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Chemo-Blended Ag & Fe Nanoparticles Effect on Growth, Physiochemical and Yield Traits of Wheat (*Triticum aestivum*)

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Abstract: The application profile of nanotechnology is increasing due to its influential effects on the environment. Recently, this field has gained tremendous magnitude in the agriculture sector as a potential improving agent for plant growth, slow-release fertilizer, and targeted delivery of agrochemicals for sustainable crop productions. A study was designed with the aim to explore the potential effects of nanoparticles mixed with organic chemicals on the growth and physiochemical properties of wheat. Synthesized silver NPs and iron NPs were characterized through SEM and a particle analyzer, which confirmed the fine particles of a size < 20 nm. The application of chemo-blended NPs enhanced plant height, shoot and root biomass and leaf area. Chlorophyll (a, b) and total chlorophyll contents were promoted with an application of blended NPs. Chemo-blended nanoparticles promoted total soluble sugars, total free amino acid contents and total protein contents of wheat. Antioxidant enzyme activities, such as superoxide dismutase, peroxidase and catalase were significantly promoted with blended NPs. Yield related attributes were also promoted in response to nanoparticles blended with organic chemicals. These results suggest that the application of chemo-blended NPs may increase plant growth and development through the improvement of the physiochemical properties of wheat.

Keywords: chemo-blended NPs; antioxidant enzyme activities; wheat

Citation: Jhanzab, H.M.; Qayyum, A.; Bibi, Y.; Sher, A.; Hayat, M.T.; Iqbal, J.; Qamar, M.; Elesawy, B.H.; Ismail, K.A.; Gharib, A.F.; et al. Chemo-Blended Ag & Fe Nanoparticles Effect on Growth, Physiochemical and Yield Traits of Wheat (*Triticum aestivum*). *Agronomy* **2022**, *12*, 757. https://doi.org/10.3390/agronomy12040757

Academic Editor: Ștefan-Ovidiu Dima

Received: 8 February 2022
Accepted: 20 March 2022
Published: 22 March 2022

1. Introduction

The demand for world food production is increasing for feeding over increasing populations. There is a dire need to increase food production by 70% by 2050 [1]. The world is demanding economically sound and eco-friendly agricultural development against population intensification and natural resources depletion [2]. Innovations in nanotechnology have provided solutions to many challenges through a sustainable, efficient, and

resilient system of agriculture [3]. Food safety and quality enhancement, the minimization of agricultural inputs, efficient utilization of nutrients from the soil and increasing the capability of plants to absorb more nutrients are some of the possible uses and benefits of this technology [4]. Usage of nanoparticles is increasing, however further exploration for the synthesis of novel and biodegradable agrochemicals to ensure food security is needed.

Nanoparticles are characterized by their small size <100 nm [5], and owing to a larger surface to volume ratio, modified their physicochemical distinctiveness in comparison with bulk material [6]. Due to its unique properties, the synthesis and utilization of NPs have raised serious concerns about their impacts on the ecosystem [7]. The size and concentration of NPs are responsible for interactions with other materials to cause toxic and adverse effects [8,9]. Plants, as a primary organism of the ecosystem, accumulate and translocate nanoparticles and can suffer oxidative stress through the production of reactive oxygen species [10]. Therefore, the potential impacts of nonmaterial on the environment should be carefully analyzed.

Previous studies reported phytostimulatory and inhibitory effects. Amongst the range of NPs, Ag NPs are widely studied nanoparticles due to their inimitable properties, such as antimicrobial applications, water purification and coating material for steel products [11]. Silver NPs increased germination and growth of *Boswellia ovalifoliolata* significantly [12]. Nanoparticles induced changes in numerous metabolic pathways that affect plant growth and succeeding development. Plant growth and biochemical attributes, such as protein and carbohydrates formation, antioxidant enzyme activities and chlorophyll contents were enhanced as a result of Ag NPs treatment in wheat and asparagus [13,14]. Silver nanoparticles promoted the root and shoot length of *Phaseolus vulgaris* and *Zea mays* [15] and increased superoxide dismutase activity in *Lycopersicon esculentum* [16]. Morphological characters of cucumber [17], carbohydrates, protein contents, SOD and CAT activities of bacopa plants were promoted in response to Ag NPs [18]. Nanophosphorous fertilizer improved photosynthetic activity and nutrient use efficiency (NUE), ultimately enhancing the quality and yield of rice [19].

