



Share Your Innovations through JACS Directory

Journal of Nanoscience and Technology

Visit Journal at <http://www.jacsdirectory.com/jnst>

Biosynthesis and Characterization of Iron Oxide Nanoparticles Synthesized using Earthworm Based Extracts

P. Suganya, P.U. Mahalingam*

Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram – 624 302, Tamil Nadu, India.

ARTICLE DETAILS

Article history:

Received 06 July 2018

Accepted 17 July 2018

Available online 04 August 2018

Keywords:

 β -Fe₂O₃ NPs*Vigna radiata*

Earthworm

ABSTRACT

Iron oxide (β -Fe₂O₃) nanoparticles were synthesized through a biological method using earthworm based extracts like *Eudrilus eugineae* vermiwash, *Eisenia fetida* vermiwash, *Eudrilus eugineae* crushed extract, *Eisenia fetida* crushed extract, *Eudrilus eugineae* warm water extract, *Eisenia fetida* warm water extract and cow dung extract as a reducing and stabilizing agents. The development of β -Fe₂O₃ nanoparticles in the reaction mixture was determined by UV-visible spectroscopy. Followed by, the synthesized β -Fe₂O₃ nanoparticles were characterized by X-ray diffraction, Fourier transforms infrared spectroscopy, scanning electron microscopy, and energy dispersive X-ray and transmission electron microscopy. Synthesized β -Fe₂O₃ NPs are characterized as crystalline structure of hexagonal shape with particle size of 2.08-94.37 nm and carrying unique functional groups. Bioefficiency of β -Fe₂O₃ was assessed through In-vitro seed germination study with green gram (*Vigna radiata*) and results revealed that 200 mg concentrations of α -Fe₂O₃ supported better seed germination and early growth in *V. radiata*.

1. Introduction

The biosynthesis of nanoparticles have been considering environment friendly, cost effective, safe and alternate to chemical and physical methods [1-3]. The nanoparticles are used for various bioapplications viz., bioseparation, detection of biological entities (cell, protein, nucleic acids, enzymes, bacteria, virus etc.), clinic diagnosis (magnetic resonance), MFH (magnetic fluid hyperthermia), targeted and biological therapy [4]. The iron oxide (Fe₂O₃) nanoparticles were synthesized by various methods as electrochemical synthesis [5], laser pyrolysis [6], chemical reduction [7] etc. Iron oxide nanoparticles has wider application in biomedicine [8] self-cleaning coatings, topical sunscreens, and antimicrobial soaps [9-13], ferrofluids [14], catalysts [15], separation process [16], batteries [17], sensors [18] and environmental remediation [19]. In recent times, green synthesis of iron oxide NPs using biological agents have become popular among the scientist across the world. The various biomaterials were already been characterized as potential agents for the synthesis of iron and iron oxide nanoparticles [20, 21]. Therefore, the present study is aimed at biosynthesis and characterization of β -Fe₂O₃ nanoparticles synthesized using earthworm based extracts.

2. Experimental Methods

2.1 Preparation of Earthworm Based Extracts

Seven different earthworm based extracts have been prepared and used in the present study. Bio-extracts are includes vermiwash that is extracted from a matured compost produced individually from two earthworms viz., *Eudrilus euginaea* and *Eisenia fetida* by soaking compost with adequate water and filtered; earthworm crushed extracts from *E. euginaea* and *E. fetida* obtained by grinding the five worms with distilled water using mortar and pestle and filtered; The warm water extracts were obtained by soaking earthworm (10 g each of *E. euginaea* and *E. fetida*) in warm water for half an hour and filtered and cow dung extract was obtained from 100 g dried cow dung soaked in 100 mL distilled water, thoroughly mixed and filtered. All these seven extracts were collected and β -Fe₂O₃ nanoparticle synthesis.

