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Effects of a foliar-applied *Saccharomyces cerevisiae*-Urea-Zn-DAP nanofertilizer blend on seed germination and biomass in radish (*Raphanus sativus* L.)

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Abstract

Radish (*Raphanus sativus* L.) is an economically sustainable root vegetable cultivated worldwide for its nutritional, pharmaceutical, and agricultural importance. However, its production can be compromised by poor seed germination, nutrient shortage, and deteriorating soil fertility, which directly affect its marketable yield. This study focuses on a nano-biofertilizer comprising the plant probiotic *Saccharomyces cerevisiae* IIUI-565, conjugated with urea-Zn-DAP nanofertilizer, to enhance radish seed germination. Herein, for the first time, indigenous mixed herbs were used to synthesize urea-Zn-DAP nanofertilizer. The mixed herbal spice methanolic extracts consisted of eugenol (93%) and caryophyllene (4.8%) as the main reducing agents, identified by Gas Chromatography-Mass Spectrometry. UV-Visible and Fourier Transform Infrared Spectroscopy confirmed mixed herb-mediated synthesis and synergism of urea-Zn-DAP nanofertilizers. X-ray diffraction (XRD) analysis provided the presence of a hexagonal crystalline structure. SEM image revealed ultra-small spherical-hexagonal-shaped particles of urea-Zn-DAP NFs with an average size of 0.98 nm. Greenhouse experiments demonstrated that the foliar application of *S. cerevisiae* (1×10^7 CFU mL^{-1}) with urea-Zn-DAP NFs (30 μM) achieved a germination rate of 95%, noticeably after 10 days in comparison with urea (70%). This comprehensive approach outlines a sustainable pathway to engineering microbial-nanomaterial consortia as next-generation nanobiofertilizers to foster resilient, eco-friendly crop production.

Introduction

Radish (*Raphanus sativus* L.) is an annual root vegetable from the Brassicaceae family, valued globally for its diverse uses and nutritional benefits [1]. Although radish is a widely consumed root vegetable, its leaves are typically not utilized as by-products in commercial, industrial, or agricultural contexts [2]. *Raphanus sativus* L. leaves (RSLs) and roots (RSR) contained most of the reported phytochemicals, including alkaloids, phenols, and flavonoid compounds. The most significant of these phytochemicals, known for their pharmacological and nutraceutical properties, were flavonoid compounds [3]. Radish has a short growth cycle, high nutritional value, including calcium, magnesium, and vitamins B6 and C, and strong market demand, emphasizing the link between healthy soil management and agricultural productivity [4, 5]. Despite its agronomic advantages, radish productivity is often limited by suboptimal seed germination, inadequate nutrient availability, and declining soil fertility, particularly in intensively cultivated agricultural systems [6]. In Pakistan, radish cultivation is hindered by several challenges, such as a shortage of quality seeds, significant pest and disease pressure, and the effects of climate change, particularly under drought environment [7]. These challenges directly affect early plant establishment and ultimately reduce marketable yield, necessitating innovative, eco-friendly farming techniques.

Dependency on traditional chemical fertilizers, while effective for boosting crop production, raises sustainable environmental risks such as soil degradation and nutrient loss [8]. In agriculture, urea-based fertilizers are frequently used due to their high nitrogen content of 46%. In 2019–2020, urea provided 76.5% of the 107.4 million tons of nitrogen required globally [9]. The demand for urea has increased significantly in the past ten years, rising from 147.5 million tons (Mt) in 2009 to 178.3 Mt in 2019. The probability of fertilizer usage efficiency by crops is low, only 10 to 25% for phosphorus-based and 20 to 50% for nitrogen-based fertilizers [10]. The U.S. Food and Drug Administration (FDA) has classified widely used urea and diammonium phosphate (DAP) as Generally Recognized as Safe (GRAS) in food items intended for human consumption, yet their efficiency and environmental impact require improvement.

Zinc, a crucial micronutrient, is often deficient in low-income populations and remains vital for both crop growth and human nutrition [11]. Recent studies revealed that integrating nanofertilizers, such as nano-hydroxyapatite and total silicon-based nanocomposites, can improve nutrient delivery, reduce losses, and enhance crop performance [12]. Nanobiofertilizers, as an emerging technology, particularly due to their ultra-small size offer additional advantages such as increased solubility, improved penetration, targeted fertilizer delivery, and thus reduced environmental toxicity [13]. In addition to minimizing the need for chemical pesticides and fertilizers, nanobiofertilizers have the potential to enhance crop production and promote sustainable agriculture [14].

The combination of nanofertilizers with plant probiotics, such as *Saccharomyces cerevisiae*, enhances seed germination, root development, and overall plant growth, and reduces reliance on chemical fertilizers [15]. *Saccharomyces cerevisiae*, also known as a probiotic strain, enhances the bioavailability of proteins, vitamins, and enzymes and is commonly found in baked and fermented foods, fruits, and plants [16]. The environmentally safe yeast has demonstrated plant growth-promoting potential through the production of phytohormones such as indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPYA), vitamins, and metabolites that enhance seed germination and root development [17]. Similarly, plant growth-promoting yeasts (PGPY) are typically incorporated into agricultural practices through soil integration, foliar spraying, and seed inoculation, and have shown potential as plant growth-promoting (PGP) bioagents [18].

Furthermore, spices are widely recognised not only for their allelopathic and medicinal properties, but also for their bioactive compounds that can influence plant seedling growth and development [19]. Additionally, these phytocompounds are used as reducing agents for nanoparticle synthesis [20].

Consequently, this study investigates for the first time the foliar application of a microbial-nanofertilizer consortium comprising *S. cerevisiae* IIUI-565 and urea-Zn-DAP nanocomposites, enhanced with 10 herbal spice extracts, to promote radish seed germination. The synergy of plant probiotic (*S. cerevisiae* IIUI-565) with ultrasmall nanofertilizers aims to provide a sustainable, eco-friendly strategy to improve radish seed germination, reduce dependency on chemical fertilizers, and advance sustainable agricultural practices.

Materials and methods

Reagents and Chemicals

Sabouraud Dextrose Agar (SDA) CM0041 (Merck Millipore, Darmstadt, Germany), Ethanol (70%), and Zinc Sulphate $ZnSO_4$ were bought from the USA: Sigma Aldrich, St. Louis, MO. Urea (H_2NCONH_2), Diammonium Phosphate (DAP). Since each ingredient was analytical grade, no other purification was necessary.

Collection of Plant Probiotic S. cerevisiae IIUI-565 Strain

Plant probiotic *S. cerevisiae* IIUI-565, a freeze-dried culture, was obtained from the IIUI research group of the Biological Science Department, International Islamic University, Islamabad, Pakistan, working on a plant probiotic strain. The strain was grown in a nutritional medium containing 1.5 grams of glucose, 0.20 grams of yeast extract, 1.5 grams of NH_4Cl, 0.65 grams of KH_2PO_4, and 1.25 grams of Na_2HPO_4 in 60 milliliters of distilled water. The exponential phase ended when *S. cerevisiae* IIUI-565 cells were incubated for 12 hours at 25°C while shaking at 70 rpm. The cultures were subsequently stored at 4°C for further use [21].