Iron (Fe) is a very important element for plant growth mechanisms, such as photosynthesis, respiration, cell metabolism and enzyme activities [20]. Iron oxide NPs promoted photosynthesis by increasing chlorophyll contents and biochemical and enzyme activities in different stages of light reactions [21]. Foliar application of iron nanoparticles increased leaf weight, pod weight and soybean yield [22]. Plant metabolic processes, such as the facilitation of the transfer of photosynthates, increased with nano-calcium carbonate NPs in peanut leaves [23]. Total chlorophyll, grain iron contents and wheat yield improved with iron oxide NPs [24,25].

Organic chemicals, such as nicotinic acid, tryptophan and myo-inositol have an imperative role in growth regulation and many other physiological functions. Exogenous application of nicotinic acid increased plant growth, protein synthesis [26] and enzyme activities. It also induced protection against oxidative stresses through DNA methylation [27]. Tryptophan acts as a physiological precursor of auxin, ultimately promoting growth, membrane stability index, relative water contents as well as yield for many crops [28–30]. Tryptophan increased the total soluble sugars, proteins and oil yield of thyme plants [31]. Myo-inositol helps with phosphate storage in seeds, translocation of messenger RNA [32], cell wall biosynthesis [33], cell to cell communication [34], transport and storage of phytohormones. Therefore, keeping in view of the above importance, the experiment was designed to determine the role of nanoparticles blended with organic chemicals on the growth, physiochemistry and yield of wheat. Individual effects of Ag NPs, Fe NPs and organic chemicals, such as nicotinic acid, tryptophan and myo-inositol have been reported. However, organic blended nanoparticles have not been reported yet.

2. Materials and Methods

2.1. Production of Ag Nano Particles (Ag NPs)

Production of Ag NPs was carried out through the reduction of silver nitrate with trisodium citrate dihydrate. A solution of 500 ppm of $AgNO_3$ (Sigma-Aldrich, Munich, Germany) and 300 ppm of $Na_3C_6H_5O_7$ (Merck, Darmstadt, Germany) were prepared. Prior to the mixing of both solutions, the $AgNO_3$ solution was heated at 80 °C on a hot plate for 10 min. Trisodium citrate solution was gradually added to the silver nitrate solution. Then, the solution was stirred at 7000× *g* (1500–1600 rpm) 80 °C for 1 h using a magnetic stirrer until a golden yellow color was attained [35]. Freshly prepared silver nanoparticles were analyzed through scanning electron microscopy (SEM) and a Zeta particle analyzer.

2.2. Production of Fe Nano Particles (Fe NPs)

Iron nanoparticles were prepared by reducing ferric chloride hexa-hydrate ($FeCl_3 \cdot 6H_2O$) through onion extract. Iron chloride solution was made by dissolving 968.37 mg of iron chloride per liter of de-ionized water. Boiling of the solution was carried out for 4 min and reduction was completed with the addition of onion extract (30 mL) until the color appeared a reddish-brown. The final product obtained was used as a stock solution (200 ppm).

2.3. Nanoparticles Characterization

The characterization of nanoparticles was carried out through SEM and a Zeta particle analyzer. The size of the nanoparticles was analyzed using a scanning electron microscope (SEM) from the University of Peshawar, Pakistan and the Zeta particle analyzer for the analysis was from the Nuclear Institute of Biology and Genetic Engineering (NIBGE) Faisalabad, Pakistan (Figure 1).