2.2 Biosynthesis of Iron Oxide Nanoparticles using Earthworm Based Extracts

Iron oxide nanoparticles being synthesized using earthworm based extract using standard procedure [22]. In a typical reaction mixture of 1 mM FeCl₃ solution (50 mL) was added with 1 mM ferric nitrate (Fe(NO₃)₃) and NaOH solutions and an equal volume of earthworm based extracts. The reaction mixture was mixed using magnetic stirrer under 80 °C for 2 hours or until the dark yellow colour was changed to black colour which indicating the formation of water soluble β -Fe₂O₃ nanoparticles. The formation of β -Fe₂O₃ nanoparticles was preliminary confirmed with UV-Vis spectrum (Jasco UV- Vis- 750) and the spectra reading interpreted using origin 8.5 version software. Then, the black precipitate was washed repeatedly with distilled water followed by ethanol to remove the impurities from the final product. Followed by a black precipitate was obtained as pure product after drying at 60 °C for an over night and the final products were used for further studies.

2.3 Characterization of Iron Oxide β -Fe₂O₃ Nanoparticles

The purified iron oxide nanoparticles (α Fe₂O₃) NPs were analyzed by X-ray diffraction analysis (Bruker ECO D8 Advance) for determining the crystallinity of nanoparticles. Then, β -Fe₂O₃ NPs were characterized by FT-IR spectral measurements in the range of 400- 4000 cm⁻¹ to identify the biomolecules that are responsible for the reduction, capping and stabilizing of nanoparticles during their potential synthesis. The measurement was carried out with Jasco FTIR 4100 instrumentation. The shape and morphology of the Iron oxide sample were analyzed by scanning electron microscope (SEM) (Vega 5 TESCAN 129ev). In SEM analysis, β -Fe₂O₃ NPs were uniformly spread on the SEM plate was placed acetone clean carbon tape and applied butter coat. Followed by in a vacuum chamber for 1 h and loaded in SEM instrument. The energy dispersive X-ray spectrum (EDAX) recorded the areas of the solid surface of iron nanoparticles to reveal the chemical composition of nanoparticles. The EDAX results were done by EDX with Bruker, nano D-12480 instrument and inorganic materials were identified by EDAX. Size and morphology of β -Fe₂O₃ NPs were determined through transmission electron microscopy (TEM) study. The TEM imaging was taken by the instrument Jeol/ JEM 2100 and operated by acceleration voltage range of 200 kV.

*Corresponding Author:pumahalingam.gri@gmail.com(P.U. Mahalingam)

2.4 Bioefficacy Characterization of β -Fe₂O₃ NPs on Seed Germination and Early Growth in *Vigna radiata* through In-vitro Study

Bioefficacy of β -Fe₂O₃ NPs was determined through seed germination with green gram, *Vigna radiata* as per standard procedure [23]. Healthy seeds were washed with 10% sodium hypochloride solution for 10 min and three times washed with deionized water for surface sterility. Followed by, the seeds were soaked in various concentration of β -Fe₂O₃ NPs solution (100 to 500 ppm) for overnight. Then, undamaged seeds were transferred onto filter paper in a petri dish and kept in a growth chamber at 25 °C under dark. Seed germination and early growth viz, shoot length, root length, fresh weight and dry weight of the whole crop were assessed on 10th day.

3. Results and Discussion

3.1 Crystallographic Structure of Iron NPs

X-ray crystallographic structures of β -Fe₂O₃ synthesized from earthworm based extracts are shown in Fig. 1 and their crystalline properties are presented in Table 1. The diffraction peaks as observed in X-ray are corresponding to metal iron oxide nanoparticles which are in hexagonal shape and the size ranges from 2.08 to 94.37 nm. From among the seven extract, vermiwash extract of *Eudrilus* showed better crystalline product with 2.08 nm (Table 1). The diffraction peaks are broad bump about 2 θ values JCPDS no 76-1821 (Fig. 1). In this study, it was confirmed that the seven earthworm based extracts are act as potential capping or stabilizing agents. These results are well supported by Mukherjee et al. [24] and they were successfully synthesized β -Fe₂O₃ NPs through the biological method and also characterized β -Fe₂O₃ NPs as a crystalline structure with hexagonal shape of 5.5 nm in size.

