of., also, in area 6, the paper is finished up.

**Literature Review:** <sup>[12]</sup> explained the improvement of germination as well as starch metabolism by exercising the nano priming technology for aged rice seeds. Rice aged seeds announcement has been primed with phyto synthesized tableware (Ag) NPs at 5 and 10 pm. According to the evaluation, AgNPs can be utilized as a nano-preparation master for rice-produced seed germination and starch handling. In any case, the nano planning procedure works without NP application to soils and gives little thought to AgNPs.

<sup>[13]</sup> Delved into the application of phyto-synthesized zinc oxide nano patches for nano-priming in ancient rice seeds, with *Cassia occidentalis* as the selected plant species, was investigated. The researchers employed various characterization techniques, including X-ray diffractometer (XRD), UV visible spectroscopy, particle size analyzer (PSA), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM), to evaluate the properties of the applied nanoparticles. When contrasted with the benchmark group and other preparing medicines, the examination uncovered that seeds treated with ZnO NPs at a centralization of 20mg/L had essentially expanded plumule length and new weight. These discoveries propose that phyto-coordinated zinc oxide nano patches utilized in nano-planning essentially affect the germination and biomass assortment of old rice seeds.

<sup>[14]</sup> Portrayed that nano organizing with zero valent iron (nZVI) gathers germination and movement in sweet, made rice cultivar <sup>[15]</sup>. After being prepared with varying amounts of nZVI (10, 20, 40, 80 and 160mg L-1)

and being allowed to grow for fourteen days, the seeds were harvested. Based on the findings, it was thought that nZVI's expertise in seed planning could help seedling growth and germination at low doses. nZVI displayed an extension in the activity of amylase and protease during germination., NADPH dehydrogenase action, chlorophyll and carotenoid content and seedling level all expanded.

<sup>[15]</sup> delved into the ocean weed grounded biogenic ZnO NPs to upgrade agro morphological characteristics and germination of aged rice<sup>[15]</sup>. Likewise, the assessment uncovered that the nano-organized rice seedlings and the foliar and applied rice crop had a reliable expansion in Zn content. The impact of the investigation was not established despite the fact that there were extensive field preliminary investigations.

<sup>[16]</sup> depicted the effects of various nanomaterials on seed germination and seedling improvement were explored through a meta-evaluation. The meta-investigation looked to decide if the utilization of nanomaterial medications especially affected the germination and improvement of agrarian plant species. The examination uncovered that the drugs with nanomaterials in a general sense chipped away at the likelihood of convincing germination (FGP). The mean distinction (MD) in FGP was viewed as 1.97 (0.96, 2.98) for the Ag-NP (silver nanoparticle) pack, 1.21 (0.34, 2.09) for the other-NP group, 1.40 (0.88, 1.92) for the joined appraisal considering various types of nanoparticles, and 1.47 (0.85, 2.09) for the idea social events. The evaluation besides showed that there was an improvement in Zn content in the nano organized rice seedlings and foliar moreover applied rice crop in a fix subordinate way. The impact of the investigation was not established despite the fact that there were extensive field preliminary investigations.

<sup>[17]</sup> examined the impact of ZnO NPs on the attributes of germination and improvement of created rice seeds (*Oryza sativa*) in vitro. The activity of ZnO NPs essentially redesigned the seed germination of created rice, with Sudu Samba seeds showing a 7.3 improvement in seed germination (at 500 mg/L ZnO) and Suwandel and Madathawalu showing updates of 20 and 17 wholeheartedly. ZnO NPs were integrated by a wet compound design by blending ethanolic consequences of NaOH and Zn (CH<sub>3</sub>COO)<sub>2</sub> 2H<sub>2</sub>O. Plant improvement appraisal wasn't finished anyway was fundamental for crop effectiveness.

Most of the below-mentioned works have concentrated on the analysis of nano priming technology for the purpose to ameliorate the seed germination as well as starch metabolism of the aged rice seeds. Many experimenters have taken NPs as well as other accoutrements for the analysis of the performance of the germination and bounce metabolism. But, considering the different combinations will form an effect and improvement in

the successful achievement of the aged rice seeds growth. Thus, it's important to examine the effect of aged rice seeds through the germination improvement test, starch agar plate analysis and different analyses when it is employed for nano priming technology. As there are many findings in the history of NPs analysis in upgrading the rice seeds growth, in this paper, adding Fe<sub>2</sub>O<sub>3</sub> as NPs accoutrements for developing the analysis of the performance of aged rice seeds will yield advanced issues.