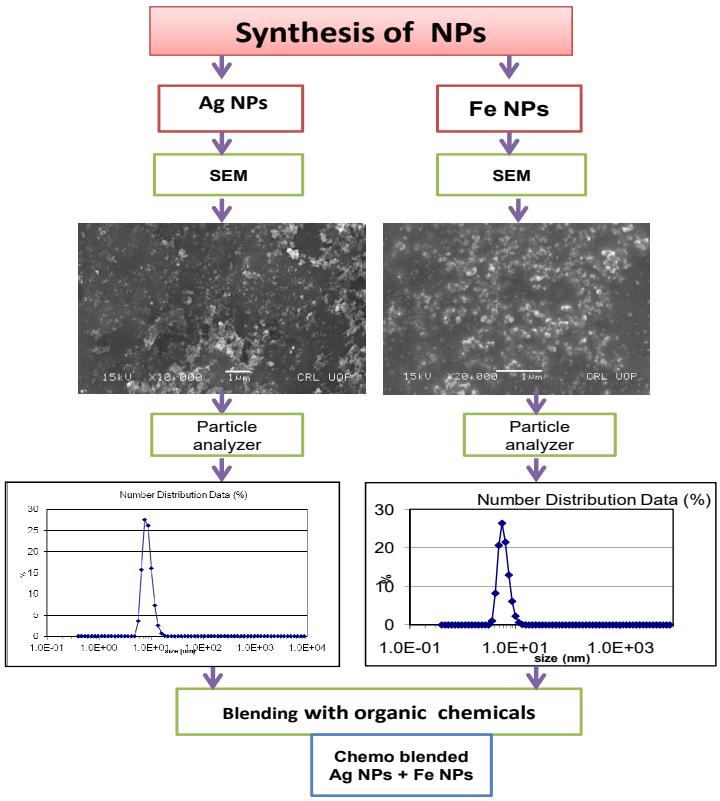

Figure 1. Synthesis, characterization and preparation of blended NPs. Ag NPs were synthesized through chemical reduction. Synthesis of Fe NPs was carried out through onion extract. Freshly prepared Ag NPs and Fe NPs were characterized through SEM and a Zeta particle analyzer. SEM analysis revealed fine NPs with less than 20 nm in size, while the Zeta particle analyzer also confirmed nanosized particles.

2.4. Experimental Procedure

The experiment was conducted to check the response of wheat growth to chemo-blended nanoparticles. Wheat variety Punjab-2011 was used in the experiment. Pre-sterilized seeds were sown in Petri dishes, followed by three layers of filter papers moistened with distilled water. After one week, ten healthy and equal length seedlings were transferred to plastic pots containing fertile soil. After a strong establishment of transferred seedlings, a foliar application of treatments was employed. Possible combinations of secondary optimization were prepared by mixing the nanoparticles with organic chemicals on the basis of best performing concentrations in primary optimization of Ag NPs, FeNPs and organic chemicals. A foliar spray of the treatments was employed after a strong establishment of transplanted seedlings at tillering stage. From previous experiments, best performing concentrations were selected and chemicals were properly mixed for making individual combinations. Ag NPs (5 ppm), nicotinic acid (10 ppm), tryptophan (25 ppm), myo-inositol (25 ppm) and Fe NPs (5 ppm) were blended according to combinations. Treatments included were (T1 = control (distilled water), T2 = Ag NPs + nicotinic acid + tryptophan, T3 = Ag NPs + tryptophan + myo-inositol, T4 = Ag NPs + nicotinic acid + tryptophan + myo-inositol, T5 = Ag NPs + nicotinic acid + tryptophan + myo-inositol + Fe NPs). A completely randomized design with three replicates was employed. To check the possible effects of blended NPs data on growth attributes, chlorophyll spad value, plant height (cm), shoot fresh and dry mass (g), root length (cm), root fresh and dry masses (g) were collected and analyzed for inferences of means. Physiological attributes, such as chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll), total amino acid contents, total soluble sugars and total soluble proteins, were analyzed. Antioxidant enzymes (SOD, CAT and POD) were also analyzed. Yield and its attributes, e.g., spike length, number of grains/spike, 100-grains weight and yield were also recorded.

2.5. Fresh and Dry Weight of Wheat Shoot

Fresh mass of shoot was recorded on an electrical balance. Shoots were dried out in an electric oven at 70 °C. Shoot dry weight was analyzed on an electrical balance after completely drying the sample.

2.6. Fresh and Dry Weights of Wheat Root

To estimate the root biomass, roots were cleaned and dried out with blotting papers. The fresh weight was recorded on an electronic balance. Air dried roots were dried out in an oven at 70 °C. Dried root mass was measured on an electronic balance.

2.7. Root Length (cm)

Randomly, three plants were chosen from every treatment and their length was recorded with a measuring scale (cm).

2.8. Plant Height (cm)

The height of the plants was analyzed after two weeks of treatment application with the measuring scale by selecting three representative plants from each treatment.

2.9. Leaf Area (cm^2)

Three representative plants were selected from each treatment and their leaf area was recorded with a leaf area meter.

2.10. Determination of Chlorophyll Contents

It was analyzed with the procedure described by [36]. Fresh leaves were added to the test tube containing 5 mL of ethanol (80%) and immersed thoroughly. Leaf extort was

heated in a water bath for ten minutes (80 °C). A dark room was used to cool the leaf extract. The optical densities were analyzed at 645 and 663 nm for chl "a" and "b", correspondingly.

$$\text{Chlorophyll a} = \frac{(12.7 \times \text{ OD at } 663) - (2.69 \times \text{ OD at } 645) \times \text{ V}}{1000 \times \text{ Shoot fresh weight (g)}}$$

$$\text{Chlorophyll b} = \frac{(22.9 \times \text{ OD at } 645) - (4.69 \times \text{ OD at } 663) \times \text{ V}}{1000 \times \text{ Shoot fresh weight (g)}}$$

$$\text{Total Chlorophyll} = \frac{(2.02 \times \text{ OD at } 643) + (8.02 \times \text{ OD at } 663) \times \text{ V}}{1000 \times \text{ Shoot fresh weight (g)}}$$