Preparation of Indigenous Herbal Spices Extract

Different indigenous herbal spices such as cardamom (*Elettaria cardamomum*), cumin seeds (*Cuminum cyminum*), black pepper (*Piper nigrum*), fenugreek (*Trigonella foenum-graecum*), coriander (*Coriandrum sativum*), cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum*), turmeric powder (*Curcuma longa*), mace (*Myristica fragrans*), and nigella seeds (*Nigella sativa*) were used for the first time to prepare the indigenous herbal spices extract. The methanolic extract was made by grinding the indigenous herbal spices [1:1] into tiny particles with a mixer grinder. In this process, 24 g of the combined material was dissolved in 100 mL of methanol, and the solution was stirred continuously for 48 hours to achieve complete dissolution. The solution was heated for 6 to 7 hours at 70°C and 700 rpm using a magnetic stirrer. The solution was filtered three times by Whatman Grade 1, 15 mm qualitative filter paper. The filtrate was collected and stored at 4°C for GC-MS screening and nanoparticle synthesis [22].

Green Synthesis of Urea-Zn-DAP Nanocomposite

Green urea-Zn-DAP-based nanofertilizers were synthesized as reported [23], using a combination of indigenous mixed herbs with certain modifications. Aliquots of 60 mL of indigenous herbal extract were added dropwise to the precursor solution (urea-Zn-DAP) in a 1000 mL beaker kept on a magnetic stirrer at 600 revolutions per minute at 70°C in a dark period for 7 to 8 h after various physical parameters were optimized for the typical synthesis of urea-Zn-DAP NFs. Once all components have been added, the solution's pH becomes neutral. Every two hours, the solution's color changed until a heavy yellow tint was apparent. After 15 minutes of centrifugation at 6000 rpm, the pellet was cleaned three times using 70% ethanol and distilled water. The pale yellow-grey pellet was then dried at 80°C and stored at 4°C for additional analysis and a pot examination. The schematic illustration of indigenous herb-based urea-Zn-DAP nanofertilizers is shown in Fig. 1.

Identification of Potential Reducing Agents in Indigenous Herbal Spices Extract using GC-MS

Indigenous herbal spices extract (15µl) was run on Gas Chromatography-Mass Spectrometric (Thermo Scientific ISQ 7000 Single Quadrupole GC-MS System) for quantitative analysis of secondary metabolites (potential reducing and stabilizing agents for nanoparticles synthesis) using methanol as a solvent through carrier gas “He”. Operating detailed conditions for examination were column length 15 m, column breadth 0.2 mm, and film thickness 1µm, beginning temperature Ti of infusion harbor 110°C for 2 minutes, part mode capillary injector, column channel weight 90kPa, Broiler temperature (GC) sloped at 10°C to 280°C for 9 minutes, and Electron ionization EI voltage 70eV. The infusion harbour was maintained at 250°C with a stream rate/velocity of 1 millilitre per second of the helium "transportation energy." Mass spectroscopy's spectral extent to show the signal was 40–500 (m/z). The metabolic items displayed in the mixed herb extract were matched with standard chemical compounds in the NIST (National Institute of Standards and Technology) 17 library on a mass range premise. The relative percentage value of each part was found by calculating its peak value over the total interval. Spectral data and chromatogram peaks were analyzed using the Turbomass program to determine the volatile identity of a compound [24].

Physiochemical Characterization of Urea-Zn-DAP-Based Nanofertilizers

Green synthesized urea-Zn-DAP-based nanofertilizers were characterized at the Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad, Pakistan. The optical properties of synthesized urea-Zn-DAP NFs were characterized using a UV-Visible spectrophotometer (Evolution 201 Thermo Fisher) in the wavelength range from 190 to 800 nm to confirm urea-Zn-DAP-based nanocomposites by applying UV light [25]. FTIR spectra were acquired in transmission mode based on Thermo Fisher Scientific Nicolet™ iS50 FTIR Spectrometer software, ranging from 400–4000 cm^{-1} at 4 cm^{-1} resolution. The infrared spectrum tests of the NFs, primarily characterization of their active functional groups and interactions, were conducted using FTIR spectroscopy [26]. The size and phase variation of the synthesized nanofertilizers were measured using X-ray diffraction spectroscopy Model: XRD Regaku (Dmax IA) at 60 kV of voltage and 60 mA of current [27]. The particle size was found using the Debye-Scherrer formula (due to the extremely sharp peak seen during diffraction analysis [28]. Scanning Electron Microscopy (SEM TESCAN MAIA3) was used to analyze the topography and particle size of all synthesized nanofertilizers. The biosynthesized urea-Zn-DAP nanofertilizers were made conductive by applying a thin layer of gold before preparing SEM slides. As a result, the samples were examined under the SEM at a voltage of 20 kV [29]. The purity and elemental analysis of manufactured urea-Zn-DAP nanofertilizers were examined using Energy Dispersive Spectrometers (EDS) with Energy Dispersive X-ray (EDX) analysis. High-energy electrons are emitted in Scanning Electron Microscopes to display morphology, and an image was created by revealing element composition through backscattered electrons [30].

Preparation of Urea-Zn-DAP Nanofertilizers and S. cerevisiae Consortia

The suspension was prepared using urea-Zn-DAP (30 µM) nanofertilizers and *S. cerevisiae* IIUI-565 (1×10^7 CFU mL^{-1}) combined with deionized water in a 50-mL reagent bottle. Urea-Zn-DAP nanofertilizers were agitated for approximately five minutes to ensure a homogeneous mixture. This microbial-nanomaterial consortium was used in a green greenhouse pot experiment.

Greenhouse Pot Experiment

Collection of Seeds

The radish seeds were sourced directly from the National Culture Collection of Pakistan (NCCP), Division National Agriculture Research Centre (NARC), Islamabad, Pakistan. They were thoroughly surface sterilized using 70% ethanol, then rinsed with deionized water and carefully air-dried on paper towels.

Preparation of Soil and Seedling Pots Setup

Garden soil, collected from a depth of 10 to 30 cm, was autoclaved and used to establish the greenhouse pot experiment. According to the designed setup, 10 radish seeds were planted in 11 × 9 cm plastic pots with 9 drainage holes at the bottom, filled with a substrate composed of 50% garden soil, 10% sand, 10% dried leaves, 10% rotten vegetables, and 20% manure. Each pot was filled with 167 g of autoclaved soil. Three replicates were made for all the treatments.

Greenhouse Pot Trial

The greenhouse pot experimental design was conducted at Sector 1/F, Wah Cantt, Rawalpindi, Pakistan (33°44'52"N, 72°46'50"E) from December 3, 2023, to February 3, 2024, to observe the impact of plant probiotic *S. cerevisiae* IIUI-565 and ultra-small-sized urea-Zn-DAP nanofertilizers on radish seed germination through foliar application. The experiment was performed under controlled environmental conditions such as temperature (16°C), relative humidity (52.2%), and photoperiod (8h light/8h dark) for 60 days. The experimental setup consisted of three treatment groups (T1, T2, and T3), each replicated 3 times. Soil supplemented with only *S. cerevisiae* IIUI-565 (T1), soil supplemented with urea-Zn-DAP nanofertilizers (T2), and soil with a combined application of *S. cerevisiae* IIUI-565 and urea-Zn-DAP nanofertilizers (T3). Conventional urea was used in a positive control group (PC), while soil without urea-Zn-DAP was considered as a negative control (NC). Foliar applications of *S. cerevisiae* IIUI-565 and urea-Zn-DAP NFs were sprayed twice during the experiment. The first application was performed 10 days after transplantation, followed by days 21, 45, and 60. The plants were harvested after a precise 60 days, to assess maximum growth and quality. The quantitative growth parameters, including shoot and root lengths, fresh and dry weights, leaf area, germination %, and relative water content, were recorded on these days [31].

Growth and Biomass Analysis

The shoot and root lengths of the harvested radish plants were measured independently for each replica using measuring tape. Biomass was recorded immediately after harvest, and samples were oven-dried at 85 °C to determine dry weight. The leaf area was measured using formula [31].