**Experimental Design:** In the agribusiness, the capacity to further develop the germination pace of matured or latent rice seeds is pivotal. One strategy that might potentially work is seed nano planning. Regularly, a compound diminishing is utilized to consolidate NPs. The majority of reducing agents, on the other hand, are extremely hazardous chemicals. Plant substitutes can take the place of such harmful reductants because they are friendly to the environment., subsequently, green union of NPs has arisen as another innovation. The essential point was to analyze the introduction of the developed rice seeds while exploring NPs with germination test assessment as well as starch processing examination. In addition, the research aims to use various assays, such as the starch agar plate-amylase assay, the antioxidant enzyme assay and the Histo chemical localization assay, to improve the growth of aged rice seeds. Initially, materials like Citrus hystrix and Fe<sub>2</sub>O<sub>3</sub> NPs had been gathered. Followed by the gathering of materials, make the preparation of chosen plant (Citrus hystrix) extract with the phyto chemical screening analysis on the plant extract. After the planning of the plant, the spotlight must be continued on toward the Fe<sub>2</sub>O<sub>3</sub> NPs. synthesis of the used NPs as an environmentally friendly and less expensive alternative to chemical and physical methods. Then, the proposed NPs should be characterized by applying different techniques like UV-VIS spectroscopy, XRD, particle size analyzer and FTIR. The excellent answer for the Fe<sub>2</sub>O<sub>3</sub> NPs had been created. In addition, the germination test on mature rice seeds was performed to anticipate the presentation of the seed and seedling in the field as well as to assess the practicality or quality of the seeds. Seed germination testing is a useful method for evaluating the display of seeds under clear development conditions. Planters can make better decisions about how many seeds to plant with this information. After the germination test, various measures like starch agar plate a-amylase examine, cell reinforcement compound measure and histochemical confinement were acted in the examination. At long last, eventually, ANOVA tests are performed for testing the effects of germplasm and formative stage, germplasm and year and germplasm and subsequent to maturing time on seed germination. (Fig. 1) explains

the overall workflow of the performance of the aged rice seeds by employing nano-priming technology.

## MATERIALS AND METHODS

The materials used in this investigation were described in this section. The significant materials are kaffir lime (*Citrus hystrix*) and  $\text{Fe}_2\text{O}_3$  NPs. Iron oxides can be handily combined in the lab and are normal regular composites. There are 16 iron oxides, including oxides, hydroxides and oxide-hydroxides. Under ideal redox and pH conditions, these minerals are the outcome of waterless reactions. One of the "three fundamental" oxides of iron is  $\text{Fe}_2\text{O}_3$ , with the other two being interesting iron (II) oxide (FeO). also, iron (II, III) oxide ( $\text{Fe}_3\text{O}_4$ ), which is usually the mineral magnetite. Hematite, a metal that comes from minerals, is used to make  $\text{Fe}_2\text{O}_3$ . The kaffir lime, a little tree in the family Rutaceae, has for some time been utilized as an enhancing in Southeast Asian food because of its specific citrus flavors' assets for and. The leaves of the kaffir lime plant actually contain significant bioactive composites (polyphenols), which are known for their remarkable capacity for disease prevention. Along these lines, the leaves of kaffir lime have been recognized as a characteristic material hotspot for the biosynthesis of  $\text{Fe}_2\text{O}_3$  NPs.

**Preparation of Selected Plant Extract and Phyto Chemical Screening Analysis:** The selected plant extract is *Citrus hystrix*. It is an ingredient that can be difficult to find anywhere other than specialty supermarkets. Its leaves boast a sharp, citrus flavor that's similar to a lime. However, they tend to lend a slightly earthier, floral note to dishes. Despite the fact that it is difficult to duplicate the distinctive flavor of these tropical fruits, many people liken the flavor to that of lemongrass. *Citrus hystrix* was obtained directly from nursery houses in the Giang area of Vietnam for the study (10° 22' 52.02" N, 105° 25' 11.58" E)<sup>[18]</sup>. Resulting to get-together, *C. hystrix* leaves are washed to take out new matter, dispose of squashed withdraws during transportation and pick amazing leaves, ideally mature ones. Clearly following washing, 20grams of its dried blooms were assessed, finely cut up and retained 250ml Milli-Q water in a 500-mL conelike compartment. To cement the mix, it was allowed to ascend for an hour on a beautiful stirrer. The procedure was then cooled and isolated with the help of Whatman No. 1 channel paper to level out. The filtrate was taken care of in a cleaned assessing glass at a temperature of 20°C for future examination purposes.

