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Agricultural applications of zinc oxide nanoparticles coated with soil humic acidon wheat (*Triticum indicum*) seed germination

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Abstract

Applications of nanoscale materials in agriculture are still in infancy compared to medical and industrial sectors. For promising applications in nanoagriculture, humic acid isolated from agriculture soils capped with zinc oxide nanoparticle (HA-ZnO NPs) were synthesizedand investigated for wheat (*Triticum indicum*)seed germination. HA-ZnO NPs were confirmed with variety of physicochemical techniques such as X-ray diffraction, Fourier transform infrared (FTIR), UV-Vis spectroscopy and scanning electron microscopy (SEM).The data confirmed the adsorption of humic acid on surface of ZnO nanoparticles.The HA-ZnO NPs exposure significantly increased germination by 26.3% root length (28.3mm), shoot length (38.0mm), fresh weight (0.79g) and dry weight (0.5g).This study reveals that HA-ZnO NPs application as co-fertilizer in suitable concentrations becomes more effective for improvement of seed germination and seedling growth of wheat plant. The germination response varies with concentration. To the best of our knowledge, this study first time reports the soil humic acid coated ZnONPs on germination of wheat.

Keywords: Zinc oxide nanoparticles, Humic acid, Seed germination, Wheat (*Triticum indicum*)

Introduction

Nanoparticles possesses unique size, physical properties and potencial applications in biomedical, agriculture , materials sciences, engineering and environmental. These are most studied materials due to their response to magnetic field, high saturation magnetization, non-toxic and biocompatible nature (Petcharoen et al., 2012).

The magnetite nanoparticles are readily aggregated in aqueous systems and are vulnerable to air oxidation. As a result, magnetite stabilization is beneficial (Carlos et al., 2012).In the natural environment, HA is formed by the biodegradation of dead organic matter (Zhang &Bai, 2002). HA generally contains –OH as well as – COOH groups (Kyzas et al., 2017).Because of its large surface area of metal ions, pH-sensitive HA has a higher adsorption potential. The adsorption of drug molecules on the carrier is accomplished by chemical interactions that are vulnerable, such as hydrogen bonds and Vander Waals forces (Huang et al., 2017).Metal NPs' properties are influenced by their size, stability, and concentration in the medium. Due to their low toxicity, low cost, and possible biocompatibility, zinc oxide nanoparticles (ZnO NPs) are promising (Lingaraju et al., 2016).ZnO NPs have a wide variety of biomedical uses, from diagnostics to therapeutics. ZnO NPs have a number of special properties, including semiconductivity, wound healing, antifungal, and antibacterial properties (Raoufi, 2013).Crop priming with HA has been shown to protect seeds in water-stressed soils while also reducing seeding and seedling growth times (Sheteiwy et al., 2017).The mechanism by which HA decreases oxidative stress in plants has been demonstrated in studies (Alenazi et al., 2016).Previous studies (Nakasato et al., 2017; Oliveria et al., 2015) have shown the importance of polymeric based materials on seed germination and seedling development of various plant species to propose nanoparticle and their effective concentrations in this field.

The applications of HA are already reported such as drug delivery, fertiliser, detergents, food and hybrid materials due to its excellent biocompatibility and biodegradability. A number of studies have worked on HA as hybrid material for other functional oxides because of its highwater content and cost effectiveness from wide sources (Akaighe et al., 2011). Seed coated with HA has been reported to protect the seeds in water deficient soils and reduce the time of seeding and seedling development (Sheteiwy et al., 2017) by reducing the oxidative stress in plants. Alenazi et al., (2016) proposed that HA can decrease transpiration rate under stress condition and increase water efficiency. Currently, various researchers are working on hybridizing the multifunctional metal oxide with biopolymers to fabricate multifaceted biomaterials with superior biological activities (Baker et al., 2017).

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In this study the HA-ZnO nanoparticles have been synthesized, characterized and their effect on wheat seed germination has been investigated.

Materials and Methods

Chemicals

Zinc acetate(>99%), potassium hydroxide (>99%) were purchased from Merck (Darmstadt, Germany). HA was purchased from Sigma-Adrich. Distilled water used in for all experiments was purified with Rephile Bioscience, (Resistance, 18 MX cm).

