Molecular Docking of Biosynthesized Zinc Oxide Nanoparticles to Screen Their Impact on Fungal Pathogen of Carrot Plant

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Abstract

Zinc plays a key role in plants growth and application of Zinc can, therefore, contribute to crop yield improvement. Nowadays, nanoparticles have received high attention because to their novel properties. The current work is done with an aim to investigate the biosynthesis of zinc oxide nanoparticles (ZnO NPs) and effect on fungus *Rhizoctonia solani* and on carrot crop. Use of nanoparticles as a nano-fertilizer requires an understanding of nanoparticles impact on crop plants We have used seed coat of almond for the synthesis of zinc oxide nanoparticles (ZnO NPs) characterized by EDS, FTIR, SEM and TEM. Spray with 50ppm and 100 ppm caused significant increase in plant growth parameter of carrot plants. It has been reported that the synthesized ZnO NPs demonstrated an inhibitory activity against plant pathogenic fungi *R. solani.* Antifungal efficiency of ZnONPs was further explained with help of Molecular docking analysis. Confirmation of the least binding energy was used to predict binding site of receptor with NPs to know mechanistic approach. ZnONPs are likely to interact with the pathogens by mechanical enfolding which may be one of the major toxicity actions against *R. solani* by ZnONPs.

Introduction

In the past years, toxic effect of pesticides has been reported on human health and environment. Pesticide in food causes diseases like cancer in humans. For example pesticide DDT (dichlorodiphenyltrichloroethane) used in agriculture causes cancer or other diseases in humans (Cohn et al., 2007). Alternate to these pesticides is required. Nanomaterials (NPs) have received high attention due to their unique properties and beneficial in agriculture and other sectors. Globally in 2010 it is estimated that 260,000-309,000 metric tons of nanoparticles were produced (Yadav et al., 2014) and worldwide consumption of nanomaterials is approximately from 225,060 metric tons to 585,000 metric tons in 2014 to 2019 (BCC Research, 2014). Global annual production of ZnO NPs estimated between 550 and 33,400 tons and the third most used nanomaterials (Bondarenko et al., 2013; Connolly et al., 2016). Nanomaterials have been reported to absorb 15–20 times higher than their bulk particles (Srivastav et al. 2016). ZnO NPs levels in environment were approximately 3.1–31 µg/kg in soil and 76–760 µg/L in water (Ghosh et al., 2016). Zinc oxide nanoparticles (ZnO NPs) are capable to enhance agriculture production and crop yield (Sabir et. al. 2014). Nanoscale treatment of zinc oxide promotes stem and root growth in peanuts (Prasad et al. 2012). ZnO NPs are found better for plant growth and provide better resistance against pathogens; these are less toxic to plants and plant growth promoting soil bacteria (Khan and Siddiqui 2019; Stampoulis et al. 2009).

Nanomaterials have attracted attention for plant disease management caused by various plant pathogens (Alghuthaymi et al. 2015). ZnO NPs can act as an effective antimicrobial agent against microorganisms (Sabir et al. 2014). Green synthesis of nanoparticles includes synthesis through plants, algae, bacteria; fungi and biological approach have limited use of expensive and toxic chemicals. Previous studies showed synthesis of ZnO NPs from different plant materials (Sangeetha 2011; Vidya 2013; Abdul 2014; Ramesh 2015; Awwad 2020). In this study an attempt is made to green synthesis of zinc oxide nanoparticles were done and their effect were examined on the growth of carrot plant and

screen their effect on plant pathogenic fungus *Rhizoctonia solani*. In addition, molecular docking was performed to understand interactions between receptor of R. solani and biosynthesized nanoparticles and estimating the binding affinities.

Materials And Methods

Green synthesis of zinc nanoparticles

Zinc nitrate GR used as such (purchased from Merck, India). 100 mL, 1 mM solution of zinc nitrate was prepared in an Erlenmeyer flask. Take 10 ml extract of seed coat of almond. After that 0.67gm of zinc acetate was added in 50ml of water after constant heating and stirring for 12 hours add 2 plates of NaOH in 25 ml of distilled water add in the reaction mixture and further heating with stirring the sample was centrifuged and then dried in oven. Zn^{+} to Zn^{0} respectively. The UV–Visible spectroscopy that comes in the range of 272 nm confirms the formation of ZnONPs respectively.