Followed by the preparation, the opportunity has arrived to figure out the phytochemical screening. Essentially, phytochemicals are a wide range of nonnutritive substances found in plant foods that may have an impact on health. Using Gas

Chromatography-Mass Spectroscopy (GCMS), phytochemicals should be subject to both subjective and quantitative investigation. Tests that are strong, fluid, or vaporous can be broke down utilizing GCMS. Phytochemical screening of the concentrates uncovered the presence of alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, tars, heart glycosides, phenolic compounds and coumarins. *Citrus hystrix* leaf dispense with was had a go at including the foundation evaluation of different phytochemicals according to the standard shows.

**Synthesis and Characterization of Chosen NPs:** The NP was chosen as  $\text{Fe}_2\text{O}_3$ . In a commonplace starter, 30 g of sodium hydroxide (NaOH) slugs were isolated in 1 L DI water at room temperature by blending to move toward a reasonable result of NaOH. After carefully adding the  $\text{FeCl}_3$  broken down result to the sodium hydroxide result, drop by drop and mixing was done by drowsy locally built moving, another result was obtained by stirring 250 milliliters of DI water at room temperature to dissolve 40 grams of  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ . In addition, the response result's pH was maintained between 7.5 and 8.5 to maintain the congruence of  $\text{Fe}_2(\text{OH})_3$ 's intense granularity. The appropriately settled rush was separated through filtration after 60 minutes and it was dried for 12 hours in a hot air roaster at 85 degrees Celsius. The earthy-colored dried patches were washed several times with deionized water until the pH reached 7.00. Before being allowed to gradually cool to room temperature, the hasten was also heated to 400 degrees Celsius for eight hours. Eventually, the earthy colored iron oxide item ( $\text{Fe}_2\text{O}_3$ ) that was shown prior was ground into a consistently fine structure and utilized in this paper as an adsorbent.

After synthesis, the obtained red brown product had been saved for characterization. The incorporated red brown product had been characterized by using different methodologies like Ultra Violet-visible spectrophotometer with a range of 200-900 nm, XRD, particle size analyzer, zeta potential and FTIR<sup>[20]</sup>. There are some of the following explanations on the characterization of the methodologies:

**UV-Visible Spectrophotometer:** A graph is plotted in UV-VIS spectroscopy depicting the electron transition from ultraviolet to visible radiation at various levels. This line chart of different absorptivity's on unambiguous degrees of radiations is a result of the retention limits of mixtures at specific levels. These levels are called districts of ingestion and the mixtures are named as chromophores.

**X-ray Diffraction:** X-ray powder diffraction (XRD) is a quick sharp technique basically used for stage ID of a glasslike material and can give information on unit cell

viewpoints. It is in like manner used to conclude the cell limits, space bundle and atomic headings of novel or in advance dark glasslike materials' (crystallographic) structure.

**Particle Size Analyzer:** A specialized strategy for deciding the size circulation of particles in a powder or fluid example is known as molecule size examination. Depicting the size dissemination of particles in a sample is used. Particle size analysis can be useful for aerosols as well as solids, suspensions and emulsions.

**Zeta Potential:** Estimating the charges, the zeta potential is an extent of the strong electric charge on the NP's surface. When a NP has a net surface charge, the charge is channelled by the grouping of particles with restricting charges near its surface.

**Ftir:** The FTIR method, a form of spectroscopy, is capable of identifying variations in the overall composition of biomolecules by analyzing alterations in functional groups. By detecting disparities in the molecular bonding structure between entities, it unveils valuable insights into the presence of their interactions.

**Preparation of Prime Solution:** Two different concentrations, 20 mg L<sup>-1</sup> and 40 mg L<sup>-1</sup>, of Fe<sub>2</sub>O<sub>3</sub> NPs are denoted as Fe<sub>2</sub>O<sub>3</sub> NPs 20 and Fe<sub>2</sub>O<sub>3</sub> NPs 40, respectively. These nanoparticles serve as nano priming agents. To create them, the NPs are dispersed in deionized water using ultrasonic vibration at a power of 100W for approximately 25 minutes. Another significant solution known as ferrous sulphate (FeSO<sub>4</sub>) is referred to as FeSO<sub>4</sub>20 and FeSO<sub>4</sub> 40 and is also dispersed in deionized water.

The departmental lab gave the early blossoming freak Citrus hystrix seeds after they had been marked. Different commonly occurring developed rice seeds were preserved for close to two years at temperatures between 25 and 30°C. The strong seed was chosen from this and 70% ethanol was used to clean the surface for about two minutes., then, washed with refined water. From that point onward, the seeds were drenched in sodium hypochlorite (NaOCl) for 10-15 minutes., likewise, along these lines in some cases cleaned with refined water after that. The most common way of absorbing sanitized seeds various groupings of FeSO<sub>4</sub>20, FeSO<sub>4</sub>40, Fe<sub>2</sub>O<sub>3</sub> NPs 20 and Fe<sub>2</sub>O<sub>3</sub> NPs 40 for as long as 24 hours is known as seed preparing.