HA preparation

HA was isolated from agriculture soil of Khairpur by IHSS method. 100 g of powdered soil soaked in 0.1 % in sodium hydroxide and after 24 hrs the supernatant was decanded and acified with 0.1 M HCl, then the suspension was sonicated in ultrasonic bath for 15 min. The solution was then filtered using 0.1 um cellulose filter. The resultant suspension was centrifuged at 4°C 4500 rmp,decalcinated and stored for respective utilization (Pirzada et al, 2012).

Synthesis of zinc oxide nanoparticles hybrid with humic acid (HA-ZnO NPs)

For the preparation of complexes, solid HA was dissolved in 0.1 M NaOH to obtain HA solution (500mg/L), the pH of solution was maintained at 5.0 by addition of 0.1 M HCl. Then the ZnO-nanoparticles (200mg) in powder form was mixed with 1 liter HA solution in a bottle and the bottle was kept in shaker for two days. The suspension was centrifuged at 3500 rpm for 30 minutes. Subsequently the precipitated material obtained was rinsed with deionized water, dried at room temperature and ground gently. Finally powder samples were stored in polyethene bags (Motameni& Alireza, 2012).

Physicochemical characterisation

FTIR spectroscopy was used to determine the chemical composition of HA and HA/ZnO nanoparticles with Sensei spectrometer. XRD pattern was obtained with XPERT-PRODiffractometerequipped with a Cu ($K\alpha$ = 1.54 A˚).The size in diameter of crystallite was calculated using Debye–Scherrer equation:

($D = K\lambda/\beta\cos\theta$),

where β (full-width at half-maximum) is radian, θ is angle of peaks at maximum of diffraction, K is constant of a value of 0.9 and λ is X-ray shining wavelength (0.15406 nm Cu $K\alpha$) (Xavier et al., 2012).The dried hybrid nanoparticles (1 g)were deposited on sample holder. The XRD patterns were determined at 10° - 80° angles along scanning rate of 1°/min.The size and surface charge of HA-ZnO NPs were determined with dynamic light scattering (DLS) MalverenZetasizer(UK). The surface area of NPs was determined through BET on Coulter SA 3100 surface area analyzer, under continuous N_2 flow.The SEM measurements were determined using Jeol JSM-840 (Xavier et al., 2012). Prior to SEM analysis, the samples were coated with gold under vacuum on sputter coating machine. The optical properties of fabricated HA-ZnO NPs were analysed using UV– Vis spectrophotometer (Shimadzu) within a 200 – 600 nm range.

Effect of HA-ZnO NPs on wheat seed germinations

Untreated and unprocessedwheat (*Triticum indicum)* seeds were purchased from Sadiqabad Punjab, Pakistan. Seed germination on cellulose filter paper was performed according to method described by Zhang et al., (2020)by using light transparent trays in presence of light. Distilled water washed seeds of wheat plant were placed on cellulose filter paper (0.45 µm), 10 seeds per plate and approximately 1 cm apart in Petri dishes. Prior distilled water was added to filter papers for humidification. 2 mL of treatment solution of HA-ZnONPs containing 5ppm, 10ppm, 15 ppmand 20 ppm were spread thoroughly on filter paper. Seeds were kept humid.Seed germination rate were recorded after interval of 24 hours. After 15 days germinated seedling were harvested and relative root elongation (RE), relative seed germination (SG), root length (RL), shoot length (SL), root and shoot fresh weight (FW) and dry weight (DW) were recorded. DW was achieved after the drying seedlings in oven at 60 °Cfor 48 hours.

Three replicates were performed for each test along with negative controls (Double distlled (DD) water). Seeds with roots longer than 5 mm were considered geminated. The germination percentage in each test was measured by root length with a ruler on filter paper.

The relative root elongation (RE), and seed germination (SG) were calculated according to formulae (Haghighi et al., 2012)

$$Root\ elangation\ = \frac{Mean\ root\ length\ with\ test\ sample}{Mean\ root\ length\ with\ control} \times 100 \qquad Eq.1$$

$$Seed\ germination\ = \frac{Seed\ germination\ with\ test\ sample}{Seed\ germination\ with\ control} \times 100 \qquad Eq.2$$

Results and Dicussion

Characterization of coated HumicAcid with ZnO NPs

The synthesized coated ZnO NPs with HA were characterized by XRD, SEM, FTIR and UV-Vis Spectroscopy.

X-ray diffraction analysis of HA-ZnO NPs

The crystalline nature of HA-ZnO NPs were investigated by X-ray diffraction (XRD).The pattern are exhibited in Fig.1.