2.11. Total Soluble Sugars

Weighted fresh leaves were placed in the test tubes containing 5 mL of (80%) ethanol. Tubes were heated on a water bath for 1 h (80 °C). After that, a sample extract of 1 mL was used in new tubes and diversed with 1 mL of (18%) phenol and de-ionized water. After that, tubes were permitted to situate in the room's warmth for 60 min. As a final point, H_2SO_4 (5 mL) was added and vortexed. Absorbance was recorded on a spectrophotometer at 490 nm. For blank reading, 80% ethanol was used [37].

2.12. Total Free Amino Acid Contents

The amino acid contents were analyzed by following the protocol documented by [38]. The sample extract was mixed with 1 mL of (10%) pyridine and 1 mL of (2%) ninhydrin solution. The O.D of samples was recorded by a UV spectrophotometer at 570 nm.

2.13. Total Soluble Protein Contents

Weighted fresh leaf was cut into tiny pieces and added to test tubes. Phosphate buffer (5 mL) was added to the tubes. Pestle and mortar were used to grind the samples. The sample extract (0.5 mL) was used in an additional set of tubes and de-ionized water (0.5 mL) was added. Finally, Bio-Rad color dye (3 mL) was added and vortexed. Absorbance was recorded on a UV spectrophotometer at 595 nm. For the blank sample, phosphate buffer was used [39].

2.14. Super Oxide Dismutase (SOD) Activity

An amount of 10 mL of phosphate buffer was taken from test tubes with pH 7.0. A weighted 0.5 g of leaf sample was mashed in pre-chilled phosphate buffer. The centrifugation of solution was conceded out at 15,000× *g* (4 °C) for 15 min. The superfluent was mixed with SOD buffer (3 mL) and riboflavin (0.1 mL). The mixture was shaken and kept beneath a fluorescent lamp at 25 °C for 8 min. The color of the tubes turned from yellow to brown or black. One additional set of the same samples was prepared and placed in the dark. The wavelength of both tubes (dark and light) was recorded with a spectrophotometer at 560 nm [40].

2.15. Catalase (CAT) Activity

The known volume of the frozen sample was homogenized in 60 µL comprised of 1 mM of DTT and 50 mM of phosphate buffer. The centrifugation of samples was carried out for five minutes at 12,000× *g*. Enzyme extract of 100 µL and 1.9 mL of distilled water were used for the reaction mixture. Enzyme activity was analyzed by recording the absorbance at 240 nm for 3 min with a spectrophotometer [41].

$$\text{Catalase } (\mu\text{g/g}) = \frac{\text{A240 nm/min } \times 1000}{43.6 \times \text{ mg protein/mL reaction mixture}}$$

2.16. Peroxidase (POD) Activity

The POD activity was analyzed with the guaiacol oxidation technique documented by [42]. The extraction process was the same as SOD and CAT. The reaction mixture comprised of 1 mL sodium phosphate buffer, 0.95 mL of 0.2% guaiacol, 1 mL of 0.3% hydrogen peroxide, and 0.05 mL of enzyme extract. An absorbance of 470 nm for POD was recorded at 30 s (half-minute interval) up to 3 min with a UV-1600 spectrophotometer.

$$\text{POD } (\mu g/g) = \frac{\text{Abs. of sample at 470 nm } \times \text{ vol. sodium phosphate buffer}}{0.01 \times \text{ reaction time } \times \text{ wt. of sample } \times \text{ vol. sample extract}}$$

2.17. Response of Wheat Yield to Chemo-Blended Nanoformulations

To check the response of chemo-blended nanoformulations on wheat yield, wheat variety Punjab-2011 was used in the experiment. The healthy and even-sized seeds were separated and sterilized in 70% ethanol for 3 min. The sowing of seeds was carried out on Petri plates with three filter papers and distilled water was used to soak the filter paper. After one week, ten healthy and equal length plants were transferred to clay pots. Pots were filled with 15 kg of fertile soil. At the time of tillering, treatments were employed as a foliar spray. At maturity, data on spike length, grains per spike, 100-grains mass and yield were recorded.