Area = Length × Width (cm)

The given formula calculated the germination percentage of all the treatments:

G.P (%) = (Number of Germinated Seeds / Total Number of Seeds Planted) ×100

The leaves were immersed in water overnight to reach full turgidity to determine their relative water content. Following that, fresh and dry weights of leaves were measured [32].

% of RWC = [(fresh weight - dry weight) / (fully turgid weight - dry weight)] × 100

This design allowed precise evaluation of the individual and combined effects of probiotic and nanofertilizer treatments on radish growth, biomass accumulation, and physiological performance.

Statistical Analysis

The three replications of the experimental investigation resulted in mean ± SE data being given. GraphPad Prism 8.4.2 (San Diego, CA, USA) was used for the statistical analysis. The relevance of *S. cerevisiae* IIUI-565 and urea-Zn-DAP nanofertilizers on radish seed development was evaluated using a two-way analysis of variance test (ANOVA). Multiple mean comparisons were performed using Tukey's HSD post hoc test, with a significance threshold of $p < 0.05$.

Results

Identification of Potential Reducing Agents in Indigenous Herbal Spices Extract using GC-MS

GC-MS analysis of the methanolic extract of indigenous herbal spices, as illustrated in (Fig. 2, Supplementary Table 1), includes eighty-six phytochemicals that act as capping and reducing agents. Among these phytocompounds, eugenol (93%) and caryophyllene (4.8%) were found as the main reducing agents during nanofertilizer synthesis. Eugenol (2-methoxy-4-prop-2-enylphenol) is a prominent constituent of clove, cinnamon, turmeric, and black pepper, with the largest abundance (93%) found in clove. Macelignan (4-((2S,3R)-4-(Benzo[d] [1, 3] dioxol-5-yl)-2,3-dimethylbutyl)-2-methoxyphenol) and caryophyllene (4.8%) (4Z)-4,11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene are other important components of clove, cinnamon, and black pepper.

Physiochemical Characterization of Urea-Zn-DAP-Based Nanofertilizers

UV light was absorbed by the sample, resulting in an energy spectrum of absorption at wavelengths ranging from 190 to 800 nm, designed for light transmission. The highest absorbance peak falls within the 190–350 nm spectrum, with a particular intensity at 206 nm. The collective oscillation of electrons in the synthesized nanofertilizer and their interaction with light wavelengths cause the urea-Zn-DAP-based NFs to show Surface Plasmon Resonance (SPR). The UV absorption was weak due to the presence of complex and insufficient biomolecules, resulting in a change in the district color. The study of the energy band gap and absorption maxima requires an analysis of the optical properties. The band gap of urea-Zn-DAP NFs was determined to be 6.0 eV using the energy equation [33], as illustrated in (Fig. 3A).

The functional groups of the active components are found by FTIR spectroscopy based on the peak value in the infrared region. The FTIR spectra of urea-Zn-DAP nanofertilizer are displayed in (Fig. 3B). The fingerprint region was confirmed by peaks below 1000, mainly at 410–460 cm^{-1}, which verified the successful production of indigenous herbal extract-mediated urea-Zn-DAP NFs. The vibrations in the graphs illustrate that different stretches at distinct levels result in diverse peaks. The peak at 433 cm^{-1} is dependent on the ZnO stretching bond, as reported by [34]. The band located at 1012.462 cm^{-1} is associated with Zn-OH stretching, while the peak at 1646.938 cm^{-1} identifies N-H vibrations, the peak at 1438.66 cm^{-1} signifies C = C and O-H bending, with peaks at 1537.013 cm^{-1} and 2030.709 cm^{-1}. Another peak, at 2360.482, was found and associated with the alkane's C-O stretching vibration. The peak at 3527.224 to 3924.423 cm^{-1} was due to the OH stretching of alcohols and phenols.

The XRD profile determined the crystalline structure of green-synthesized urea-Zn-DAP NFs (Fig. 3C). The hexagonal structure of the synthesized nanofertilizers was represented by all the sample's diffraction peaks with no sign of impurities. The Scherer equation proved that the average crystallographic size of the ultra-small-sized urea-Zn-DAP NFs was 0.682 nm. The dimensions of urea-Zn-DAP nanofertilizers were determined using FWHM data, revealing measurements with prominent and well-defined peaks at a d-spacing of 9.123 Å. The strong peak of the pattern shows a larger crystalline size.

The surface morphology of ultra-small-sized urea-Zn-DAP-based NFs is shown in (Fig. 3D). The forms of the NFs were hexagonal and almost spherical, respectively, with some irregularities signifying non-homogeneity. Green-synthesized urea-Zn-DAP NFs have a particle size of 0.682 nm, and there is a visible tendency toward mild agglomeration is present. Spherical/granular, floral-like, triangular, nano-sheet, nano-flakes, needle-like, and spot-like shapes were present in the surface morphology of synthesized NFs. The distinct morphology of synthesized urea-Zn-DAP nanofertilizers was considerably affected by the respective indigenous mixed herbal spices applied for the first time in this research.

The elemental composition of synthesized nanofertilizers and the percentage of each element in the resulting materials can be found by EDX spectroscopy, a mode of operation of SEM (Fig. 3D). The elements zinc, carbon, oxygen, and phosphorus were found in the EDS spectrum of urea-Zn-DAP nanofertilizers. The proposed structure is consistent with the stoichiometric ratios in the spectra, which show aluminium content of 0.1%.

Impact of Plant Probiotic *S. cerevisiae* IIUI-565 and Ultra-Small Urea-Zn-DAP-Based Nanofertilizers on Radish Seed Germination

In the current greenhouse study, at 10 days post-germination, foliar application of all treatment groups (T1, T2, and T3) exhibited measurable seedling growth compared to urea (standard), showing minimal development. Among all treatments nanomicrobial consortium i.e. T3 (*Saccharomyces* cerevisiae 1 × 10^7 CFU mL^{-1} and urea-Zn-DAP nanofertilizers-30 μM) demonstrated enhanced seed growth ($p < 0.05$) throughout the study as illustrated in (Fig. 4), confirming synergistic effect between *Saccharomyces* cerevisiae (probiotic yeast) and urea-Zn-DAP nanofertilizers.

The study conducted a comprehensive analysis of variance (Two-way repeated measures ANOVA) to assess the influence of various concentrations of *S. cerevisiae* IIUI-565 (1 × 10^7 CFU mL^{-1}) and ultra-small-sized urea-Zn-DAP NFs (30 μM) on the growth and development of radish seeds. Notably, the obtained p-value for the significance test was below 0.0001, showing statistically significant length variations. According to Tukey's technique, post hoc comparisons, the untreated control group consistently displayed noticeably shorter mean length at every time interval.

Radish Growth Parameters

Foliar application of T3 (combination of soil, *S. cerevisiae* IIUI-565, and urea-Zn-DAP NFs) at 1 × 10^7 CFU mL^{-1} and 30 μM concentrations resulted in notable enhancements in several plant growth indicators when compared to both positive and negative controls (Fig. 5). The enhanced growth parameters included increases in shoot length (7.7 cm, 7.0 cm, 9.5 cm, 10.5 cm, and 12 cm), and root length (2.55 cm, 2.25 cm, 3.44 cm, 2.65 cm and 3.22 cm), plant fresh weight (3.8 g, 2.5 g, 4.3 g, 5 g, and 5.8 g), plant dry weight (1.8 g, 1.4 g, 1.9 g, 2.3 g, and 2.6 g), leaf area (0.75 cm^2, 0.24 cm^2, 0.8 cm^2, 1.0 cm^2, and 1.5 cm^2), germination percentage (70.7%, 60.5%, 80.9%, 90.0%, and 95.0%), and relative water content (28.968%, 17.857%, 31.48%, 37.5%, and 44.03%). The NC (negative control) showed the lowest values for shoot length, root length, plant fresh weight, plant dry weight, leaf area, germination percentage, and relative water content.