Plant culture

Carrot seeds were sown in pots separately after seed surface sterilization by 0.1% sodium hypochlorite for 5–7 minutes and washed with distilled water. After ten days of seed germination plants grown in each pot were sprayed with 50 ppm and 100 ppm ZnO NPs. Each treatment was replicated five times and pots were arranged on a greenhouse bench at 26 ± 5°C and watered regularly. The experiment was terminated 50 days. Plant growth parameters were analysed and total chlorophyll and carotenoids content of carrot leaf also estimated.

Fungus culture

Rhizoctonia solani fungus was cultured on potato dextrose agar (PDA) medium at 25°C. Fungal mycelium after culture was treated with synthesized ZnO NPs. SEM and confocal analyses were done to analyze the effect of ZnO NPs.

Molecular docking

Using Auto Dock method (Morris et al. 2009) molecular docking study was performed to know the preferred binding mode and binding sites of ZnONPs with pathogenic receptor. 3D protein structure of Rhizoctonia solani pathogenic receptor was retrieved from the PMDB database and allocated PMBD Id is PM0079487.The Crystallographic Information File (CIF) of ZnO was downloaded from the website of the materials project. The CIF of ZnO was converted and saved into PDB format and used as ligand for docking study. Before docking simulation, Gasteiger partial charges, adding Kolman charges, polar, non-polar hydrogen atoms and Lamarckian genetic terminology were applied to ZnONPs and receptor. In this docking study AutoGrid produced a large grid map to cover the entire surface of the protein. The pose showing the least binding energy (more negative binding energy) was the best docked model which was further exercised to visualize binding sites using the BIOVIA software [BIOVIA,2015].

Quality control and quality assurance

The analytical grade of chemicals and reagents was used for overall analysis. The deionized water was used for reagent preparation and dilution. The chemicals and reagents were purchased from Merck, India. A total of three replicas of samples were investigated to eliminate the error during sample collection and preparation.

Results And Discussion

In the last decade several papers reported for biosynthesis of zinc oxide nanoparticles (ZnONPs) from plants (Rao, 2016; Choudhary, 2018). After reviewing previous literature we synthesized zinc oxide nanoparticles (ZnO NPs) from seed coat of almond. We carried out the biosynthesis of ZnONPs from the extract of seed coat of almond. The finally formation of ZnONPs are identified on the basis of spectral (UV-vis, EDS, FTIR, SEM and TEM).

Characterization of synthesized ZnO NPs

UV–Vis spectroscopy

Several papers have reported the biosynthesized zinc oxide nanoparticles have UV-vis absorption spectrum from 200 to 300nm with Nanodrop 2000c spectrophotometer (Themo Scientific, Walthman MA, USA) (Choudhary, 2018). If we increase the concentration of extract then increase in wavelength up to 448 nm. The UV-vis spectra show a peak at range at 272 nm and it was shown the formation of ZnO nanoparticles.

FTIR analysis

FTIR spectroscopy analysis was performed to ascertain the involvement of possible plant bio-compound responsible for reduction of Zn + ions and capping and stabilization of bio-reduced ZnO NPs synthesized by using plant extract. Figure 2 shows the aqueous and synthesized ZnO NPs using almond seed coat extract where the absorption spectrum manifests prominent transmittance located at 3434 (NH), 1638, 1563, 1416 and 528 cm^{-1} in the region (Fig. 2). The strong show to the –C□C– stretches (flavanones) and broad peaks indicating the –N–H– stretches (amide group) and cyclic CH_2 stretches (aliphatic group). The prominent band at 528 cm^{-1} confirms the formation of ZnO NPs. The FTIR of ZnO NPs and exist band at 3434 (NH), 1638, 1563, 1416 and 528 cm^{-1}, the occurrence of these peak in the FTIR spectrum of ZnO NPs evidently indicates the dual role as a green reducing agent and also as a stabilizing agent.