3. Results

3.1. Effect of Blended NPs on Morphological Attributes of Wheat

The experiment was conducted to evaluate the growth of wheat in response to nanoparticles mixed with organic chemicals. In this experiment, possible combinations were formulated from the best performing concentrations of silver nanoparticles, nicotinic acid, tryptophan, myo-inositol and iron nanoparticles. Pre-sterilized seeds of Punjab-2011 were germinated in the lab and plants were transplanted to pots crammed with fertile soil. Foliar application of blended NPs was employed when seedlings were well established. Data pertaining to the growth parameters are presented in Table 1. The highest chlorophyll spad value was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos as compared with the control. The application of blended nanoparticles significantly promoted leaf area. Leaf area was recorded at its maximum with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos.

Table 1. Effect of different treatments of blended NPs on morphological attributes of wheat.

Treatments	Chlorophyll (Spad Value)	Leaf Area (cm^2)	Plant Height (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Length (cm)	Root Fresh Weight (g)	Root Dry Weight (g)
Control	40.4 ± 0.88 b	14.9 ± 0.67 c	29.43 ± 0.33 c	0.79 ± 0.11 c	0.35 ± 0.69 c	12.71 ± 0.89 e	0.49 ± 0.03 d	0.09 ± 0.01 b
AgNPs+N.A+Try	45.4 ± 0.97 ab	19.5 ± 0.76 b	34.56 ± 0.31 b	2.41 ± 0.14 b	0.67 ± 0.08 a	15.23 ± 0.87 d	1.32 ± 0.10 ab	0.11 ± 0.01 b
AgNPs+Try+myo-inos	44.6 ± 1.18 ab	21.1 ± 0.51 ab	34.50 ± 0.47 b	2.35 ± 0.17 b	0.42 ± 0.05 b	16.10 ± 0.93 c	1.043 ± 0.11 c	0.13 ± 0.01 b
AgNPs+N.A+Try+myo-inos	43.8 ± 0.88 ab	22.0 ± 0.48 ab	35.46 ± 0.45 b	2.9 ± 0.18 a	0.72 ± 0.08 a	16.83 ± 1.01 b	1.21 ± 0.14 bc	0.12 ± 0.01 b
AgNPs+N.A+Try+myo-inos+FeNPs	46.9 ± 1.27 a	23.7 ± 0.41 a	38.63 ± 0.59 a	2.80 ± 0.20 a	0.71 ± 0.08 a	17.63 ± 1.11 a	1.52 ± 0.14 a	0.22 ± 0.02 a
LSD	5.04	6.912	1.774	0.608	0.58	0.418	0.386	0.055

The data are presented as mean (±SE) from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test ($p \leq 0.05$).

Data regarding plant height in response to different treatments are presented in Table 1. The height of the plants' maximum response was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos. The minimum response was recorded with the control. Shoot fresh weight was recorded as the maximum value with AgNPs+N.A+Try+myo-inos followed by AgNPs+N.A+Try+myo-inos+FeNPs. The minimum value of the plant height was recorded with the control.

Dry mass of shoot was recorded at its maximum with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos. The minimum was recorded with the control. The highest root length was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos. The maximum root fresh weight was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try. The minimum was recorded with the control. Similarly, the maximum root dry weight was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+Try+myo-inos. The minimum trend was recorded with the control.

3.2. Effect of Chemo-Blended Nanoparticles on Chlorophyll Contents of Wheat

Data regarding the effect of blended nanoformulations on the chlorophyll contents of wheat is presented in Table 2. The results indicate highly significant differences among various treatments. The highest chlorophyll a was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+Try+myo-inos. Similarly, chlorophyll b and total chlorophyll contents' maximum were obtained with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos, as compared with the control.

Table 2. Effect of different treatments of blended NPs on chlorophyll contents of wheat.

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
Control	0.17 ± 0.01 b	0.20 ± 0.01 c	0.37 ± 0.02 d
Ag NPs+N.A+Try	0.31 ± 0.02 a	0.27 ± 0.01 abc	0.58 ± 0.03 c
AgNPs+Try+myo-inos	0.33 ± 0.02 a	0.24 ± 0.01 bc	0.57 ± 0.03 bc
AgNPs+N.A+Try+myo-inos	0.27 ± 0.02 ab	0.35 ± 0.02 ab	0.62 ± 0.03 b
AgNPs+N.A+Try+myo-inos+FeNPs	0.36 ± 0.02 a	0.36 ± 0.02 a	0.72 ± 0.03 a
LSD	0.7538	0.1174	0.0918

Data are presented as mean (±SE) from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test ($p \leq 0.05$).