As shown in (Fig. 5a, 5b, Supplementary Table 2, 3), the interaction effect of concentration (treatment) and time was less than 0.0001 in the variables "shoot length and root length" (ANOVA, $F (12, 40) = 11.32$, and $F (12, 40) = 8.366$, $p < 0.0001$ correspondingly. Multiple analyses did not reveal any discernible changes between the treatments over the 10-day assessment period. In contrast to both controls (PC and NC), the treatments T1, T2, and T3 of the *S. cerevisiae* IIUI-565 (1 × 10^7 CFU mL^{-1}) and the urea-Zn-DAP NFs (30 μM) and their combination demonstrated a statistically significant distinction. Notable differences were also observed over the 60-day study.

The repeated measures analysis of variance (ANOVA) test showed a noteworthy interaction between treatments and period for the variables "radish fresh and dry weight," with p-values below 0.0001 (ANOVA, $F (12, 40) = 3.589$ $P = 0.0011$, and $F (12, 40) = 8.590$, $p < 0.0001$, respectively). This interaction effect is clear in (Fig. 5c, 5d, Supplementary Table 4, 5), which displays the data. Over the 60-day evaluation period, treatments T1, T2, and T3 of *S. cerevisiae* IIUI-565 and urea-Zn-DAP NFs (1 × 10^7 CFU mL^{-1} and 30 μM concentrations) showed notable increases in fresh and dry weight compared with the untreated plants (PC and NC).

The "leaf area" exhibited significant differences on day 60 among the different treatments, as shown in (Fig. 5e Supplementary Table 6). Treatment T3 (1×10^7 CFU mL^{-1} of *S. cerevisiae* IIUI-565 and 30 μM of urea-Zn-DAP nanofertilizers) resulted in a statistically significant (ANOVA, $F (12, 40) = 311.2$, $p < 0.0001$) increase in leaf area per plant (1.5 cm^2) compared to the conventional urea (PC) and soil without urea-Zn-DAP (NC).

In the variable "germination %", the p-value is less than 0.0001 (ANOVA, $F (12, 40) = 3531$, $p < 0.0001$) as illustrated in (Fig. 5f, Supplementary Table 7). The pot (T3) treated with *S. cerevisiae* IIUI-565 (1×10^7 CFU mL^{-1}) and urea-Zn-DAP nanofertilizers (30 μM) showed the highest germination percentages (95%). In contrast, the pot that was not treated with nanofertilizers (negative control) showed the lowest germination percentage (60.5%). T1 (1×10^7 CFU mL^{-1} of *S. cerevisiae* IIUI-565) and T2 (30 μM of urea-Zn-DAP NFs) treatments showed 85 to 90% seed germination percentages, respectively, while the pot without *S. cerevisiae* IIUI-565 applications showed 70.7% seed germination.

The analysis of "relative water content" revealed a statistically significant Tukey's p-value for the post hoc test below 0.0001 (ANOVA, $F (12, 40) = 755.6$, $p < 0.0001$), showing an interaction between various treatments and time, as shown in (Fig. 5g, Supplementary Table 8). There were notable variations across different periods during the 60 days, showing a substantial interaction effect. The use of *S. cerevisiae* IIUI-565 (1×10^7 CFU mL^{-1}) and urea-Zn-DAP nanofertilizers (30 μM) in various treatments had a promising impact on the relative content of water of the radish plant. Higher concentrations of these components resulted in reduced water content of leaf tissue. Figure 5g shows that the water content in radish leaf tissue fluctuated significantly across treatment levels. Radish leaf tissue exhibited significant fluctuations in water content based on treatment levels. *S. cerevisiae* IIUI-565 (1×10^7 CFU mL^{-1}) and urea-Zn-DAP nanofertilizers (30 μM), which were comparable to the 37.5% observed in T2 (urea-Zn-DAP NFs) under optimal conditions. In comparison, the NC group (negative control) showed the lowest percentage at 17.857%.

Discussion

Global food security is increasingly threatened by soil degradation and the depletion of agricultural resources. Nevertheless, chemical fertilizers are indispensable for sustaining crop productivity in a world with a burgeoning population, given their association with nutrient loss, soil fixation, and water pollution resulting from improper application [35]. However, nano-based fertilizers improve plant growth more efficiently than conventional fertilizers [36]. Therefore, the current study highlights the production of urea-Zn-DAP, a novel, highly versatile green nanofertilizer, specifically to improve radish seed germination. Urea-Zn-DAP NFs were synthesized using a wide range of biomolecules as reducing and capping agents, employing extracts from indigenous herbal spices. Plant probiotic *S. cerevisiae* IIUI-565 pertains to beneficial microorganisms on the soil surface to improve plant health and growth. On the other hand, green-synthesized urea-Zn-DAP nanofertilizers exploit nanotechnology to deliver nutrients more efficiently, potentially reducing the environmental impact of traditional fertilizers. The combination of plant probiotic *S. cerevisiae* IIUI-565 and urea-Zn-DAP NFs was used to boost plant development.

In this study, indigenous mixed herbal spices were used for the first time to synthesize the urea-Zn-DAP nanofertilizers. The change in colour from light brown to pale yellow-grey indicates the reduction of N, Zn, and P during the green synthesis of urea-Zn-DAP NFs, as reported previously [37]. For this purpose, GCMS analysis was performed, which showed 86 different reducing and capping agents in the methanolic extract of mixed herbal spices. Eugenol and caryophyllene are the most prominent phytocompounds in our extract. However, a previous study by [38] found that clove oil contains 20 distinct components, with caryophyllene and eugenol accounting for 93% and 4.80% of the total, respectively, based on GC-MS analysis.

The synthesized urea-Zn-DAP nanofertilizers were characterized by UV-Vis spectroscopy, FTIR, XRD, and SEM. The UV-vis spectroscopy shows absorption peaks ranging between 190 and 800 nm, indicating the formation of urea-Zn-DAP NFs. In a similar study by [39], they reported a significant absorption peak at 360 nm in their investigation of biological ZnO nanoparticles produced using *W. coagulans*. Tauc plots show direct band gaps of 3.43 eV for green ZnO NFs [40]. The outcomes revealed that green-synthesized urea-Zn-DAP nanofertilizers raised the energy band gap because they can absorb a greater amount of light from the whole spectrum of light. According to previous research, the existence of the ZnO stretching vibration confirms the creation of the ZnO structure [41]. Likewise, a wide range of absorption spectra at different wavelength confirm successful conjugation of *S. cerevisiae*, urea and DAP with ZnO NPs. The irregular patterns of urea-Zn-DAP nanofertilizers indicated that a large surface area may have triggered particle aggregation [42]. Structural morphology using SEM confirmed varied size and shape of synthesized nanofertilizer that might be due to a range of reducing agents in mixed herbs for the reduction of urea, zinc and DAP. This structural diversity leads to wide range of applications in agriculture to improve plant growth [43].

Seed germination is a critical developmental stage that is highly sensitive to nutrient availability and beneficial microbial interactions. In this study, the combined application of the plant probiotic *S. cerevisiae* IIUI-565 and ultra-small urea-Zn-DAP nanofertilizers improved radish seed germination, compared to untreated plants. These synergisms further reduce the toxicity of nanofertilizer due to its biobased active compounds.