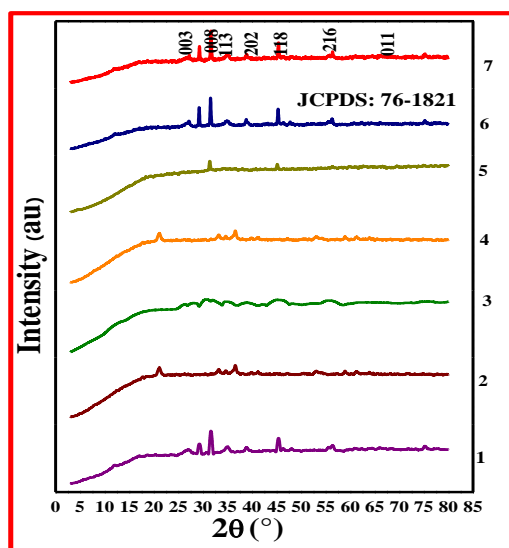


Fig. 1 XRD pattern of β -Fe₂O₃ NPs synthesized using earthworm based extracts

Table 1 Crystalline properties of β -Fe₂O₃ NPs synthesized using seven earthworm based extracts

S.No.	Samples	Crystalline shape	Crystalline size (nm)
1	<i>Eudrilus</i> vermiwash	Hexagonal	2.08
2	<i>Eisenia</i> vermiwash	Hexagonal	54.68
3	<i>Eudrilus</i> crushed extract	Hexagonal	94.37
4	<i>Eisenia</i> crushed extract	Hexagonal	45.66
5	<i>Eudrilus</i> warm water extract	Hexagonal	2.16
6	<i>Eisenia</i> warm water extract	Hexagonal	3.82
7	Cow dung extract	Hexagonal	86.03

3.2 Fourier Transform Infrared Spectroscopy Study

The FTIR analysis is used for the identification of the possible biomolecules which are responsible for the reduction of Fe ions. This is because of the biological extracts act as a capping agent and reducing agent for the stabilization of nanoparticles [25]. FTIR spectrum for β -Fe₂O₃ NPs synthesized using earthworm based extracts is shown in Fig. 2 and the specific functional groups found in β -Fe₂O₃ NPs were presented in Table 2. Among the seven extracts, β -Fe₂O₃ synthesized using *Eudrilus* vermiwash showed unique functional groups which are responsible for better reduction and stabilization properties (Table 2).

<https://doi.org/10.30799/jnst.140.18040414>

Table 2 Absorption bands and the functional groups for β -Fe₂O₃ nanoparticles synthesized using earthworm based extracts

S.No.	Number of extracts	Absorption bands (cm ⁻¹)	Functional groups
1	<i>Eudrilus</i> vermiwash	621.08 & 464.84	β -Fe ₂ O ₃
		2904.09 & 1843.95	CH & C=O group
		1745.58 & 1653.00	C=O group in a secondary group of amides
		1543.05 & 1514.12	NO ₂ aliphatic nitro compounds & triazine compounds
2	<i>Eisenia</i> vermiwash	619.15 & 464.84	β -Fe ₂ O ₃
		2926 & 1653	CH aliphatic compounds & C=O primary amide group
		1026	OH group
3	<i>Eudrilus</i> crushed extract	698.23 & 464.84	β -Fe ₂ O ₃
		2924.09 & 1653	OH group & C=O secondary amides
		1516.05 & 1460.11	CH ₃ aliphatic compounds & C-O group
		1033.85	C-O group
4	<i>Eisenia</i> crushed extract	464.84	β -Fe ₂ O ₃
		2924.09 & 1745.58	OH group & C=O group
		1658 & 1460.11	C=O in secondary amides & CH ₃ in aliphatic compounds
5	<i>Eudrilus</i> warm water	624.94 & 457.13	β -Fe ₂ O ₃
		1799.59 & 1745.58	C=O group
		1653	C=O secondary amide group
		1458.18 & 1425.40	CH ₂ & OH Group
6	<i>Eisenia</i> warm water	694.37 & 621.08	β -Fe ₂ O ₃
		2926.01 & 1743.65	C=O group
		1543.05 & 1517.98	C=O stretch of secondary amides & NH ₃ amino acids
7	Cow dung extracts	1460.11 & 1423.47	CH ₃ & OH Groups
		451.34, 671.23 & 612.15	β -Fe ₂ O ₃
		3151 & 1742	NH ₂ primary amides & C=O esters
		1402 & 1266	C-N Amide group & C-O group
		1132 & 1074	C-O & S=O group