**Germination Test and Measure of Seedling Growth:** Because it is required by seed regulations to appear on the marker, the warm germination test is the most widely used test. The chance creating seeds in a warm germination test ought to be disseminated on the

marker of the seed in case it is to be conveyed as seed. The Warm germination test reflects the field improvement probability of a seed part under ideal laying out conditions. Generally, 400 seeds from each seed package are placed under wettish conditions on smearing surfaces, moved napkins, or sea side and stayed aware of around 75-85 degrees F for about seven days in the most limit of cases. The seedlings are circulated as typical, strange, unhealthy, dead, or hard seeds at the finish of this period. This test will furnish farmers with a fair beginning of what will fill in the field. If the test reveals low germination, discard the seeds and purchase more. A general water take-up (RWU) germination test was completed following 24 hours of brooding. Simultaneously, the seeds were permitted to develop and the germination was covered for the six days that were going to start<sup>[19]</sup>. The radicle and plumule length, seedling weight and germination trial duration were all measured. The formula in equation number one was used to calculate RWU, and the formula in equation number two is used to evaluate Mean Germination Time (MGT).

$$RWU (\%) = \{Fw - Dw / Tw - Dw\} * 100$$

$$MGT = G_1 / N_1 + G_2 / N_2 + \dots + G_n / N_n$$

Where, is the fresh weight *Dw*, is the dry weight and *Tw* is the turgid weight in the equation number (1). and *G*<sub>1</sub>, *G*<sub>2</sub>.....*G*<sub>*n*</sub>, is the number of aged rice seeds germinated first, second and final count: *N*<sub>1</sub>, *N*<sub>2</sub> and *N*<sub>*n*</sub> is the number of days in the first, second and final count.

**Starch Agar Plate A-Amylase Assay:** After the germination test, it's time to talk about how to make the starch metabolism work better. Starch agar is a segregation medium that tests the capacity of an organic entity to create the extracellular proteins (exo-chemicals) α-amylase and oligo-1, 6-glucosidase that is covered out of the microorganisms and verbose into the Starch agar. Starch agar was additionally covered out of the microorganisms and it got diffused. These proteins hydrolyze skip by breaking the glycosidic contact between glucose subunits and permit the results of starch hydrolysis to enter the cell<sup>[20]</sup>.

Totally, 1gram of seeds was formalized in help possible result of 10mM pH5 that was centrifuged at 12,000g for 16min at 4°C. Starch-iodide complex had the absorbance worth of around 570nm. The absolute enzymatic effort had been assessed on the foundation of starch corrupted g-1 new tissue min-1<sup>[21]</sup>.

**Antioxidant Enzyme Assay:** Superoxide dismutase (CAT) and other antioxidant enzyme systems, primarily, catalyze responses to counterpoise free radicals and reactive oxygen species. These structure the body's



endogenous safeguard instruments to assist guard against free progressive persuaded cell with harming. The all-out cell reinforcement limit measure offers a technique to evaluate the viability of cancer prevention agents by inspecting the development of the phosphor molybdenum complex. In order to create a green phosphate Mo(V) complex in acidic conditions, this study relies on the standard procedure of reducing Mo (VI) to Mo(V) with a model analyte. Feline's typical effort consisted of briefly separating H<sub>2</sub>O<sub>2</sub> at 240 nm (annihilation ratio of 39.4 mM<sup>-1</sup> cm<sup>-1</sup>). For ferric oxide particles, the probabilities of DPPH scavenging were calculated. The particles were gotten up in a position show cell reinforcement energy<sup>[22]</sup>.

**Histo Chemical Localization:** The theme for biochemical assay of enzymes and histochemical localization is routine, but combining both still creates complexes excluding special favorable circumstances. It seems that the rigor of localization and quantitation have a kind of complementary relationship, whereby any increase in one, beyond a certain limit, requires a corresponding drop in the other. Basically, uncovering a substrate film to towel segments, which leaves the substrate stained and, in the film, is the way substrate film procedures for the histochemical confinement of compound molding seem to be. In vivo, the identification of O<sub>2</sub>-conformation was achieved by treating seeds with a staining solution<sup>[23]</sup>.

**Statistical Analysis:** Each treatment had three replications and was arranged in a randomized block design. ANOVA was led to decide the meaning of the information. To contrast the method for the medicines and the control, Dunnett's numerous examination tests were utilized at an importance level of 5%. A P-worth of under 0.05 was thought of as measurably huge.