The radish plant's shoot and root length, fresh and dry mass, leaf area, percentage of germination, and relative water content all increased significantly due to the foliar spray method compared to plants with no treatment. The synergistic effect is attributed to known growth-promoting effects of *S. cerevisiae* (especially rhizosphere) and ultra-small size of urea-Zn-DAP nanofertilizer (~ 0.682 nm), which likely facilitates rapid root uptake, increased bioavailability, and improved nutrient solubilization and translocation during early seedling metabolism. Collectively, these results demonstrate that integrating ultra-small nanofertilizers with a plant probiotic consortium is more effective than individual applications, particularly during early growth phases. The findings highlight the potential of nano-microbial consortia as a sustainable and efficient strategy for improving crop growth under greenhouse conditions.

Plant physiology, NF composition, NP size, and cell wall pore diameter (5–20 nm) all influence how nutrients released from NFs are transported and accumulate in plants [44]. Previous studies [45] have evaluated various methods of applying NPs, including hydroponics, direct soil application, seed priming, and seed coating. They have found that foliar application is the most effective method for plant growth and biofortification under both normal and abiotic stress conditions [46]. Foliar application of NFs has been seen to raise the concentration of the corresponding micronutrients in wheat, rice, and barley, resulting in improved quality and yield [47]. Through the stomata or cuticle of the leaf, foliar-applied NPs penetrate the cells. They are subsequently carried into the seeds via apoplastic and symplastic pathways, where they enter the vascular system [48].

In the present study, seed germination was ensured manually under both growing environments, and micronutrients (urea, Zn, and DAP) were applied as a nanofertilizer through foliar fertigation in radish plants [49] to investigate their physio-biochemical role in improving radish growth and development. Similar study by [50] confirmed similar findings for cucumbers, while [51] and [52] disclosed similar outcomes for potatoes. Previous studies have shown contradictory findings, suggesting that higher soil concentrations of arsenic (As) (0 μM, 60 μM, and 120 μM) significantly reduced the growth of plant organic matter, pigments used for photosynthesis, and gas exchange features $P < 0.05$ [53]. The present results are consistent with those investigated by [54], who found that even at lower application levels of their traditional analogs, foliar spraying of nano-fertilizer, either alone or in combination with fertilizers, improved vegetable yields throughout critical crop growth periods. According to studies by [55], the application of superficial nano-fertilizer significantly improved plant growth metrics and wheat yield. Additionally, researchers suggested that zinc and nitrogen interact synergistically [56]. As zinc enhances nitrogen absorption, using nitrogen fertilizer with urea and ZnO nanoparticles simultaneously resulted in improved stalk length, leaf development, and root sprouting [57].

The physical characteristics of NFs, such as their small size (less than 100 nm), high surface area, reactivity, solubility, penetration capacity, and surface-to-volume ratio, allow controlled and targeted delivery and improve absorption compared with applying higher doses of micronutrients. The foliar and root application of *Saccharomyces cerevisiae* IIUI-565 and urea-Zn-DAP NFs, followed by their application as a nano-biofertilizer, boosted the plant growth in terms of length of the shoot and root, fresh and dry weight of the plant, area of the leaf, germination percentage, and relative water content percentage compared to the non-treated control group. This might be due to the increased content of N, Zn, and P in soil (Fig. 6).

Conclusion

This research assessed the synergistic interaction between a microbial-nanofertilizer combination that encompasses indigenous herbal spice extract-mediated urea-Zn-DAP nanofertilizers and *Saccharomyces cerevisiae* IIUI-565 (a plant probiotic), aiming to enhance radish seed germination and early developmental stages under controlled greenhouse conditions. The results provide preliminary evidence supporting the feasibility of this synergistic strategy. X-ray diffraction (XRD) analysis confirmed the purity and crystalline structure of the urea-Zn-DAP nanofertilizers. Scanning Electron Microscopy (SEM) images revealed an average particle size of 0.682 nm for the urea-Zn-DAP nanofertilizers, exhibiting diverse morphologies such as spherical, floral, needle-shaped, and nanoflakes, which were markedly affected by the indigenous mixed herbal spice extracts utilized during synthesis. Foliar application of *Saccharomyces cerevisiae* IIUI-565 at a concentration of 1×10^7 CFU/mL and ultra-small urea-Zn-DAP nanofertilizers at 30 μM significantly improved radish seed germination and early growth parameters after 10 days. The combined treatment T3 (*Saccharomyces cerevisiae* IIUI-565 and urea-Zn-DAP) resulted in the most substantial enhancements, including enhancements in shoot length (12 cm), root length (3.22 cm), fresh weight (5.8 g), dry weight (2.6 g), leaf area (1.5 cm^2), germination percentage (95.0%), and relative water content (44.03%), compared to both positive (PC) and negative controls (NC). This confirms a synergistic effect of the microbial-nanofertilizer consortium in fostering early plant establishment and physiological performance. These findings suggest that vegetative characteristics such as shoot and root length, fresh and dry biomass, leaf area, germination percentage, and water retention collectively contribute to enhanced radish germination. The results position *Saccharomyces cerevisiae* IIUI-565 and urea-Zn-DAP nanofertilizer consortium as a sustainable alternative to conventional chemical fertilizers, with substantial potential for application in other high-value crops to promote sustainable agricultural practices. Subsequent studies should investigate the prolonged impacts, optimal application rates, impact on soil health, and environmental risk mitigation strategies to maximize the benefits of utilizing *Saccharomyces cerevisiae* IIUI-565 and urea-Zn-DAP nanofertilizers in agricultural practices.

Limitations and Future Directions

This study was conducted using radish (*Raphanus sativus L*.) as a model species under controlled greenhouse conditions. Radish was deliberately selected owing to its rapid and uniform germination, well-documented sensitivity to nutrient amendments, and its established use as a standard crop indicator fertilizer-efficacy bioassay. Nevertheless, these controlled conditions inherently constrain the exploration of our findings to diverse crop species and open field environments. The translational relevance of this nano-microbial synergism (*S. cerevisiae* IIUI-565 and urea-Zn-DAP nanofertilizer) can be strengthened by incorporating multispecies evaluations, diverse soil matrices, and field-scale trials across varying agro-climatic regions.

Declarations

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CRediT authorship contribution statement

Musfirah Hanif: Writing – original draft, Methodology, Investigation, Visualization, Validation, Formal analysis, Data curation. **Bushra Uzair:** Writing – review & editing, Project administration, Conceptualization, Supervision. **Rashid Nazir Qureshi:** Resources (GC-MS, UV, FTIR, XRD, SEM), Formal analysis, Data curation. **Syeda Asma Bano:** Writing – review & editing, Visualization, Validation, Formal analysis **Zukhra Abbasi:** Methodology, Writing–review & editing, Investigation, Formal analysis, Visualization, Validation, Data curation. **Nazia Jamil:** Resources, Funding acquisition, Visualization. **Apori Samuel Obeng:** Funding acquisition, Resources. **Furong Tian:** Funding acquisition, Resources, Validation.

Data availability

All data generated or analysed during this study are included in this article and its supplementary information files.