3.3. Effect of Chemo-Blended Nanoparticles on Physiological Attributes of Wheat

To assess the physiological attributes of wheat, an experiment was conducted to evaluate different blended formulations of nanoparticles. Data pertaining to the physiological parameters of wheat is presented in Table 3. The maximum total free amino acid content was recorded with AgNPs+N.A+Try+myo-inos followed by AgNPs+N.A+Try+myo-inos+FeNPs. The minimum response was recorded with the control.

Table 3. Effect of different treatments of blended NPs on physiological parameters of wheat.

Treatments	Total Free Amino Acid Contents (mg/g)	Total Soluble Sugars (mg/g)	Total Soluble Proteins (mg/g)
Control	0.31 ± 0.11 c	1.2167 ± 0.77 c	0.22 ± 0.01 c
Ag NPs+N.A+Try	0.59 ± 0.13 ab	3.0567 ± 0.84 ab	0.35 ± 0.01 b
Ag NPs+Try+myo-inos	0.47 ± 0.10 bc	2.6367 ± 0.81 bc	0.34 ± 0.01 b
Ag NPs+N.A+Try+myo-inos	0.853 ± 0.10 a	2.223 ± 0.80 bc	0.32 ± 0.01 b
Ag NPs+N.A+Try+myo-inos+Fe NPs	0.590 ± 0.12 ab	3.180 ± 0.85 a	0.64 ± 0.02 a
LSD	0.277	1.5034	0.4539

The data are presented as mean (±SE) from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test ($p \leq 0.05$).

The application of different blended formulations greatly affected the physiological attributes of wheat. A maximum total soluble sugar was recorded with AgNPs+N.A+Try+myo-inos+Fe NPs followed by AgNPs+N.A+Try+myo-inos. Similarly, total soluble proteins were recorded with Ag NPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try.

3.4. Effect of Blended Nanoformulations on Antioxidant Enzyme Activity

The response of different antioxidant enzyme activities to the application of blended nanoparticles is presented in Table 4. Antioxidant enzyme activities were promoted with an application of blended nanoparticles. The maximum superoxide dismutase activity was recorded with the application of AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+Try+myo-inos. The minimum antioxidant enzyme activity was recorded with the control. Catalase activity was highest recorded with the application of AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Tryp+myo-inos. The minimum response was recorded with the control. Significantly higher peroxidase activity was obtained with AgNPs+N.A+Tryp followed by AgNPs+N.A+Tryp+myo-inos+FeNPs. The minimum antioxidant enzyme activity was recorded with the control where no treatment was applied.

Table 4. Effect of different treatments of blended NPs on antioxidant enzyme activities of wheat.

Treatments	SOD (µg/g)	Catalase (µg/g)	POD (µg/g)
Control	2.783 ± 0.71 c	0.941 ± 041 c	0.333 ± 0.11 c
Ag NPs+N.A+Try	3.29 ± 0.79 bc	1.3731 ± 0.51 bc	1.32 ± 0.27 a
Ag NPs+Try+myo-inos	5.39 ± 0.89 ab	1.8767 ± 0.55 ab	0.633 ± 0.13 b
Ag NPs+N.A+Try+myo-inos	3.22 ± 0.75 c	2.3167 ± 0.59 a	0.687 ± 0.13 b
Ag NPs+N.A+Try+myo-inos+Fe NPs	5.93 ± 0.94 a	2.5433 ± 0.61 a	0.741 ± 0.13 a
LSD	2.1459	0.8082	1.6106

The data are presented as mean (±SE) from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test ($p \leq 0.05$).

3.5. Effect of Chemo-Blended Nanoparticles on Yield and Yield Attributes of Wheat

Different blended nanoparticles enhanced the growth, physiological and yield parameters of wheat. The data pertaining to yield attributes are presented in Table 5. The maximum spike length was recorded with AgNPs+Try+myo-inos followed by AgNPs+N.A+Try+myo-inos+FeNPs. Different blended formulations greatly affected the grains/spike of wheat. The maximum grains/spike was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos. The 100-grains weight was also affected with an application of different blended formulations. The weight of grains was highest recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by Ag NPs+Try+myo-inos. The yield of wheat was greatly affected by the application of blended nanoparticles. The maximum yield was recorded with AgNPs+N.A+Try+myo-inos+FeNPs followed by AgNPs+N.A+Try+myo-inos in comparison with the control.

Table 5. Effect of different treatments of blended NPs on yield of wheat.