## RESULTS AND DISCUSSIONS

This part makes sense of the results of the Phyto compound screening examination, different portrayal investigations, germination test investigation, different measure examinations and ANOVA results to break down the impact of the tests. The pre-arranged concentrate of the Citrus hystrix plant was utilized to test different phytoconstituents present in them. Different compound reagents were ready and explicit test for explicit phytochemicals was finished. Since each of these tests was qualitative, they were referred to as phytochemical screening. (Table 1) makes sense of the underlying subjective screening investigation of Citrushystrix separate.

(Table 1) made it abundantly clear that the important phytoconstituents of the Citrus hystrix extract are flavonoids, phenols, saponins and alkaloids. The plant extract's phytomaterials bind superficially to FeCl<sub>2</sub> to accelerate the formation of Fe<sub>2</sub>O<sub>3</sub> NPs and alter their dimensions.

Subsequent to investigating the phyto synthetic screening, the portrayals of Fe<sub>2</sub>O<sub>3</sub> NPs had been finished by various techniques like UV-VIS spectroscopy, XRD, molecule size analyzer, FTIR and zeta potential. The study of how the physical and chemical properties of NPs determine their biological effects and the reproducibility of toxicology studies both require the characterization of an NP's properties. (Fig. 2a-2e) show how UV-VIS spectroscopy, XRD, a particle size analyzer, FTIR and zeta potential were used to characterize Fe<sub>2</sub>O<sub>3</sub> NPs.

From (fig. 2a), it was set up that subsequent to confronting decrease, the rough resonances express at an inundation pinnacle of around 251nm. This occurred as a result of the development of Fe<sub>2</sub>O<sub>3</sub> NPs and the interbond transition of core iron essence electrons. From (fig. 2b), the various pinnacles were connected regarding 2 $\theta$  qualities = 29.79° (110), 34.22° (198), 39.52° (209), and 47.50° (210) of the Fe<sub>2</sub>O<sub>3</sub> NPs that assist the nature with being in translucent. Thus, the XRD scheme indicated that the Fe<sub>2</sub>O<sub>3</sub> NPs are attained by dwindling ionic iron through the Citrus hystrix factory excerpt. From (fig. 2b), it was found that unallocated tops were connected and named with indicators and it uncovered the crystallization of the bio-natural stage accomplished on the NPs. The refined inquiries uncovered that the normal iota fringe is portrayed as 28nm and the resultant molecule size goes from 20-80nm for the molecule size analyzer assessment displayed in (fig. C.) (Fig. 2d) shows that the mixing and changing of the NPs was brought about by the plant isolates utilized and the dispersing of Fe<sub>2</sub>O<sub>3</sub> NPs. The IR bunches seen at 2918cm<sup>-1</sup> and 2850cm<sup>-1</sup> exhibit sweet and aliphatic C-H protections. In a similar vein, brand names are associated with various parts and oxygen in the 400-850 cm<sup>-1</sup> region. The extending vibrations of Fe-O-H and Fe-O are, respectively, responsible for the tops at 683 cm<sup>-1</sup> and 438 cm<sup>-1</sup> and the extending vibrations of water particles that are adsorbed at a shallow level are shown by the social occasions at 3281 cm<sup>-1</sup> and 1628 cm<sup>-1</sup>. (Fig. 2e) shows the NPs' zeta capacity, which tells us about their stability in the dispersal medium. The zeta expected evaluation of the Fe<sub>2</sub>O<sub>3</sub> NPs uncovered a negative worth of -22.5mV (Fig. 2e). Because the biomolecules in the green blend construction have the ability to influence the zeta expected respect, it's possible that the presence of -COOH factors is to blame for the negative charge on the NP surface.

After the examination of portrayal, germination test examination is finished to decide the level of seeds that are alive in the matured rice seed parcel. At first, seed water take-up examination is finished in the germination test. After 24 hours of hindrance, the effects of various nano priming treatments on seed water uptake are summarized in (Table 2). As per (Table 2), it was seen that Nano-prepared seeds displayed a higher pace of water imbibition contrasted

with the other preparing bunches following 24 hours.  $\text{Fe}_2\text{O}_3$  NPs 20 and  $\text{Fe}_2\text{O}_3$  NPs 40 showed an excitement of more than half. Seeds treated with nano-priming continued to have a higher capacity for absorbing water than those treated with bulk-priming and the control. Subsequent to breaking down the water take-up of the seeds, the following stage is to look at the germination pace of matured rice seeds following preparing with various preparing specialists. (Fig. 3) gives a graphical portrayal of the germination pace of matured rice seeds subsequent to being prepared for a term of roughly 6 days.