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Figures

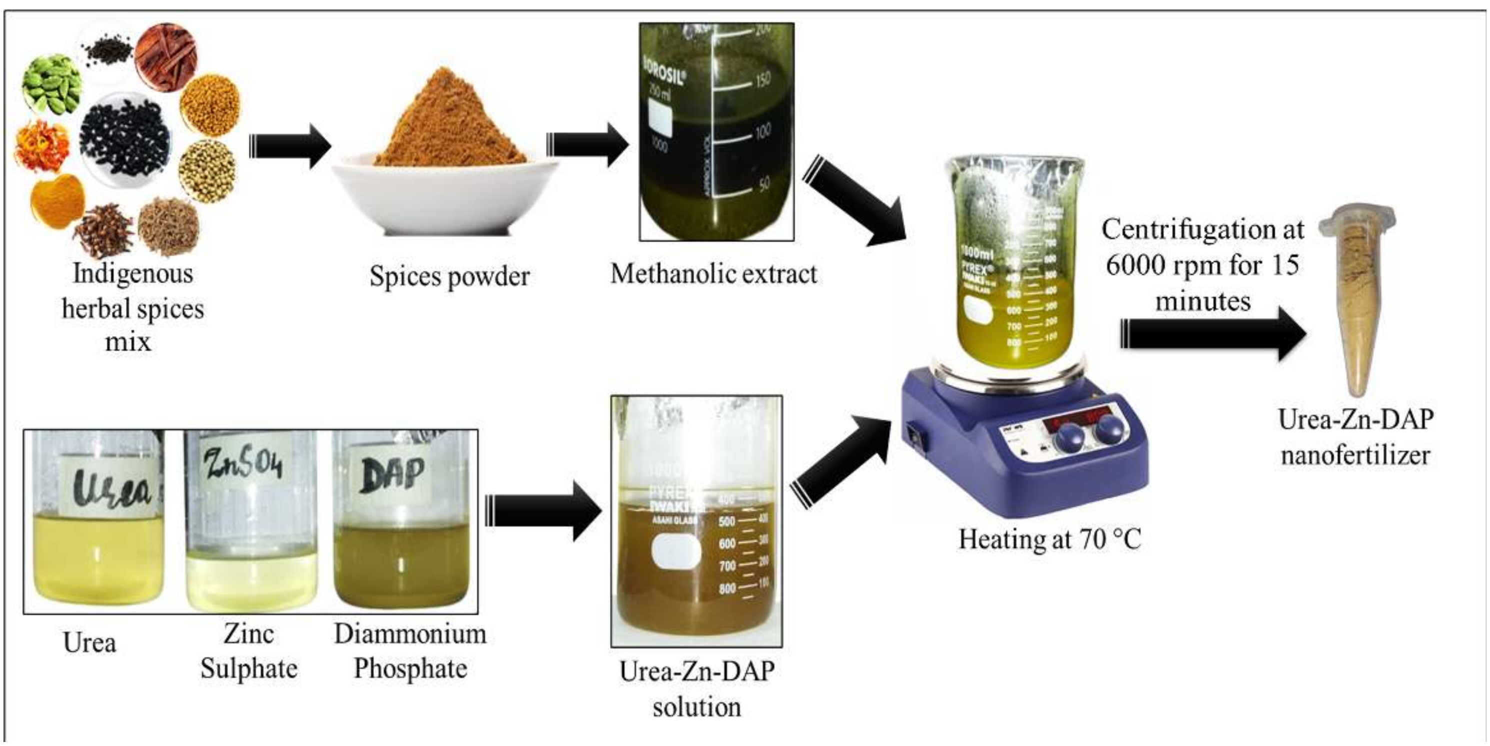

Figure 1

Diagrammatic illustration of green synthesized urea-Zn-DAP-based nanofertilizers.

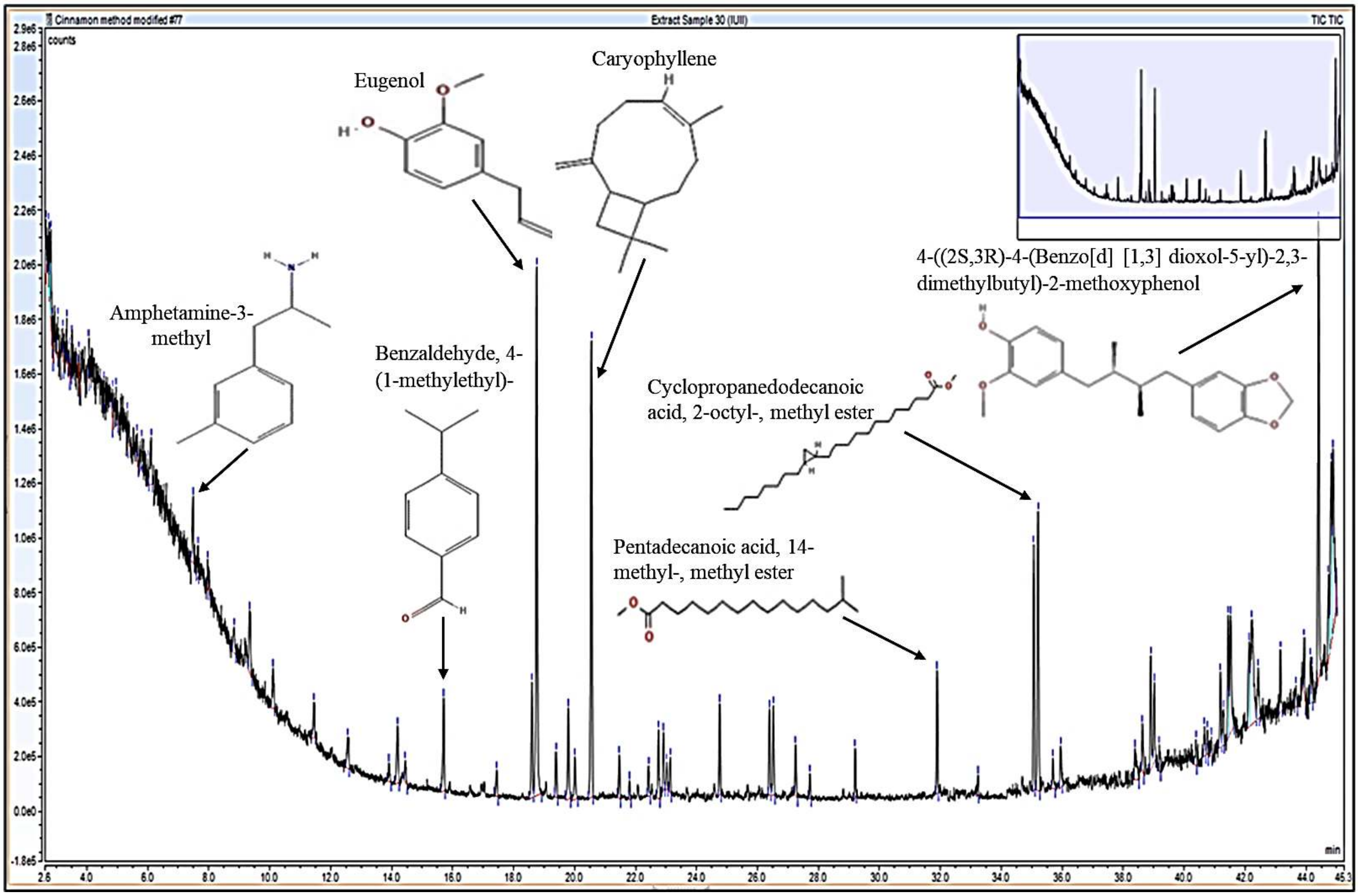

Figure 2

GC-MS chromatogram of the indigenous herbal spices extract used for green NFs synthesis.