Treatments	Spike Length (cm)	No. Grains/Spike	100-Grains Weight (g)	Yield (g)
Control	11.43 ± 1.19 c	38.07 ± 1.05 d	3.562 ± 0.33 b	19.75 ± 1.67 b
Ag NPs+N.A+Try	15.06 ± 1.32 ab	40.4 ± 1.19 cd	4.78 ± 0.47 a	24.23 ± 1.93 b
AgNPs+Try+myo-inos	15.7 ± 1.24 a	41.4 ± 1.22 bc	4.90 ± 0.51 a	21.325 ± 1.85 b
AgNPs+N.A+Try+myo-inos	15.08 ± 1.22 ab	43.46 ± 1.60 b	4.74 ± 0.71 a	32.12 ± 2.21 a
AgNPs+N.A+Try+myo-inos+FeNPs	14.47 ± 1.15 b	45.00 ± 1.67 a	4.93 ± 0.82 a	35.6 ± 2.56 a
LSD	0.8160	2.7163	0.2599	6.2142

The data are presented as mean (±SE) from three independent biological replicates. Mean representative values with different letters are significantly different according to Tukey's multiple range test ($p \leq 0.05$).

4. Discussion

The experiment was conducted to estimate the potential role of Ag NPs and Fe NPs mixed with organic chemicals on wheat growth attributes. Wheat growth parameters were significantly promoted with the foliar application of different treatments in comparison with the control. Advancements in nanotechnology have led to an increase in the production and utilization of NPs. Nanoparticles, as magic bullets, possess unique physiochemical properties and have tremendous effects on the biological system.

4.1. Characterization of NPs

Freshly prepared Ag NPs and Fe NPs were characterized through a scanning electron microscope (SEM) and a Zeta particle analyzer. Silver NPs were chemically synthesized through the reduction of $AgNO_3$ with $Na_3C_6H_5O_7 \cdot 2H_2O$. Results depicted that NPs were spherical, uniform in shape, and the size ranged from 15–20 nm (Figure 1). Similarly, Zeta particle analyzer peaks revealed that Ag NPs were <20 nm in size (Figure 1). The chemical reduction method is efficient for the preparation of exact size NPs with the same physiochemical properties. Ag NPs were prepared through chemical reduction and their size was confirmed through X-ray diffraction analysis (10–20 nm) [14]. In a related study, the same procedure was followed, and size was established through X-ray diffraction analysis (<20 nm) [35].

Iron NPs were synthesized through onion extract as a reducing agent. Synthesized Fe NPs were analyzed through SEM and a Zeta particle analyzer (Figure 1). From both results, it was confirmed that Fe NPs were <20 nm in size with regular symmetry and a round shape. Green synthesis of Fe NPs was carried out and analyzed through SEM and EDX. It was reported that NPs were spherical in shape and their size ranged between 20–30 nm [43]. Many methods were proposed for the production of nanoparticles, such as biological, chemical, and physical. In the chemical reduction method, metal in the solution was reduced in such a way that no metal ions were present in the system. The chemical reduction method comparatively produced a low cost, high yield and easy synthesis of nanoparticles [44]. Therefore, together, the nanoparticles produced were very fine in size and shape.

4.2. Effect of Chemo-Blended NPs on Growth and Physiochemical Properties of Wheat

Blended nanoparticles significantly promoted growth-related attributes of wheat. Among various NPs, Ag NPs were extensively used because of their diversified uniqueness and properties [45]. Silver NPs promoted the growth and chlorophyll contents of mustard [46] and wheat [14]. Treatment with Ag NPs promoted the length of shoots and roots of *Zea mays*, *Phaseolus vulgaris* [15] and cucumber [17]. Foliar application of iron oxide nanoparticles enhanced leaf, pod weight and soybean yield in comparison with the control [22]. Plant metabolic processes, such as the facilitation of the transfer of photosynthates, increased with iron oxide NPs in the peanut leaves [47]. Iron oxide nanoparticles

augmented total chlorophyll contents, grain iron contents, and the yield of wheat in comparison with the control [24]. Ag NPs increased germination and the root and shoot growth of maize [48]. Blended nanoparticles significantly promoted the growth and antioxidant enzyme activities of wheat [49].