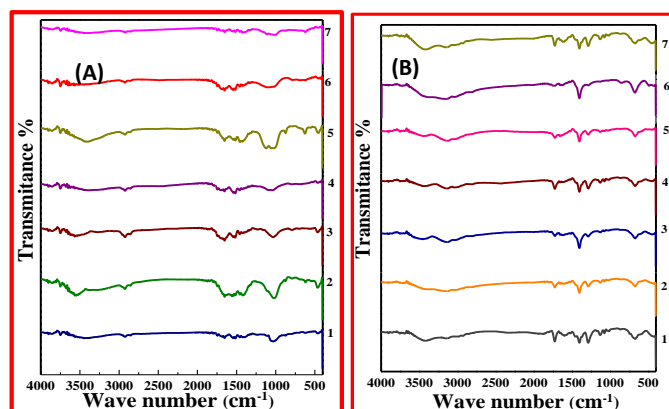


Fig. 2 FTIR spectrum A) crude earthworm based extracts and B) β -Fe₂O₃ NPs synthesized using earthworm based extracts

3.3 UV-Vis Absorption Study

The preliminary confirmation of developing β -Fe₂O₃ nanoparticles was monitored using UV-Vis spectrophotometer. The reduction of ferric chloride solution under earthworm based extracts indicates the change of color from yellow to black. The UV-Vis spectrum as shown in Fig. 3 confirmed the formation of iron oxide as an absorbance peaks were obtained at 200- 800 nm and the strong broad surface plasmon resonance at 292 nm. The results are in accordance with earlier report on iron oxide (β -Fe₂O₃) nanoparticles synthesized from biological materials like tea extracts [26, 27] and they were also consider UV- Vis spectrum as tool to monitor the purity of β -Fe₂O₃ NPs.

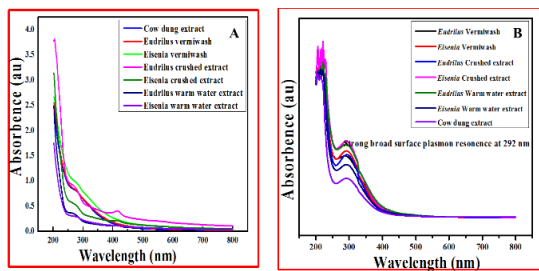


Fig. 3 UV- visible spectrum A) crude earthworm based extracts and B) β -Fe₂O₃ NPs synthesized from earthworm based extracts

3.4 Scanning Electron Microscopic Study

SEM images of β -Fe₂O₃ synthesized using seven different earthworm based extracts showed different morphologies (Fig. 4). The shape and particle size of β -Fe₂O₃ NPs are presented in Table 3. The β -Fe₂O₃ nanoparticles are appeared as spherical structure with the average size of 12±0.31 to 29±0.31 nm. SEM imaging also revealed that the *Eudrilus* vermiwash extracts has high potential in production of β -Fe₂O₃ NPs with the standard size of 12±0.31 nm. According to Afsheen et al. [28], biological extracts has strongly responsible for reducing and capping of β -Fe₂O₃ NPs. The chemical composition of iron oxide NPs was determined by energy dispersive X-ray spectroscopy and the EDAX spectrum reveals the presence of both iron and oxygen as shown in Fig. 5. The earthworm based extracts used in the present study are capable of absorbing the surface of iron nanoparticles which play key role in the stabilization and reduction of iron oxide nanoparticles.