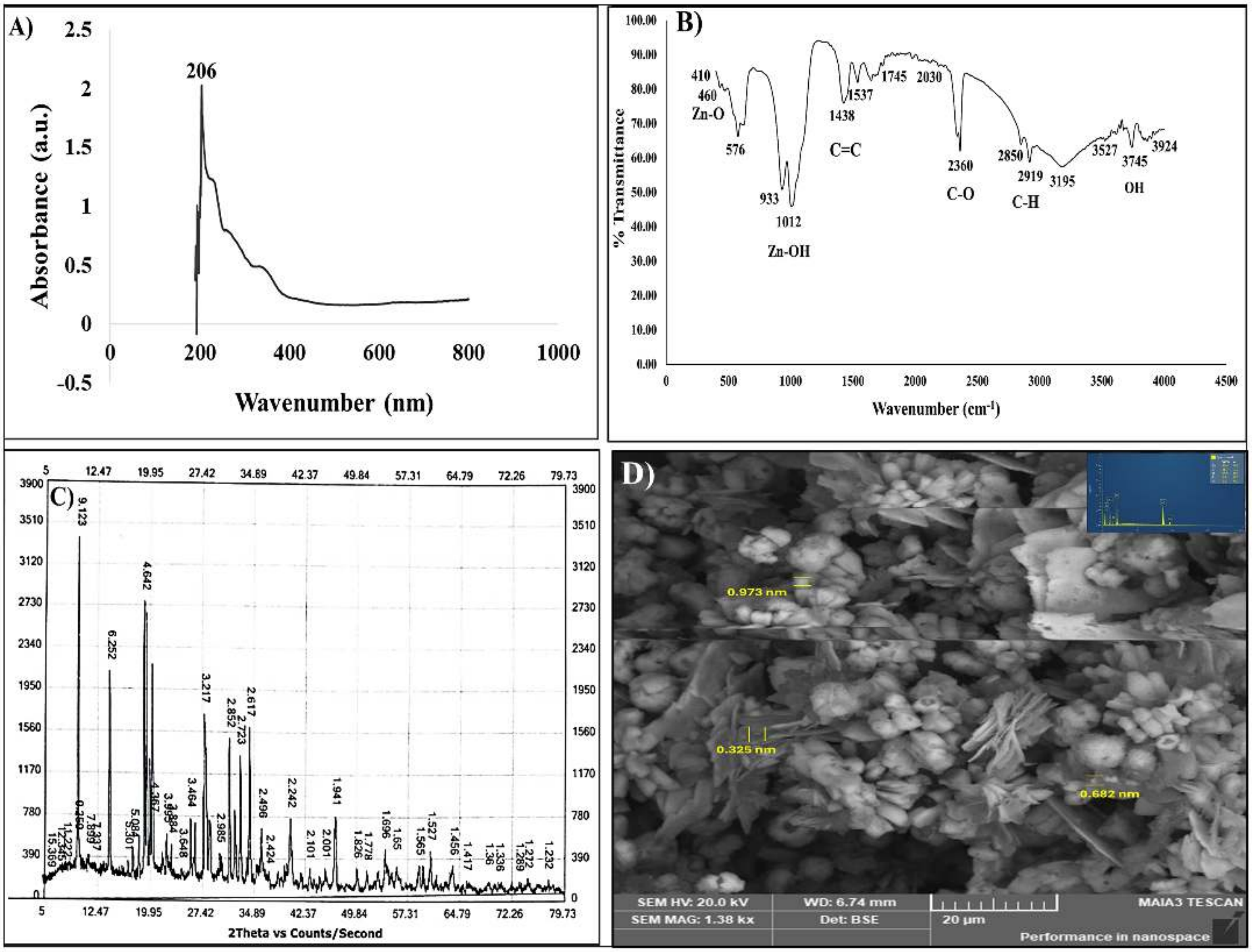

Figure 3

Characterization of indigenous herbal spices extract-based ultra-small-sized Urea-Zn-DAP NFs. **A)** UV spectroscopy, **B)** FTIR analysis, **C)** XRD spectrum, **D)** SEM with EDX profile.

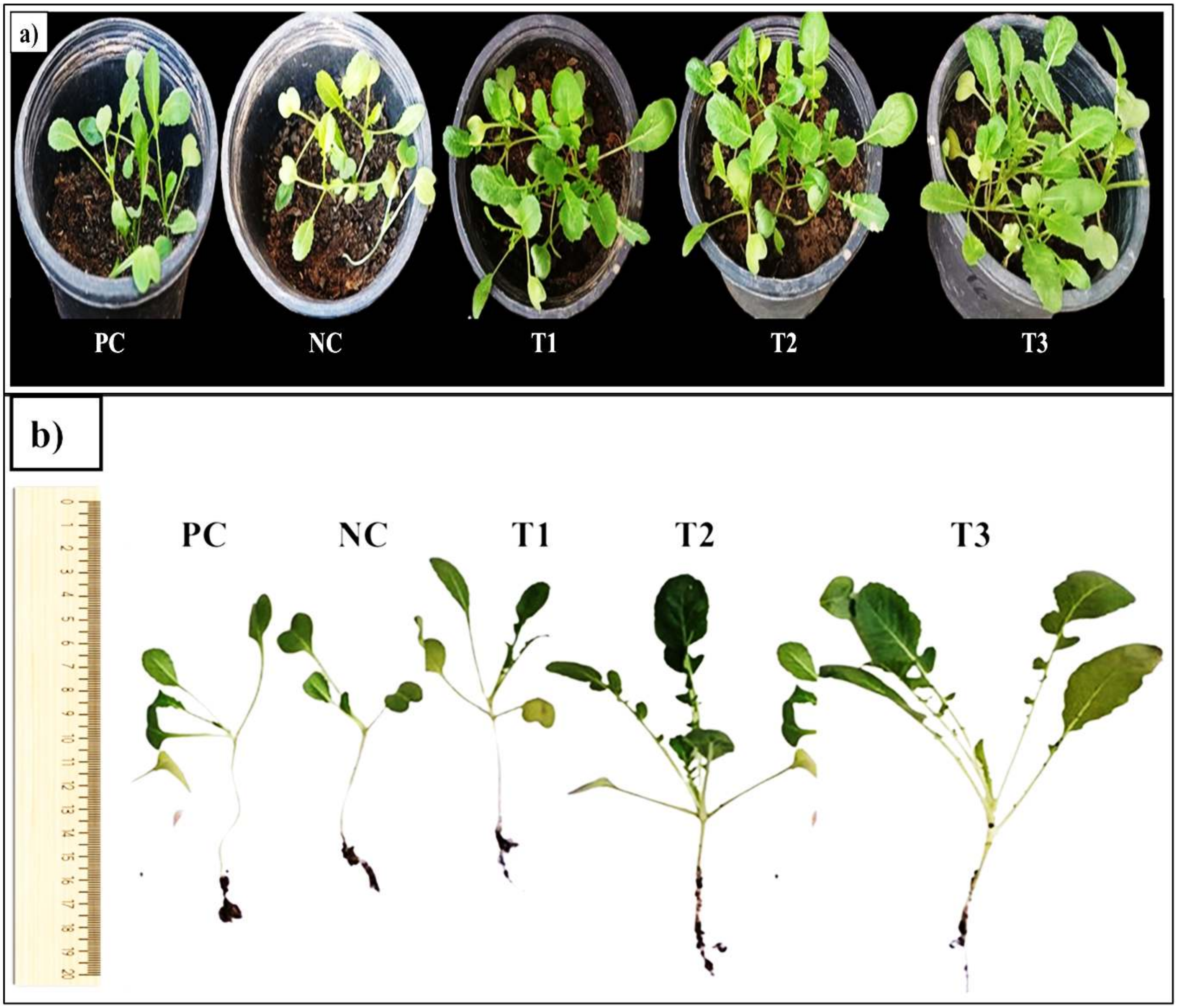

Figure 4

Effect of the *S. cerevisiae* IIUI-565 (1×10^7 CFU mL^{-1}) and urea-Zn-DAP nanofertilizers on the 60th day of seed germination in a greenhouse (**a**) plant growth under potted conditions and (**b**) *in vitro* seedling growth.