Energy dispersive spectrum (EDS); Analysis of synthesized ZnO nanoparticles

EDS analysis shows the presence of zinc and oxygen in synthesized nanoparticles. The labeling shows the names and percentages of the elements for the ZnO sample. Obviously, Zn and O are the main

constituents of the sample, and within EDX detection limits there is no visible evidence of impurities

Scanning electron microscopy (SEM)

SEM images show the morphology of synthesized ZnO NPs. SEM analysis done at magnification ×3500 magnification.

TEM and SAED

Transmission electron microscopy (TEM) analysis showed the spherical dispersed nanoparticles of size 20 nm. The Selected Area Electron Diffraction (SAED) method have been employed to gain knowledge into how the ZnO morphology depends on the Ultrasonic Spray Pyrolysis (USP) process. SAED pattern analysis (Fig. 5B) shows the crystalline nature of ZnONPs in around 20 nm sizeshowing the crystalline nature of the nanoparticles.

Effect on carrot plant growth

Spraying of 50 ppm and 100 ppm ZnO NPs causes a significant increase in plant growth parameters, chlorophyll and carotenoid contents (Table 1 and Fig. 6.). Application of ZnO NPs at 100 ppm as foliar spray caused a highest significant increase in plant growth, chlorophyll and carotenoid contents in plants. Raja et al. (2019) found that ZnO NPs improve seed germination in *Vigna mungo.* Thunungunta et al. (2018) found that ZnO NPs improve brinjal growth. Faizan et al. (2018) found ZnO NPs improve tomato plant growth.

Table 1
Effect of spraying of different concentration of ZnO NPs on carrot plant

Treatment	Plant length (cm)	Plant fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Chlorophyll in fresh leaves (mg/g)	Carotenoid in fresh leaves (mg/g)
C (No ZnO NPs)	46.30c	57.43c	1.89c	2.35 a	0.228c	0.0502c
50 ppm ZnO NPs	49.89b	60.12b	2.08b	2.96 a	0.259b	0.0523b
100 ppm ZnO NPs	53.67a	63.91a	2.97a	3.38 a	0.298a	0.0541 a

Values within a column and same type of treatment followed by the same letter are not significantly different with DMRT test at $P \leq 0.05$.

Antifungal activity

The antifungal activity of prepared ZnO NPs was investigated against fungus *R. solani.* It was evident from SEM analysis that ZnO NPs disturb the fungus mycelium. This was due to the binding of prepared ZnO NPs to the outer membrane of fungus shown in Confocal image (Fig. 7). ZnO NPs inhibit the growth of fungi by causing deformation in fungal hyphae (He et al. 2011). Khan and Siddiqui (2018) reported inhibitory effect of ZnO NPs on bacterium *Ralstonia solanacearum*, fungus *Phomopsis vexans* and plant parasitic nematode *Meloidogyne incognita.*

Molecular docking analysis

In order to understand the in vitro efficiency of ZnO NPs, the ligand protein model was used in the molecular docking study. Docking of ZnO NPs into a modeled receptor, endochitinase (PM0079487) was performed to know proper orientation of nanoparticles with in receptor including non covalent interactions between the active site of receptor and ZnO NPs leading to the design of new drugs for further biological research. Docking pose with binding energy (-8.60 kcal/mol) was considered a best model for describing interactions. The potential optimal combination between the ligand and the receptor protein is illustrated as can be seen in the Fig. 8a-8e. A conventional hydrogen bond was established between oxygen of ZnO NPs and the amino hydrogen of HIS57 with a distance of 2.3512Å (Fig. 8c and 8d).Other non-covalent bonds between Zn of ZnO NPs and hydroxyl hydrogen of TRP52, TRP392 and THR395 were formed with distances of 1.92541, 2.43842 and 2.53757 Å, respectively. Residues of amino acids such as PHE53, HIS57, TRP52, MET396 and TRP392 are involved to make the active amino acids cavity around ZnONPs interacted with ZnONPs forming weak interactions such as wan der Waals interactions, pi-donor hydrogen Bond and polar interactions (Fig. 8e).These interactions of the receptor with ZnONPs involve stabilizing the docked compound in the amino acid cavity of the receptor to disturb the proliferation of fungal mycelium. In vitro experimental study may be in good agreement with the binding interaction of ZnONPs with the receptor carried out by Molecular docking study. In addition, nanoparticles are thought to interact with the fungal mycelium by mechanical coating (Dharni et al. 2015), such influence could be one of the main toxicity actions of ZnONPs against *R. solani* to prevent endochitinase of *R. solani* leads to inactivation of enzymes.