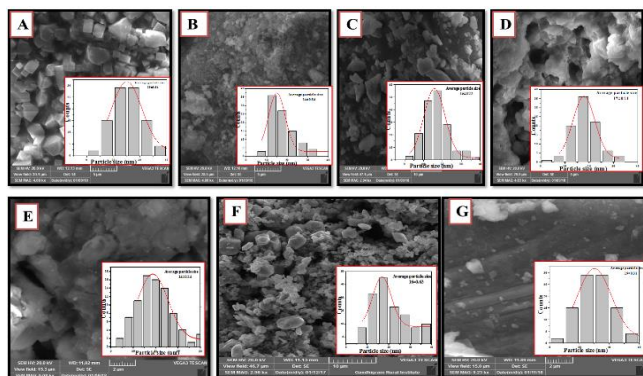


Fig. 4 SEM imaging of β -Fe₂O₃ NPs synthesized from seven different earthworms based extracts including A) Cow dung extract B) *Eudrilus* vermiwash extract C) *Eisenia* vermiwash D) *Eudrilus* crushed extract E) *Eisenia* vermiwash extract F) *Eudrilus* warm water extract G) *Eisenia* warm water extract

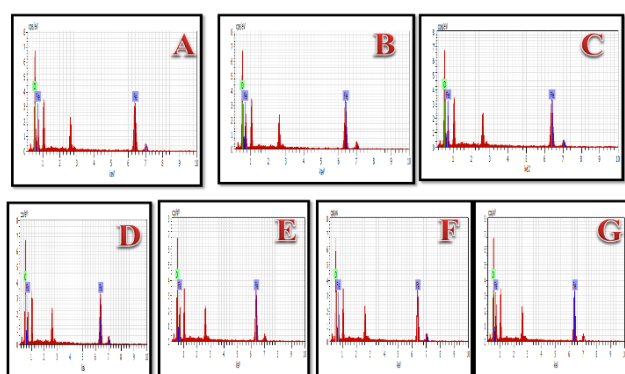


Fig. 5 EDAX graph showing elemental composition of β -Fe₂O₃ NPs synthesized from earthworm based extracts A) cow dung extract B) *Eudrilus* vermiwash C) *Eisenia* vermiwash D) *Eudrilus* crushed extract E) *Eisenia* vermiwash extract F) *Eudrilus* warm water extract G) *Eisenia* warm water extract

Table 3 Shape and size of the β -Fe₂O₃ NPs synthesized using earthworm based extracts in SEM analysis

S.No.	Extracts	Shape	Size of nanoparticles
1	<i>Eudrilus</i> vermiwash	Cubic	12±0.31
2	<i>Eisenia</i> vermiwash	Spherical	14±0.31
3	<i>Eudrilus</i> crushed extract	Spherical	15±0.22
4	<i>Eisenia</i> crushed extract	Spherical	17±0.21
5	<i>Eudrilus</i> warm water	Spherical	14±0.24
6	<i>Eisenia</i> warm water	Spherical	29±0.31
7	Cow dung extract	Spherical	16±0.63

<https://doi.org/10.30799/jnst.140.18040414>

3.5 Morphology of Iron Nanoparticles

TEM images employed to examine the size and morphology of the nanoparticles. TEM micrograph of β -Fe₂O₃ NPs obtained in the present study is shown in Fig. 6. The better particle size of 21±0.85 nm was recorded in *Eudrilus* vermiwash extract than other extracts (Fig. 6d). The present results are in accordance with TEM imaging of iron nanoparticles synthesized obtained using biological extract from *Terminalia chebula* [20].