According to (Fig. 3), arranging rice seeds with  $\text{Fe}_2\text{O}_3$  NPs 20 and  $\text{Fe}_2\text{O}_3$  NPs 40 results in a significant increase in both the speed of early germination and, more generally, when compared to the benchmark bundle and other preparing agents. The  $\text{Fe}_2\text{O}_3$  NPs 20 and  $\text{Fe}_2\text{O}_3$  NPs 40 nano-organized seeds showed germination speeds of 25% and 13%, exclusively, on

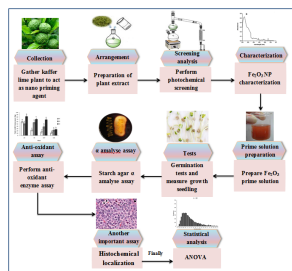


Fig. 1: Overall workflow of the performance of the aged rice seeds by employing nano priming technology

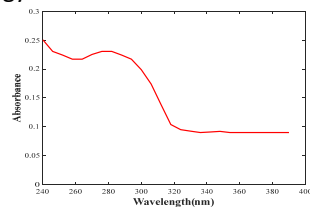


Fig. 2 (a) Wavelength (nm)

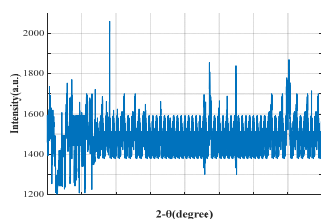


Fig. 2 (b) Intensity(a.u)

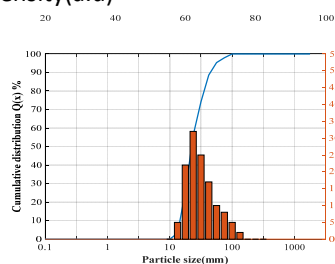


Fig.2 (c) Cumulative distribution Q(x)%

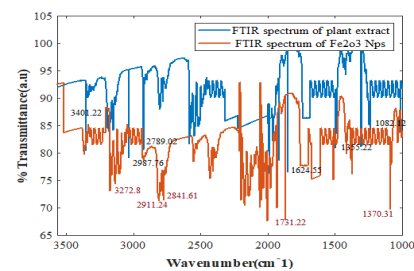


Fig.2 (d) %Transmittance (a.u.)

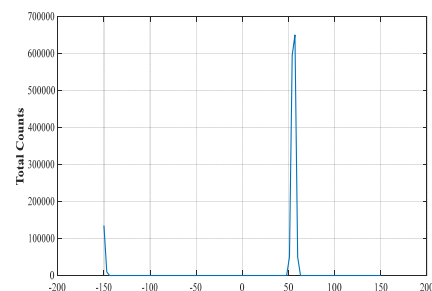


Fig.2 (e) Apparent zeta potential (Mv)

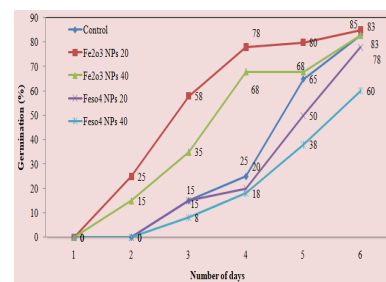


Fig. 3: Graphical representation of germination rate of aged rice seeds after priming for about 6 days

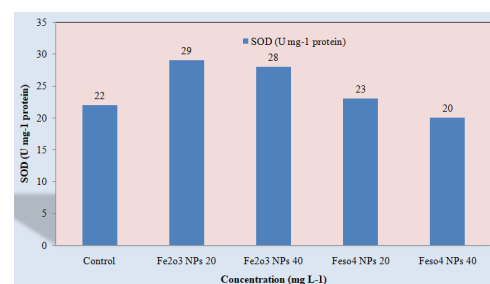


Fig 4 (a) Concentration (mg L-1)

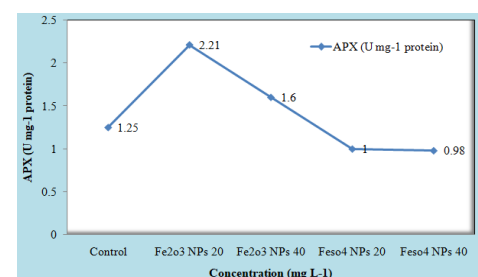


Fig. 4 (b) APX (U mg-1 protein)