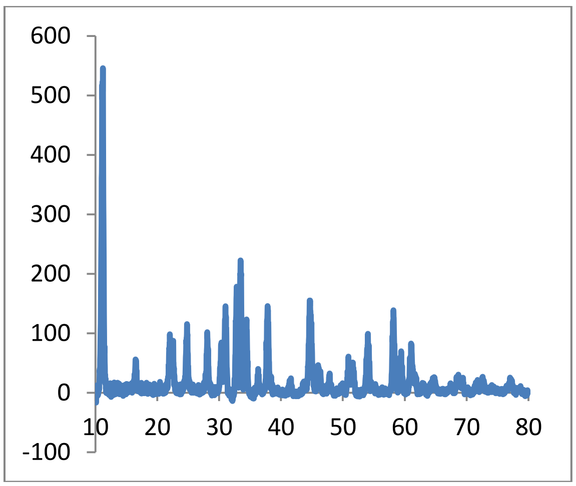

Figure 1. The XRD pattern of synthesized ZnO NPs-H

The pure HA has semi crystalline in nature, it was functionalized with ZnO NPs formingcomplete crystalline structure .TheXRD pattern of ZnO NPs-HA showed intensity peaksat11.25°, 16.6°,22.6°,24.9°,8.1°,0.45°,31.1°,33.5°,34.5°,36.3°,37.9°,41.6°,44.7°,47.8°,50.9°,51.7°˒54.2°,58.2°,59.4°,61.1° 63.1°, 64.7°, 68.7°,72.7oand 77.7°. From the data, it was observed that HA-ZnONPsshow peaks which confirmed little crystallite size when compared to ZnO NPs with much higher and narrow peaks. Thus, the addition of HA in the synthesis of ZnO NPs would help in the size reduction of ZnO NPs. The crystal size was calculated from XRD pattern by using Debye-Sherrer's equation given below.

D=kλ/βCosθ

where k is Sherrer constant(1.54A), β is the full width at half-maximum, λ is the X-ray wavelength, and θ is the Bragg diffraction angle. The average crystal size of ZnO NPs-HA was calculated 39.5x10^{-9}nm.

Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) can produce very highresolution images of a
sample surface, indicating details about less up to nm in size. Due to the very narrow electron beam, SEM micrographs is useful for understanding the surface structure of a sample having a large depth of field yielding a characteristic three-dimensional appearance. The sub-microscopic images i.e. micrographs of ZnO NPs-HAare shown in Fig. 2. with magnification of 5000X and 10 KV, respectively.

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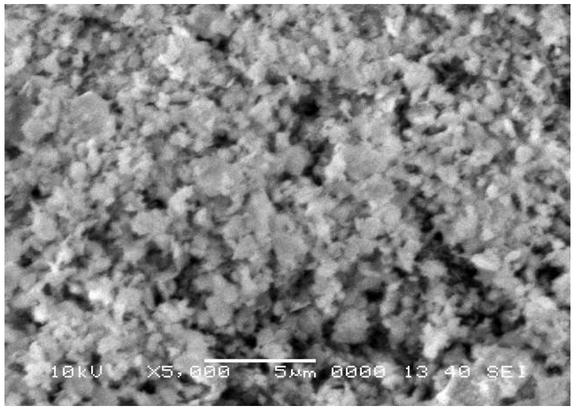

Figure2.SEM micrograph of HA-ZnO NPs

The morphology of HA-ZnO NPs was confirmed using SEM spectroscopy. SEMprovided information on the morphology of HA-ZnO NPs exhibited the well-aggregated plate like structure shows the spherical shape of HA-ZnO NPs with diameters in the range 1 to 5 µm are shown in Fig.2. ZnO NPs have higher electrostatic attraction, hence without coating their monodespersity is highly unlikely, Fig.2 confirms the aggregation of ZnO NPs and their interactions.

Fourier Transform Infrared Spectroscopy (FT-IR)

The humic acid functionalized with ZnO NPs was investigated by using the FT-IR.The structure of HA-ZnO NPs was confirmed by FT-IR spectra(Fig. 3).