Conclusion

Overall conclusion is that the ZnO NPs exhibit broad spectrum biocidal activity towards different plant pathogenic bacteria and fungi. In this study, we have demonstrated the green synthesis of ZnO nanoparticles from plant part. Synthesized NPs improve the plant growth of carrot plants. Based on the current results it proved antifungal activity of synthesized ZnONPs. The binding interactions between nanoparticles and receptors were analyzed using a molecular docking study. It can be concluded that the ZnO nanoparticles constitute an effective antimicrobial agent against pathogenic microorganisms and can improve the plant growth.

Declarations

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Authors Contributions

MK investigated the samples for ICP-AES, XRF, and EDS, and prepared original draft of the manuscript. Material preparation, data collection, analysis and supervision were performed by AUK. JA and AP, investigated and interpreted XRD, FTIR, and PSA results. IM, and MA critically evaluated the manuscript. MMSCP analyzed and interpreted FESEM micrographs. MA analyzed and interpreted TEM micrographs. VKY prepared original draft of the manuscript. HK and KKY prepared original draft and carefully checked the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Figures

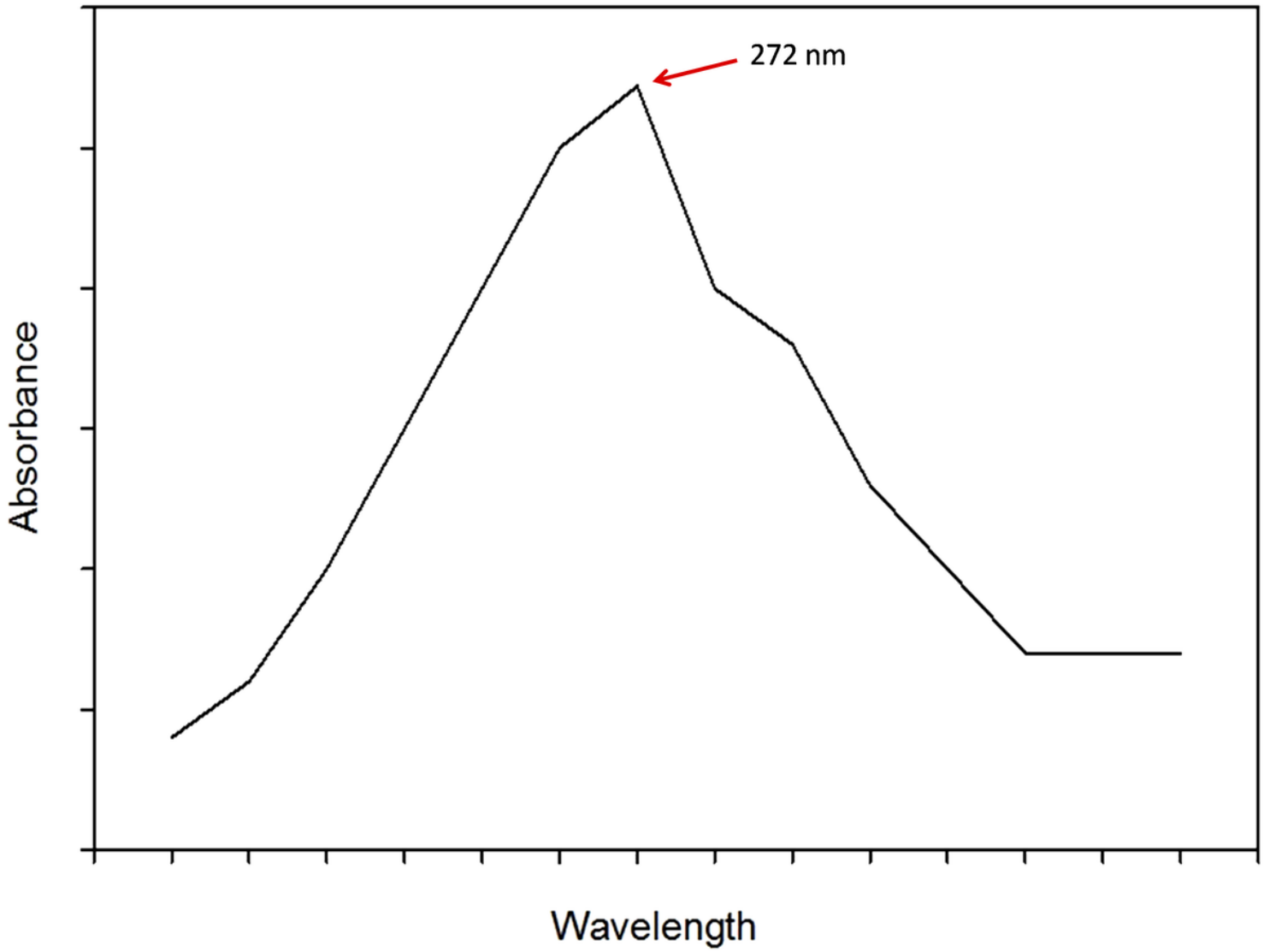

Figure 1

UV-Vis plot

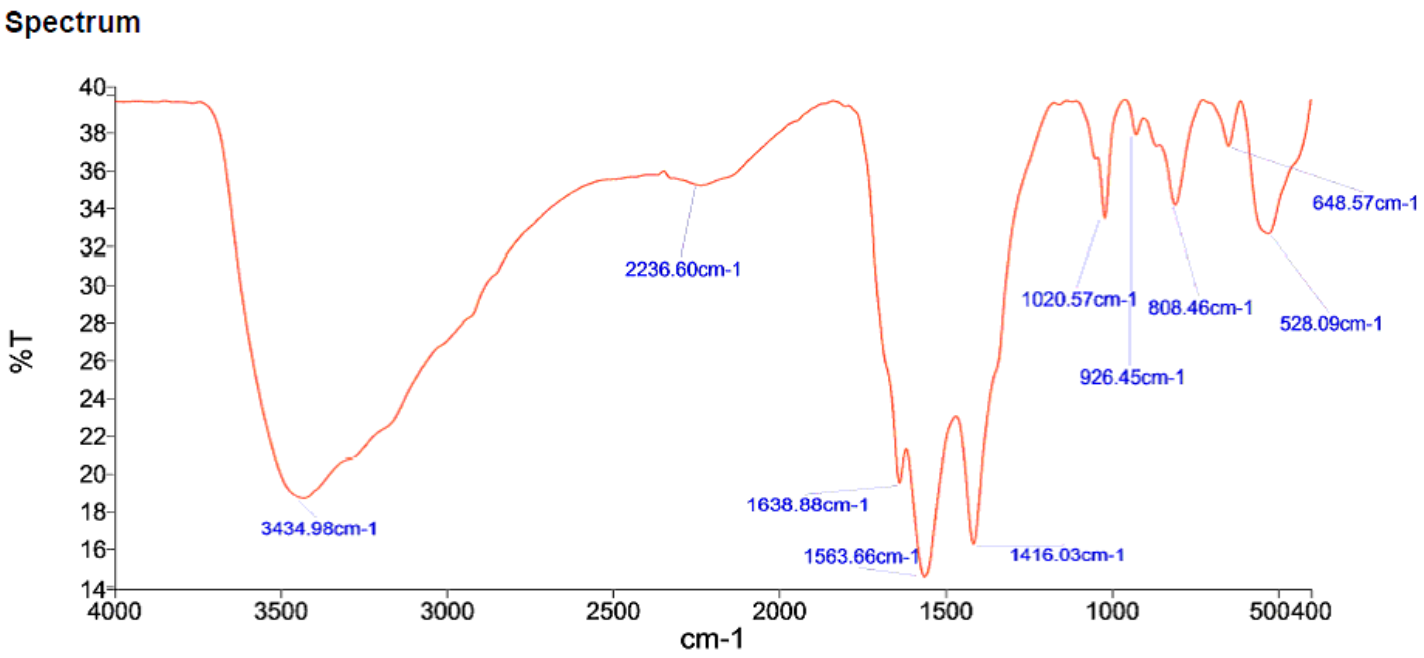

Figure 2

FTIR spectrum of zinc oxide nanoparticles of seed coat of almond

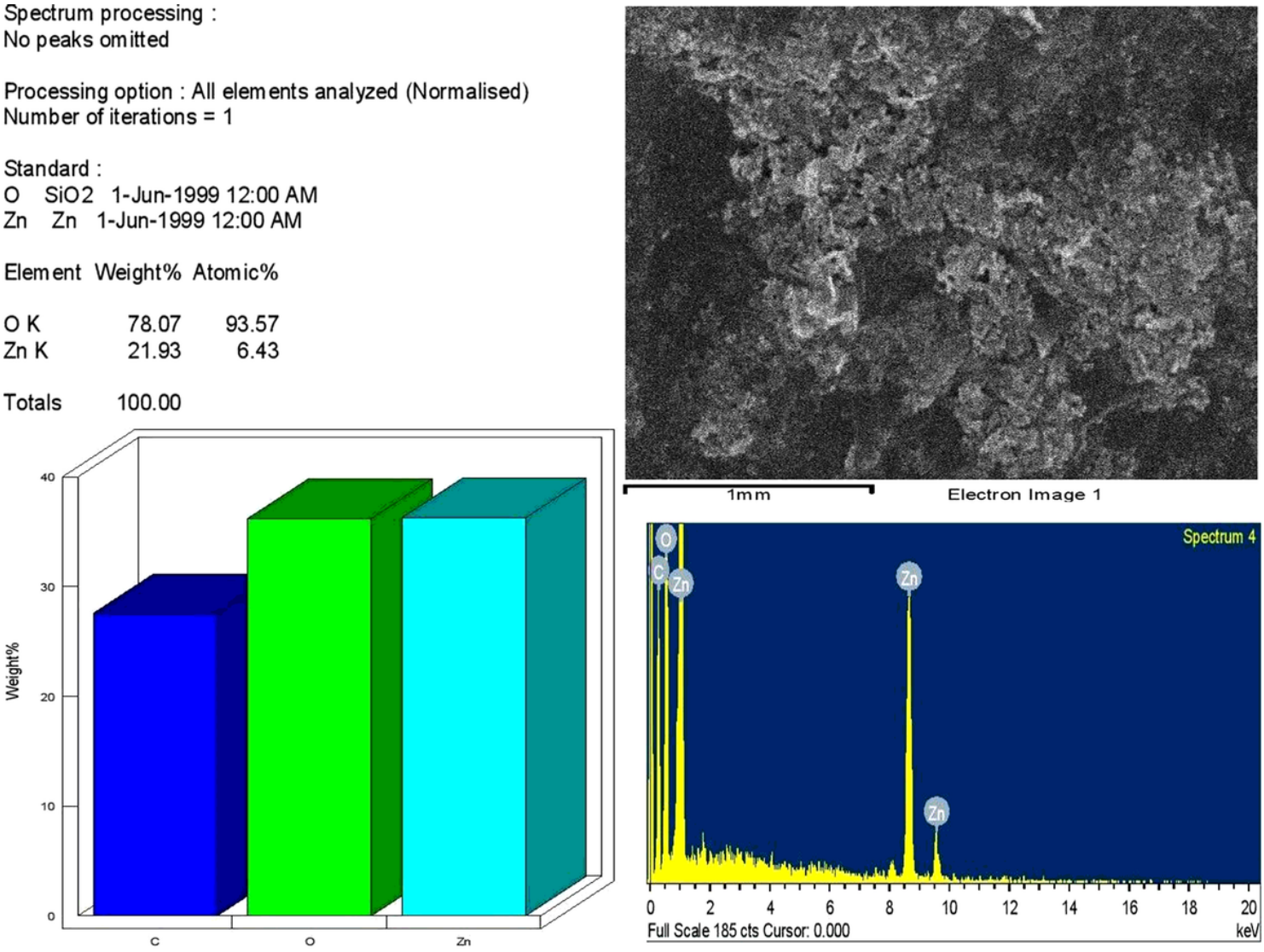

Figure 3

EDS analysis of synthesized ZnONPs

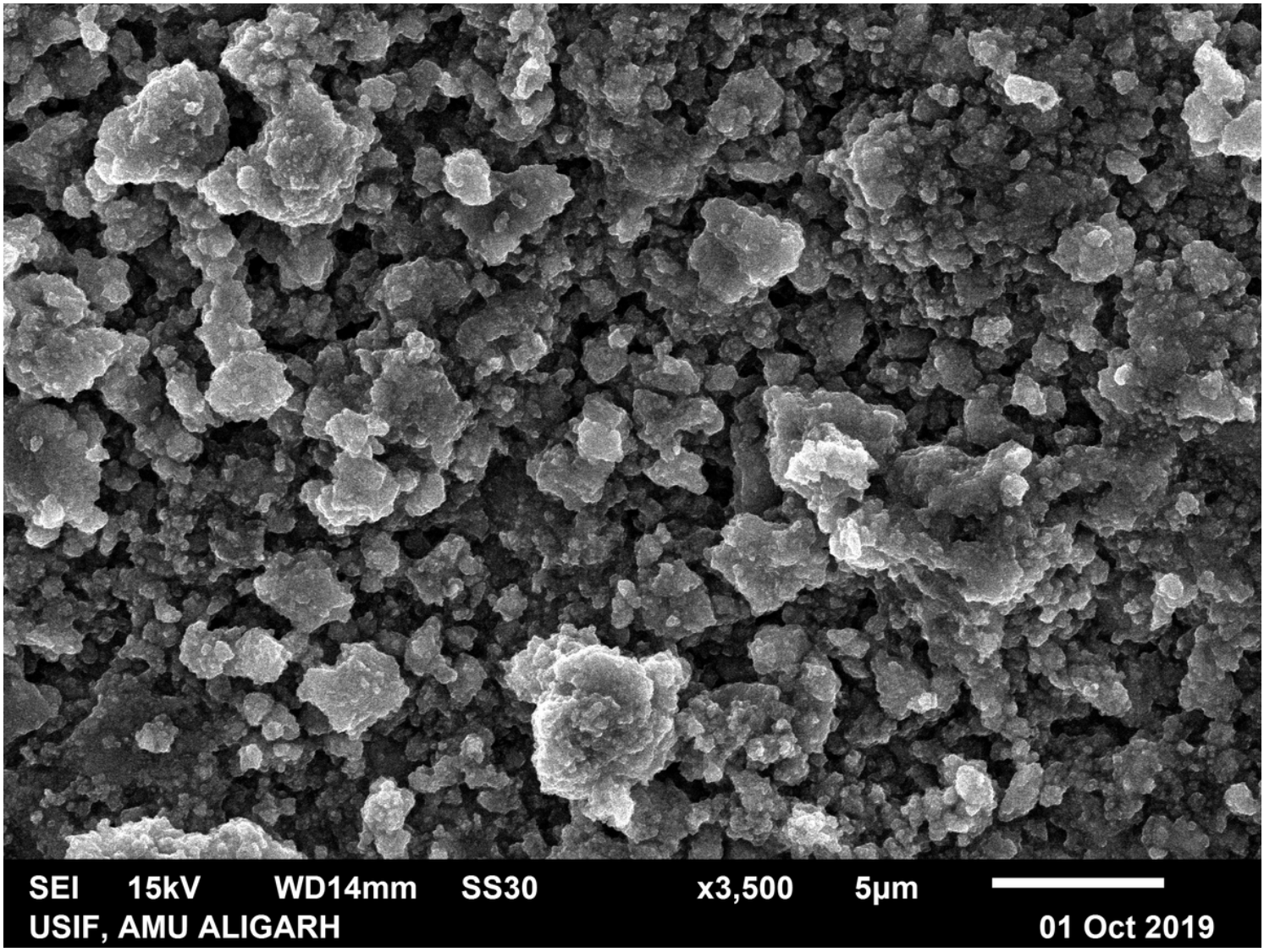

Figure 4

SEM images of ZnO NPs at different magnification.