**Table 1: Initial qualitative screening analysis of Citrushystrix extract**

Types of test	Compounds	Attained formation	Outcome
Saponins	Foam	Foam appeared for about 10 minutes	(+)
Phenols	FeCl <sub>3</sub> (Ferric chloride)	Blackish-green precipitate is formed	(+)
Flavonoids	Pb(C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub> (lead acetate)	Color of the solution changes to yellowish-brown	(+)
Terpenoids	Salkowski's	The obtained color was not the attained form color	(-)
Alkaloids	Hager's	The red precipitate is obtained	(+)
Steroids	Liebermann-Burchard	Brown ring should be formed but it was not obtained	(-)
Carbohydrate	Benedict's reagent	Yellow color is obtained	(+)
Proteins	HNO <sub>3</sub> (Nitric acid)	Yellow color should obtained but the result was not formed	(-)

**Table 2: Effect of different nano priming treatments on seed water uptake after 24 hours of inhibition**

Concentration (mg L <sup>-1</sup> )	Seed water uptake (%)
Control	30
Fe <sub>2</sub> O <sub>3</sub> NPs 20	50
Fe <sub>2</sub> O <sub>3</sub> NPs 40	48
FesO <sub>4</sub> NPs 20	27
FesO <sub>4</sub> NPs 40	20

**Table 3: Influence of various nano priming treatments on total soluble sugar content**

Concentration (mg L <sup>-1</sup> )	Total soluble sugar (mg g <sup>-1</sup> FW)
Control	11
Fe <sub>2</sub> O <sub>3</sub> NPs 20	13
Fe <sub>2</sub> O <sub>3</sub> NPs 40	12.5
FesO <sub>4</sub> NPs 20	7
FesO <sub>4</sub> NPs 40	6

**Table 4: CAT enzyme activity of germinating aged rice seeds**

Concentration (mg L <sup>-1</sup> )	CAT (U mg <sup>-1</sup> protein)
Control	0.12
Fe <sub>2</sub> O <sub>3</sub> NPs 20	0.22
Fe <sub>2</sub> O <sub>3</sub> NPs 40	0.15
FesO <sub>4</sub> NPs 20	0.1
FesO <sub>4</sub> NPs 40	0.09

**Table 5: Mean square and p values from the ANOVA of data obtained through Fe2O3 NPs application on aged rice seeds**

Source	Degree of freedom (Df)	Chlorophyll amount	Length of stem	Stem diameter	Number of fruit branches per plant	Hydrogen peroxide	Malondi aldehyde
H2o stress (A)	1	11.989	131.57	854.35	9.541	5.398	41.012
Nano priming analysis (B)	4	1.197	15.961	886.96	2.59	111.01	1.761
AB	4	0.095	0.451	889.89	0.129	1.491	0.756
Error	20	0.01	0.239	0.891	0.231	0.081	0.012
Source	Df	Number of capsule per plant	Number of seed per capsule	Total fresh yield	Dry stem yield	Per oxidase	Catalase
H2o stress (A)	1	5539.33	9.593	22.172	5.612	54123.5	9.532
Nano priming analysis (B)	4	431.012	1.112	3.459	1.081	2551	0.81
AB	4	6.708	0.04	0.069	0.068	509.12	0.0202
Error	20	3.12	0.231	0.039	0.025	37.5	0.008
Source	Df	Plant height	Plant fresh weight	Plant dry weight	Super oxide dismutase		
H2o stress (A)	1	7.508	1472.32	29.825	1673.25	-	-
Nano priming analysis (B)	4	0.519	211.38	0.721	91.389	-	-
AB	4	0.024	23.083	0.132	4.635	-	-
Error	20	0.007	4.039	0.017	1.031	-	-

the resulting day, while the control seeds showed germination on the third day following anguishing. On the last insight day, the last germination rates were seen as 83% for the Fe<sub>2</sub>O<sub>3</sub> NPs 20 and 40 prescriptions, 78 percent for the FeSO<sub>4</sub> 20 treatment and 60 percent for the FeSO<sub>4</sub> 40 treatment. These results indicate that the priming with Fe<sub>2</sub>O<sub>3</sub> NPs 20 and Fe<sub>2</sub>O<sub>3</sub> NPs 40 resulted in higher final germination percentages compared to the other treatments.

Followed by the germination test, the effect of various nano-priming treatments on total soluble sugar content had been analyzed. Dissolvable sugars don't just capability as metabolic assets and underlying constituents of cells, they likewise act as signs directing different cycles related with plant development and improvement. The impacts of different nano-preparing medicines on all out solvent sugar content are nitty gritty in (Table 3).