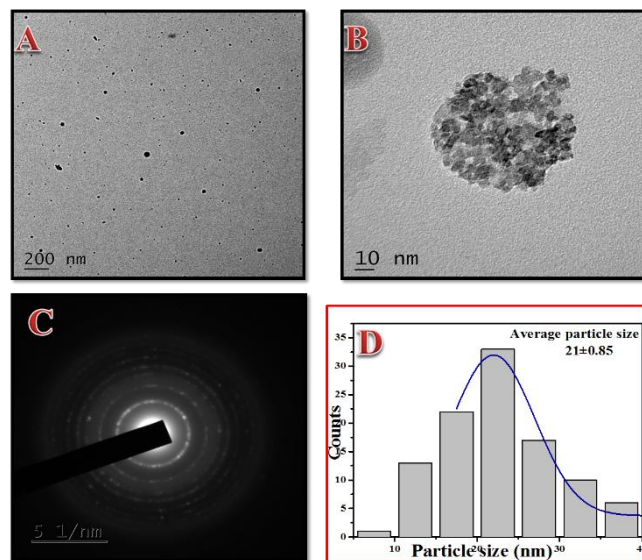


Fig. 6 TEM Micrograph of β -Fe₂O₃ NPs C) SAED pattern of β -Fe₂O₃ NPs D) Gaussian profile of β -Fe₂O₃ NPs

3.6 Seed Germination Study

The bioefficacy of β -Fe₂O₃ NPs synthesized using seven different earthworm based extracts was determined through in-vitro seed germination study using green gram *V. radiate* and the results are shown in Fig. 7a. The influence of β -Fe₂O₃ NPs on early growth of *V. radiate* was recorded in Figs. 7b to 7e. Similar kind of work was carried out by several authors to determine the bioefficacy of various nanoparticles [29, 23, 30, 31].

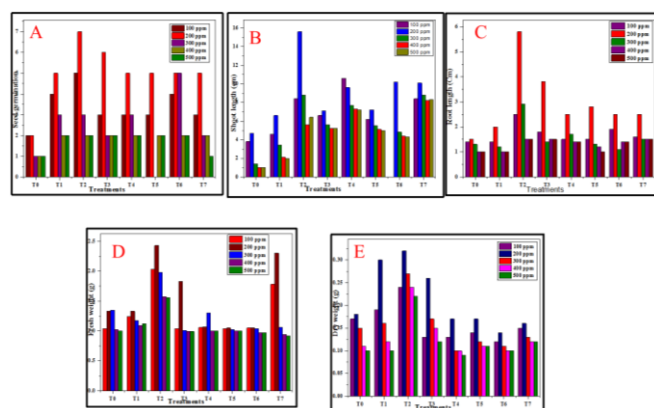


Fig. 7 Effects of NPs on green gram *Vigna radiata* A) seed germination B) shoot length C) root length D) fresh weight of whole plant E) dry weight of whole plant

4. Conclusion

In summary, cubic and spherical structure of Fe NPs were successfully prepared via co-precipitation method using seven different earthworm based extracts. These aqueous extracts of seven different earthworms based extracts were acted as potential reducing and stabilizing agents. The β -Fe₂O₃ nanoparticles appeared in different morphological structures and methods used in the present study are simple, efficient and non-toxic. Characterization studies reveals that synthesized β -Fe₂O₃ NPs are found to be hexagonal shape with better particle size and also possess number of potential biomolecules which are strongly responsible for capping and stabilizing of β -Fe₂O₃ NPs. β -Fe₂O₃ NPs produced in the present study promotes better seed germination with early growth in green gram *V. radiata*.