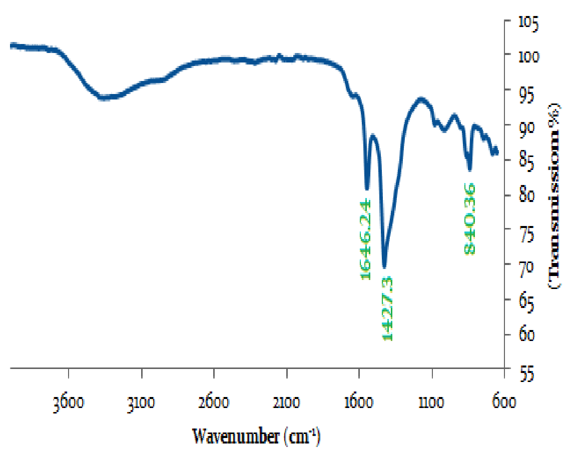

Figure 3. FTIR spectra of HA-ZnO NPs

The FT-IR spectra of the samples measured in the range of 3600–600 cm^{-1} are presented in Fig. 3. The ZnO NPs coated with HA were characterised by FTIR spectroscopy. The FTIR spectra of HA-ZnO NPspeaks show the 1600 cm-1 and 1400 cm-1 which indicates the carbonyl amide ($CONH_2$) and OH stretching C=O vibrating in phenolic group which is shown Fig. 3, the peaks are noted 2000 cm^{-1} to 1800 cm^{-1} owing to the aliphatic stretching vibration. The FTIR spctra of synthesized HA-ZnO NPs showed peaks at 700 cm^{-1} and 900 cm^{-1} which indicates the HA-ZnO NPs are synthesized.

UV-Vis Spectroscopic analysis

The HA-ZnO NPs were analyzed by using UV-Vis spectrophotometric techniques. The wavelength was scanned from 800 to 200nm giving the expected spectra (Fig.4).The formation of isolated humic acid from soil was observed at 300nm and the synthesized HA-ZnO NPs was found broad peak at 357nm by using spectrophotometric technique

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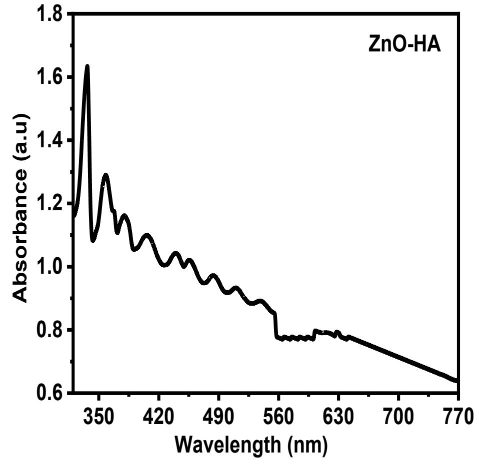

Figure 4. UV-Vis absorption spectra of HA-ZnONPs

Effect of HA-ZnO NPs on wheat seed germination

In order to investigate the effectiveness of using hybrid NPs in enhancing plant growth and development, HA-ZnO NPs were applied to the seeds of wheat plants. More pronounced results were obtained by HA- ZnO NPs when compared with NPs.

Germination

Germination of Seed started from the fourth day after cultivation and recorded on the same day continued for 14 days. Germination and seedling of wheat increased with increasing concentration as shown in Fig.5.Growth of plant was found highest (26.3%) by applying continuously 20ppm solution of prepared HA-ZnO NPs, whereas minimum growth was recorded with control (DD water). These results are in agreement with previous studies (Parsad et al., 2012; Yang et al., 2015). It has been reported by several workers that seed treatment with NPs helps in the germination process, such as breaking of dormancy, hydrolysis or metabolization of inhibitors, imbibitions and enzyme activation (Solanki& Laura, 2015) .

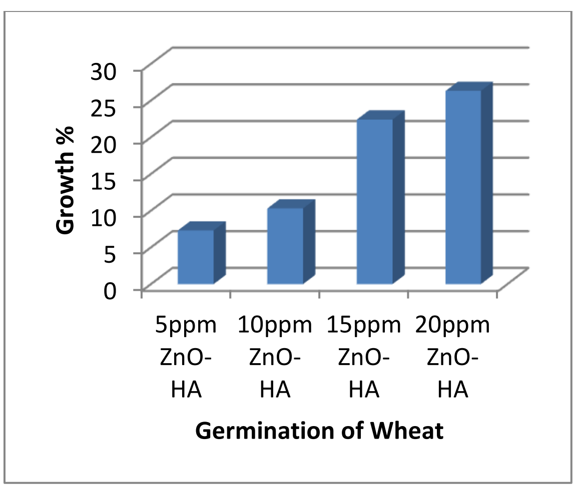

Figure 5. The effect of different concentrations of synthesized HA-ZnO NPson seed germination of wheat (*Triticum indicum*)