Key: PC (Positive control: conventional urea 30 μM), **NC** (soil without urea-Zn-DAP), **T1** (soil + *S. cerevisiae* IIUI-565, 1×10^7 CFU mL^{-1}), **T2** (soil + urea-Zn-DAP NFs 30 μM), **T3** (soil + *S. cerevisiae* IIUI-565 1×10^7 CFU mL^{-1} + urea-Zn-DAP NFs 30 μM).

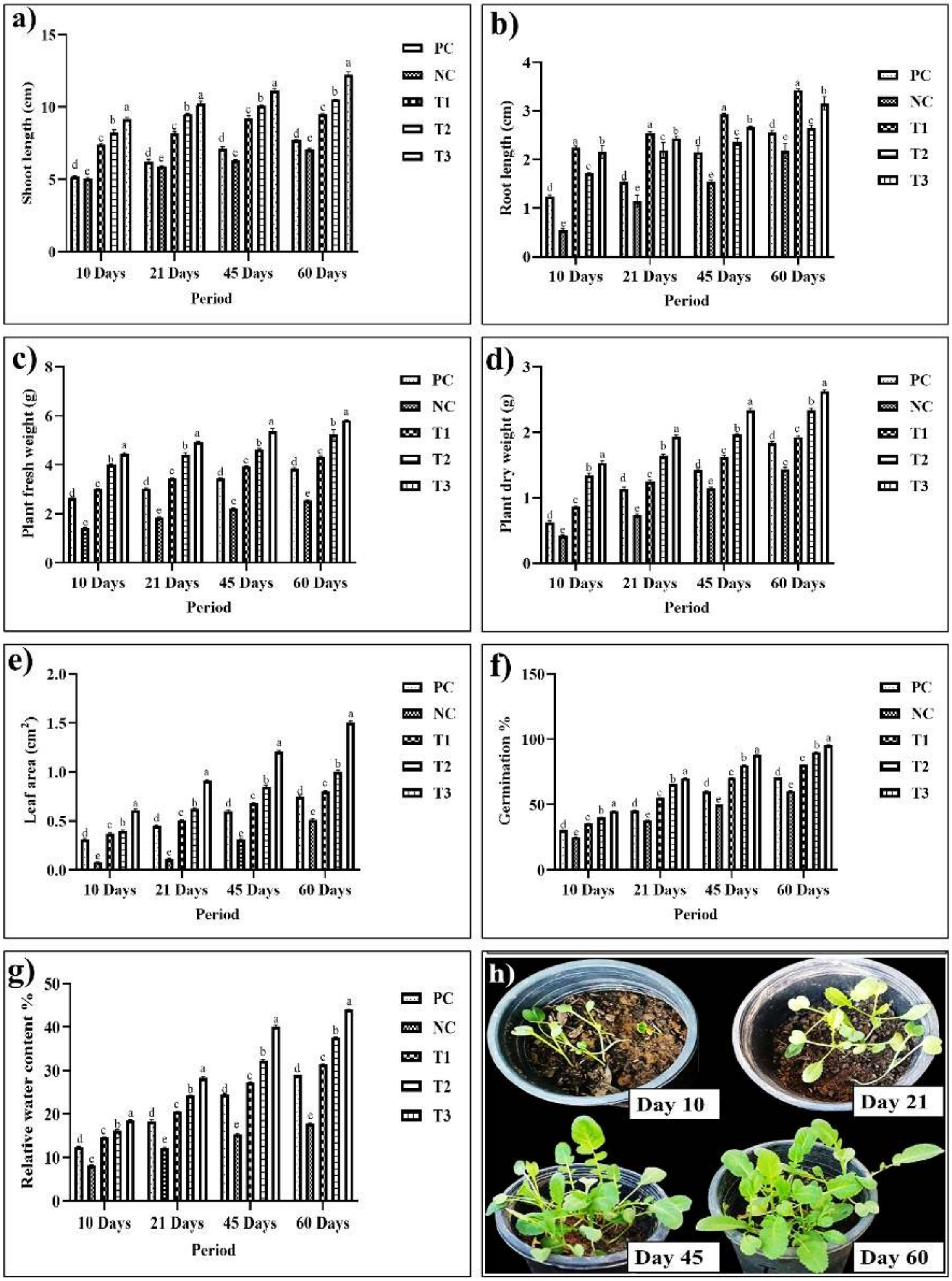

Figure 5

Effect of microbial-nanofertilizer combination of *S. cerevisiae* IIUI-565 (1×10^7 CFU mL^{-1}) and urea-Zn-DAP (30 μM) on radish crop germination at various time intervals.

Key: (a) Shoot length (cm), (b) Root length (cm), (c) Plant fresh weight (g), (d) Plant dry weight (g), (e) Leaf area (cm^2), (f) Germination % (g) Relative water content (h) T3 (*S. cerevisiae* IIUI-565 (1×10^7 CFU mL⁻¹) and urea-Zn-DAP NFs 30 μM composite). The lowercase characters on the variance bars indicated substantial distinctions between treatments.

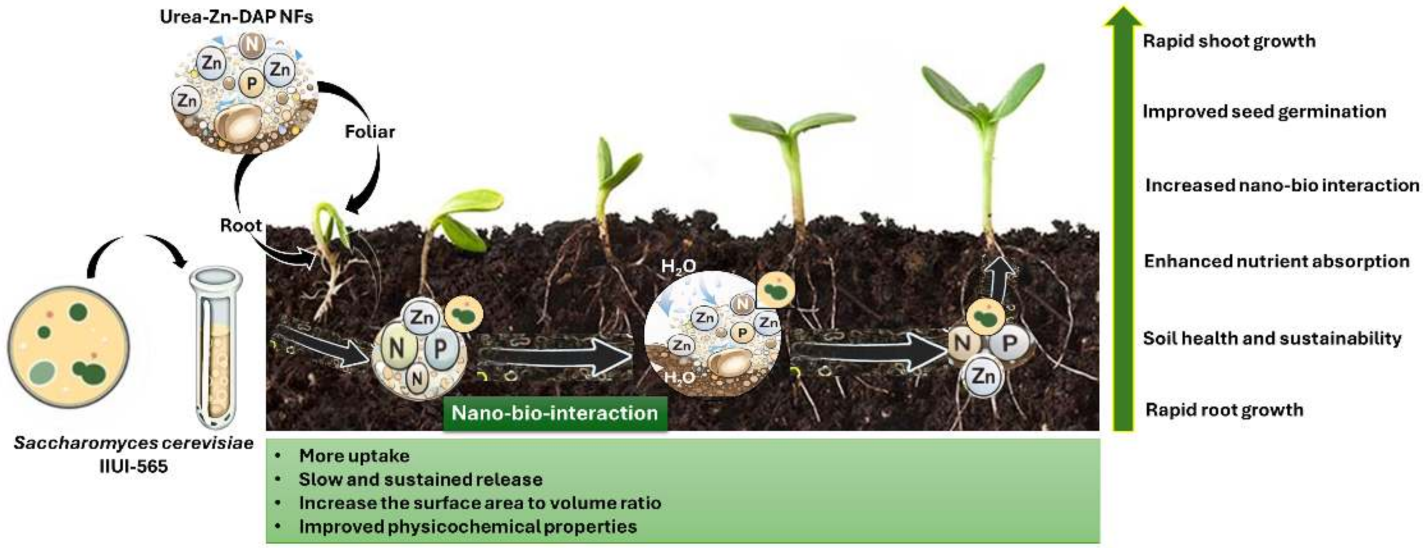

Figure 6

Hypothetical mechanism of the moisture-induced controlled nutrient release from urea-Zn-DAP nanofertilizers combined with *Saccharomyces cerevisiae* IIUI-565 and its role in improving radish seed germination.

Supplementary Files

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