References

- [1] P. Raveendran, J. Fu, S.L. Wallen, Completely “green” synthesis and stabilization of metal nanoparticles, *J. Am. Chem. Soc.* 125(46) (2003) 13940-13941.
- [2] H.S. Sharma, F.S. Ali, S.M. Hussain, J.J. Schlager, A. Sharma, Influence of engineered nanoparticles from metals on the blood-brain barrier permeability, cerebral blood flow, brain edema and neurotoxicity. An experimental study in the rat and mice using biochemical and morphological approaches, *Jour. Nanosci. Nanotech.* 9(8) (2009) 5055-5072.
- [3] S. Narayanan, B.N. Sathy, U. Mony, M. Koyakutty, S.V. Nair, D. Menon, Biocompatible magnetite/gold nanohybrid contrast agents via green chemistry for MRI and CT bioimaging, *ACS app. Mater. Interf.* 4(1) (2011) 251-260.
- [4] A.K. Gupta, M. Gupta, Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications, *Biomater.* 26(18) (2005) 3995-4021.
- [5] C. Pascal, J.L. Pascal, F. Favier, M.L. Elidrissi Moubtassim, C. Payen, Electrochemical synthesis for the control of γ -Fe₂O₃ nanoparticle size, Morphology, microstructure, and magnetic behavior, *Chem. Mater.* 11(1) (1999) 141-147.
- [6] O. Bomati-Miguel, L. Mazeina, A. Navrotsky, S. Veintemillas-Verdaguer, Calorimetric study of maghemite nanoparticles synthesized by laser-induced pyrolysis, *Chem. Mater.* 20(2) (2008) 591-598.
- [7] K.C. Huang, S.H. Ehrman, Synthesis of iron nanoparticles via chemical reduction with palladium ion seeds, *Langmuir* 23(3) (2007) 1419-1426.
- [8] C.C. Berry, A.S. Curtis, Functionalization of magnetic nanoparticles for applications in biomedicine, *Jour. Phys. D: App. Phys.* 36(13) (2003) R198-R202.
- [9] X. Ma, J. Geiser-Lee, Y. Deng, A. Kolmakov, Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation, *Sci. Total Environ.* 408(16) (2010) 3053-3061.
- [10] T.M. Benn, P. Westerhoff, Nanoparticle silver released into water from commercially available sock fabrics, *Env. Sci. Tech.* 42(11) (2008) 4133-4139.
- [11] S.J. Klaine, P.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy, D.Y. Lyon, et al., Nanomaterials in the environment: behavior, fate, bioavailability, and effects, *Environ. Toxicol. Chem.* 27(9) (2008) 1825-1851.
- [12] K. Sobha, K. Surendranath, V. Meena, T. Keerthi Jwala, N. Swetha, K.S.M. Latha, Emerging trends in nanobiotechnology, *Biotech. Mol. Biol. Rev.* 4(1) (2010) 1-12.
- [13] M. Rai, A. Ingle, Role of nanotechnology in agriculture with special reference to management of insect pests, *App. Microbiol. Biotech.* 94(2) (2012) 287-293.
- [14] B. Jeyadevan, C.N. Chinnasamy, K. Shinoda, K. Tohji, H. Oka, Mn-Zn ferrite with higher magnetization for temperature sensitive magnetic fluid, *Jour. Appl. Phys.* 93(10) (2003) 8450-8452.
- [15] M.M. Miller, G.A. Prinz, S.F. Cheng, S. Bounnak, Detection of a micron-sized magnetic sphere using a ring-shaped anisotropic magnetoresistance-based sensor: a model for a magnetoresistance-based biosensor, *Appl. Phys. Lett.* 81(12) (2002) 2211-2213.
- [16] J. Zhang, Y. Wang, H. Ji, Y. Wei, N. Wu, B. Zuo, Q. Wang, Magnetic nanocomposite catalysts with high activity and selectivity for selective hydrogenation of ortho-chloronitrobenzene, *Jour. Catal.* 229(1) (2005) 114-118.