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Seedling growth
Shoot and root length

After ten days, the shoot and root of seedlings were measured with a ruler. The highest shoot length of 38.0 mm was observed with the application of 20ppm HA-ZnO NPs (Fig.6 and 7) comparedwith control. The shoot length significantly observed increasing with concentration of HA-ZnO NPs, whereas the root length (28.3mm) found increasing with concentration of HA-ZnO NPs and lowest growth recorded under control. Majority of root shoot length differences appeared after the long period. Similar findings were reported in literature where ZnO NPs concentration effects on the root and shoot length of other plants precursor activity of Zn, especially ZnO NPs in auxin production (Solanki & Laura, 2018).

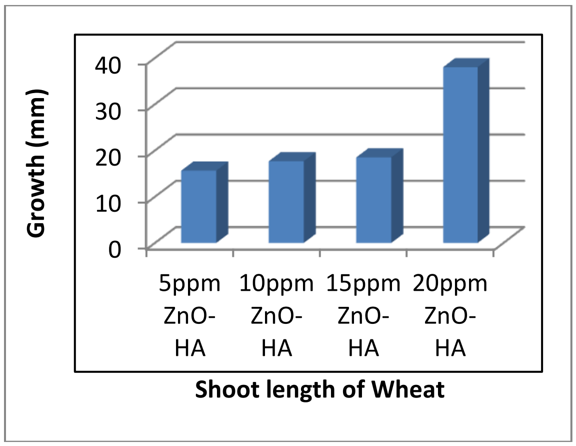

Figure 6. The effect of different concentrations of synthesized HA-ZnO NPson Shoot length of wheat (*Triticum indicum)*

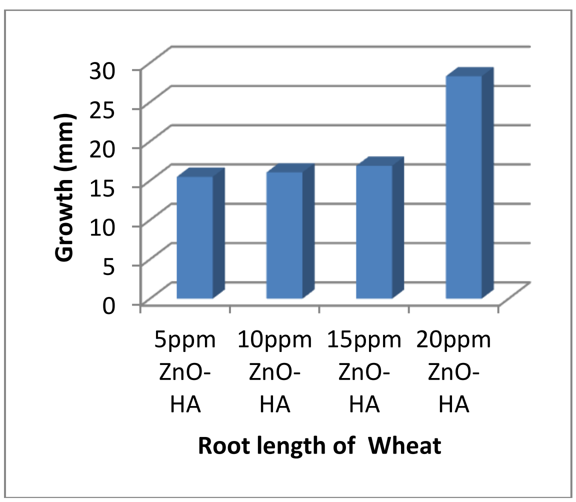

Figure 7. The effect of different concentrations of synthesized HA-ZnO NPs on Root length of wheat (*Triticum indicum*)

Fresh and dry weight
The wheat plant fresh as well dry weight increased with concentration of HA-ZnO NPs 5-20 ppm. At 20 ppm maximum fresh and dry weight of seedlings 0.79and 0.5g were calculated. However, the lowest was observed under control (Table 1). Previous study conducted by Lin and Xing et al., (2007) also found same trend.

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Table 1. Effect of different concentration HA-ZnO NPs on fresh and dry weight wheat in petri dishes

Parametrs	5ppm	10ppm	15ppm	20ppm
Fresh weigth/gm	0.63	0.7	0.74	0.79
Dry weigth/gm	0.05	0.07	0.1	0.5

Conclusion

The data obtained by FTIR, UV-Vis, XRD and SEM confirmed the coating of ZnONPs with soil humic acid. HA adsorption on nano-oxides depended largely on surface properties of nano-oxides. Significant HA adsorption was observed on nanosized ZnO, due to high adsorption capacity of HA on ZnONPs. As a result, the increase in the potentials of ZnONPs by HA coating (i.e. increase in electrostatic repulsion) suggests that HA-coated nano particles could be dispersed and suspended and have higher stability than uncoated ones in the environment. The effect of HA-ZnO NPs was observed on the wheat seed germination and its growth. Various (5-20ppm) concentrations of HA-ZnO NPs tested in this study revealed that 20ppm HA-ZnO NPs had highest positive effects on seedling growth over control on seed germination.

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