Organic chemicals, such as nicotinic acid, tryptophan, and myo-inositol acted as a growth regulator; exposure of organic chemicals to wheat increased chlorophyll contents, as well as shoot and root biomasses. It has been experimented that nicotinic acid increased the growth and biochemical characteristics of thyme plants [50]. Ascorbic acid promoted germination and tomato growth significantly [51]. The application of tryptophan promoted growth, chlorophyll contents and the yields of okra [52] and chickpea significantly [53]. It also augmented relative water contents, the membrane stability index and potassium contents of maize [30]. Treatment with tryptophan augmented plant growth and the production of wheat [54]. It has been documented that exogenous appliances of myo-inositol reduced H_2O_2 levels through regulating proline contents and antioxidant enzymes, i.e., ascorbate peroxidase and catalase activity. Its application may regulate reactive oxygen species levels within optimum limits through proline signal transduction and the maintenance of cell turgor through binding water molecules [55]. Detrimental effects of oxidative stresses can be avoided through DNA methylation by application of nicotinic acid [27]. Gibberellins and auxins, as synthetic growth regulators, have a significant impact on vegetative growth, fruit settings and the fruit yield of cucumber [56]. Inositol has significant importance in regulating several metabolic functions of the plant, such as phosphate storage in seeds [33].

Blended Ag & Fe NPs increased plant growth significantly. Nanoparticles have a greater capability to promote the metabolism of plants through increasing enzyme activities. Application of Ag NPs increased germination, chlorophyll contents of rice, maize and peanut [57]. Ag NPs, with the treatment of 1 mg/kg in soil did not affect growth and amino acid contents in wheat [58] although pea seeds treated with Ag NPs significantly promoted root length [59]. Treatment with iron oxide nanoparticles improved total chlorophyll contents, grain iron contents and wheat yield in comparison with the control [43]. Plant metabolic processes, such as the facilitation of the transfer of photosynthates, increased with iron oxide NPs in peanuts [23]. Morphological attributes, chlorophyll contents, carotenoid contents, protein and iron contents were promoted with Fe NPs [60]. Furthermore, the interaction of NPs with plants induced modifications in morphological and physiological parameters depending upon size, physical and chemical properties and concentration of NPs, however, plant species are also important in this regard [61].

Silver nanoparticles increased chlorophyll a, chlorophyll b and carotenoid contents of wheat significantly [62]. Treatment with iron nanoparticles upregulated the proteins related to photosynthesis and protein metabolism [63]. In a related study, it was recognized that silver nanoparticles promoted germination, growth, protein contents and carbohydrates syntheses. It also increased SOD and catalase activities in *Bacopa monnieri* significantly [64]. Foliar application of iron oxide nanoparticles augmented leaf and pod weight in soybean. The maximum grain yield was recorded with 0.5 g L^{-1} of iron oxide nanoparticles that expressed a 48% increase in yield in comparison with the control [22]. The application of silver NPs promoted the root growth of rice and arabidopsis significantly [65,66]. Metal nanoparticles have the ability to activate enzyme activity for the production of secondary metabolites [67]. Yield and yield attributes of wheat were prompted with the application of Ag NPs [68], Fe NPs, and Cu NPs increased the number of grains/spike, 100-grains weight, and yield of wheat [43]. Blended nanoparticles not only increased the yield of wheat but also proteins related to photosynthesis and protein synthesis were increased [49]. These outcomes advise that Ag NPs and Fe NPs can promote plant growth and development. Therefore, blended NPs have more potential for boosting crop growth, which can greatly affect the overall productivity of the crops.

Previous studies have been reported to express the possible role of nanoparticles on the growth of plants. However, studies related to blended NPs have not been reported yet. In this experiment, Ag NPs and Fe NPs were mixed with organic chemicals. Ag NPs and Fe

NPs blended with organic chemicals promoted all parameters under study. Therefore, an insightful and comprehensive investigation is needed to explore more avenues of blended nanoparticles.

5. Conclusions

The application profile of nanoparticles is increasing tremendously. Silver and iron nanoparticles have growth-promoting effects. Finer nanoparticles were blended with organic chemicals to evaluate plant growth. Chemo-blended nanoparticles significantly promoted morphology, physiology, biochemical and yield attributes of wheat. The enhanced growth of wheat in response to nanoblended formulations might be the result of an increase in the metabolic activities of wheat. Further, insight analysis will be helpful in determining the correct mode of action for chemo-blended nanoparticles.

Author Contributions: H.M.J. and Y.B. conceived of the idea; H.M.J. conducted the experiment; B.H.E., K.A.I., A.E.A. and A.F.G. collected the literature review; A.Q., A.S. and J.I. provided technical expertise; M.Q. and M.T.H. helped in the statistical analysis; A.Q. proofread and provided intellectual guidance. All authors read the first draft, helped with the revision, and approved the article. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Taif University Researchers Supporting Project number (TURSP-2020/127), Taif University, Taif, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is available on request from the authors.

Acknowledgments: The authors acknowledge the support of Taif University Researchers Supporting Project number (TURSP-2020/127), Taif University, Taif, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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