- [17] B. Koo, H. Xiong, M.D. Slater, V.B. Prakapenka, M. Balasubramanian, P. Podsiadlo, et al., Hollow iron oxide nanoparticles for application in lithium ion batteries, *Nano. Lett.* 12(5) (2012) 2429-2435.
- [18] B. Fang, G. Wang, W. Zhang, M. Li, X. Kan, Fabrication of Fe₃O₄ nanoparticles modified electrode and its application for voltammetric sensing of dopamine, *Elect. Anal.* 17(9) (2005) 744-748.
- [19] V. Madhavi, T.N. Prasad, A.V. Bhaskar Reddy, B. Ravindra Reddy, G. Madhavi, Application of phytochemical zerovalent iron nanoparticles in the adsorption of hexavalent chromium, *Spectrochem. Acta Part A: Mol. Biomol. Spectr.* 116 (2013) 17-25.
- [20] K.M. Kumar, B.K. Mandal, K.S. Kumar, P.S. Reddy, B. Sreedhar, Biobased green method to synthesis palladium and iron nanoparticles using *Terminalia chebula* aqueous extract, *Spectrochem. Acta Part A: Mol. Biomol. Spectr.* 102 (2013) 128-133.
- [21] K.S. Siddiqi, A.U. Rahman, A. Husen, Biogenic fabrication of iron/iron oxide nanoparticles and their application, *Nanosc. Res. Lett.* 11(1) (2016) 498-501.
- [22] L. Huang, X. Weng, Z. Chen, M. Megharaj, R. Naidu, Synthesis of iron-based nanoparticles using oolong tea extract for the degradation of malachite green, *Spectrochem. Acta Part A: Mol. Biomol. Spectr.* 117 (2014) 801-804.
- [23] Q.B. Ngo, T.H. Dao, H.C. Nguyen, X.T. Tran, T.V. Nguyen, T.D. Khuu, T.H. Huynh, Effects of nanocrystalline powders (Fe, Co and Cu) on the germination, growth, crop yield and product quality of soybean (Vietnamese species DT-51), *Adv. Natural Sci.: Nanosci. Nanotech.* 5(1) (2014) 015016-1-7.
- [24] D. Mukherjee, S. Ghosh, S. Majumdar, K. Annapurna, Green synthesis of α -Fe₂O₃ nanoparticles for arsenic (V) remediation with a novel aspect for sludge management, *J. Environ. Chem. Eng.* 4(1) (2016) 639-650.
- [25] C. Devatha, P. Arun Kumar Thalla, S.Y. Katte, Green synthesis of iron nanoparticles using different leaf extracts for treatment of domestic waste water, *J. Clean. Prod.* 139 (2016) 1425-1435.
- [26] H. Jung, H. Park, J. Kim, J.H. Lee, H.G. Hur, N.V. Myung, H. Choi, Preparation of biotic and abiotic iron oxide nanoparticles (IONPs) and their properties and applications in heterogeneous catalytic oxidation, *Environ. Sci. Tech.* 41(13) (2007) 4741-4747.
- [27] L. Huang, F. Luo, Z. Chen, M. Megharaj, R. Naidu, Green synthesized conditions impacting on the reactivity of Fe NPs for the degradation of malachite green, *Spectrochem. Acta Part A: Mol. Biomol. Spec.* 137 (2015) 154-159.
- [28] S. Afsheen, M.B. Tahir, T. Iqbal, A. Liaqat, M. Abrar, Green synthesis and characterization of novel iron particles by using different extracts, *J. Alloy. Compd.* 732 (2018) 935-944.
- [29] J. Lv, S. Zhang, L. Luo, J. Zhang, K. Yang, P. Christie, Accumulation, speciation and uptake pathway of ZnO nanoparticles in maize, *Environ. Sci.: Nano* 2(1) (2015) 68-77.
- [30] N. Salem, L.S. Albanna, A.M. Awwad, Seed germination and growth of cucumber (*Cucumis sativus*) effect of nano-crystalline sulfur, *Jour. Agri. Sci.* 8(10) (2016) 219-234.
- [31] T. Adhikari, S. Kundu, A.K. Biswas, J.C. Tarafdar, A.S. Rao, Characterization of zinc oxide nano particles and their effect on growth of maize (*Zea mays* L.) plant, *J. Plant Nutr.* 38(10) (2015) 1505-1515.