(Table 3) presents the appraisal of starch processing in developed rice seeds by taking a gander at the total dissolvable sugar content. When contrasted with the control, the nano-prepared seeds,

explicitly those that were treated with Fe<sub>2</sub>O<sub>3</sub> NPs 20 and 40, displayed a critical expansion in the all-out solvent sugar content. Specifically, contrasted with the benchmark group, Fe<sub>2</sub>O<sub>3</sub> NPs 20 nano-prepared seeds had an all-out solvent sugar content that was 24% higher, while Fe<sub>2</sub>O<sub>3</sub> NPs 40 nano-prepared seeds had a complete sugar content that was 19% higher.

(Fig. 4) a and 4 b indicate the graphical representation of Super Oxide Dismutase (SOD) enzyme activity and Ascorbate Per oxidase (APX) enzyme activity and table 4 explains the catalase (CAT) enzyme activity. From 4a, it is highlighted that α-amylase, an impetus pressing for carb (starch) processing in seedlings, expects a basic part. Contrasted with different prescriptions and the benchmark group, the outcomes show a critical expansion in-amylase movement in nano-arranged rice seeds treated with Fe<sub>2</sub>O<sub>3</sub> NPs 20 and Fe<sub>2</sub>O<sub>3</sub> NPs 40, with energy of 47% and 34%, separately. To additionally approve these discoveries, α-amylase examine was led by deciding the creation and



discharge of  $\alpha$ -amylase from undeveloped organism less half-seeds utilizing a starch agar plate. After staining the agar plate with iodide, a transparent zone was observed, indicating the hydrolysis of starch and the presence of  $\alpha$ -amylase. This observation confirms the enhanced  $\alpha$ -amylase activity resulting from the nano-priming treatment.

As ought to be noticeable in (Fig. 4d-f), the response of arranged seeds to various seed getting ready meds moved in the cell support enzymatic activities of Grass (superoxide dismutase), APX (ascorbate peroxidase) and Cat (catalase). The enzymatic exercises of the control and arranged seeds were at first equivalent in dry seeds (following one hour of ingestion) (data not shown). However, 24 hours after use, significant changes were observed in the enzymatic exercises for cell reinforcement. Specifically, sensation of 29% in Turf activity, half in APX development and 61% in Cat activity were seen in  $\text{Fe}_2\text{O}_3$  NPs 20 nano-arranged seeds, when stood out from mass arranged seeds and the benchmark bunch. The enzymatic activities of SOD, APX and CAT were significantly enhanced by the  $\text{Fe}_2\text{O}_3$  NPs 20 nano-priming treatment, indicating an enhanced antioxidant defense system in the seeds.

ANOVA ought to be used as the final analysis at this point. To assess the impacts of preparing strategies on seed fortification and field execution of matured rice seeds with  $\text{Fe}_2\text{O}_3$  NPs, laboratory tests and a field explore were executed. The ANOVA results are detailed in (Table 5).

From (Table 5), it had been seen that a gigantic relationship of complete chlorophyll things' association transport was found with plant new loads (FW), plant dry weight (DW) and plant level (PH) of 0.9214, 0.9638 and 0.9596, uninhibitedly and that suggests that improvement in chlorophyll contents prompts expanded biomass creation. Seed preparing medicines lead to a huge reduction in MDA item in matured rice plants both in charge and dry spell pressure conditions. The experimental rice pots' exposure to an environment devoid of water has an impact on their levels of  $\text{H}_2\text{O}_2$  and malondialdehyde, respectively. Water pressure prompts harm in the lipid bilayer construction of natural films and subsequently, MDA aggregation happens

Seed priming with  $\text{Fe}_2\text{O}_3$  NPs brings about a 53% decrease in Malondi aldehyde items in water pushed rice plants. The results of the aforementioned ANOVA indicated that  $\text{Fe}_2\text{O}_3$  NPs significantly reduced Malondi aldehyde levels.

## CONCLUSION

In this exploration, the improvement of the germination test examination and starch digestion

examination of matured rice seeds by using the  $\text{Fe}_2\text{O}_3$  NPs were researched. This study attempted to assess the  $\text{Fe}_2\text{O}_3$  NPs analysis in relation to aged rice seeds. The portrayal of  $\text{Fe}_2\text{O}_3$  NPs had been done effectively through various strategies. In germination studies, the observation time was used to analyze data collected at each seed's germination time and the number of seeds germinating at each time interval. Assays like the starch agar plate-amylase assay, the antioxidant enzyme assay and histochemical localization had improved seed growth. The ability to demonstrate the best application of  $\text{Fe}_2\text{O}_3$  NPs on aged rice seeds was uncovered through the analysis of ANOVA, which was also included in the research. Even though  $\text{Fe}_2\text{O}_3$  NPs helped seed germination and growth, more research is needed to understand exactly how they affect plants.

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