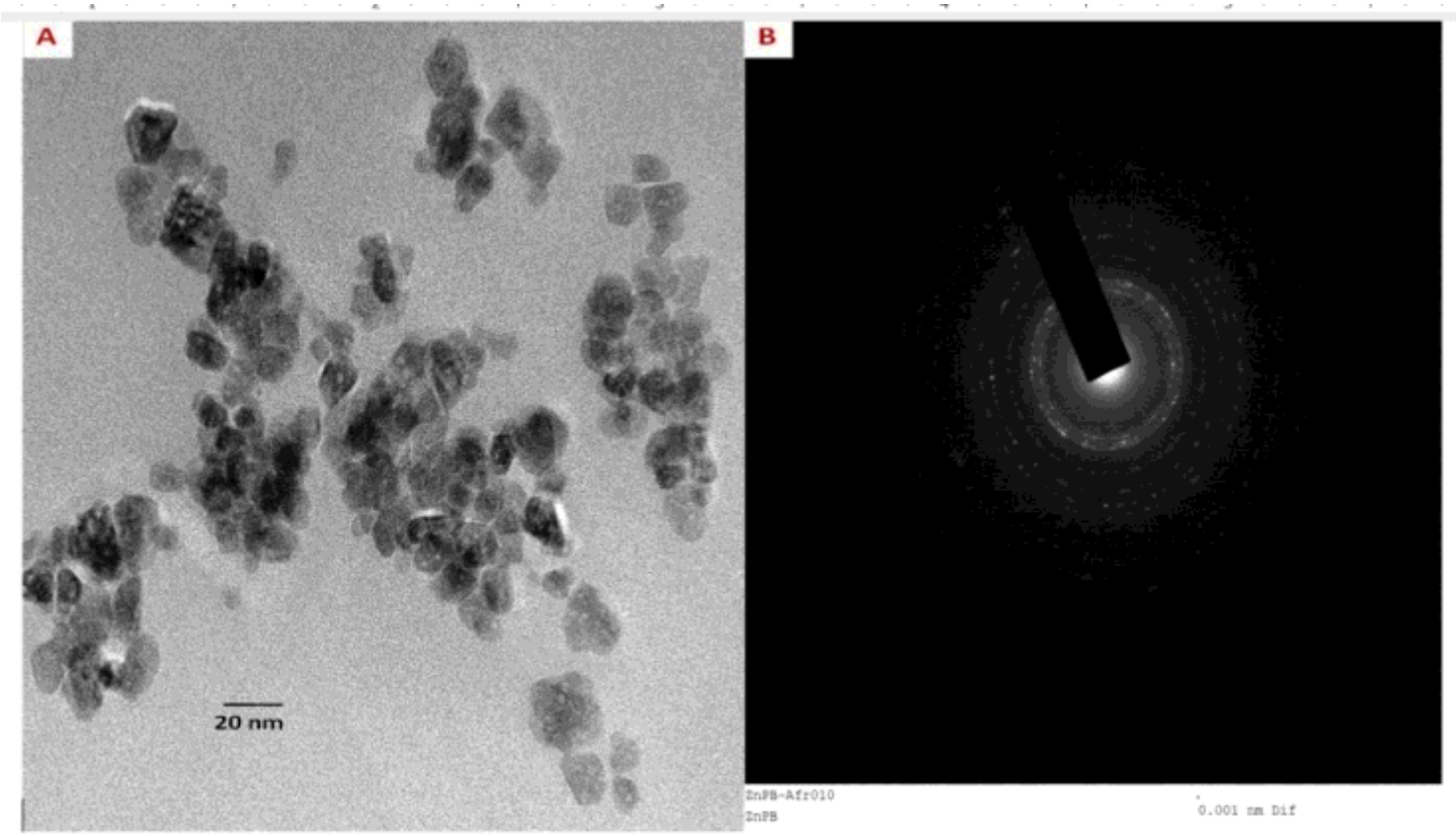

Figure 5

A- TEM of synthesized ZnO NPs. B- SAED pattern of ZnO NPs

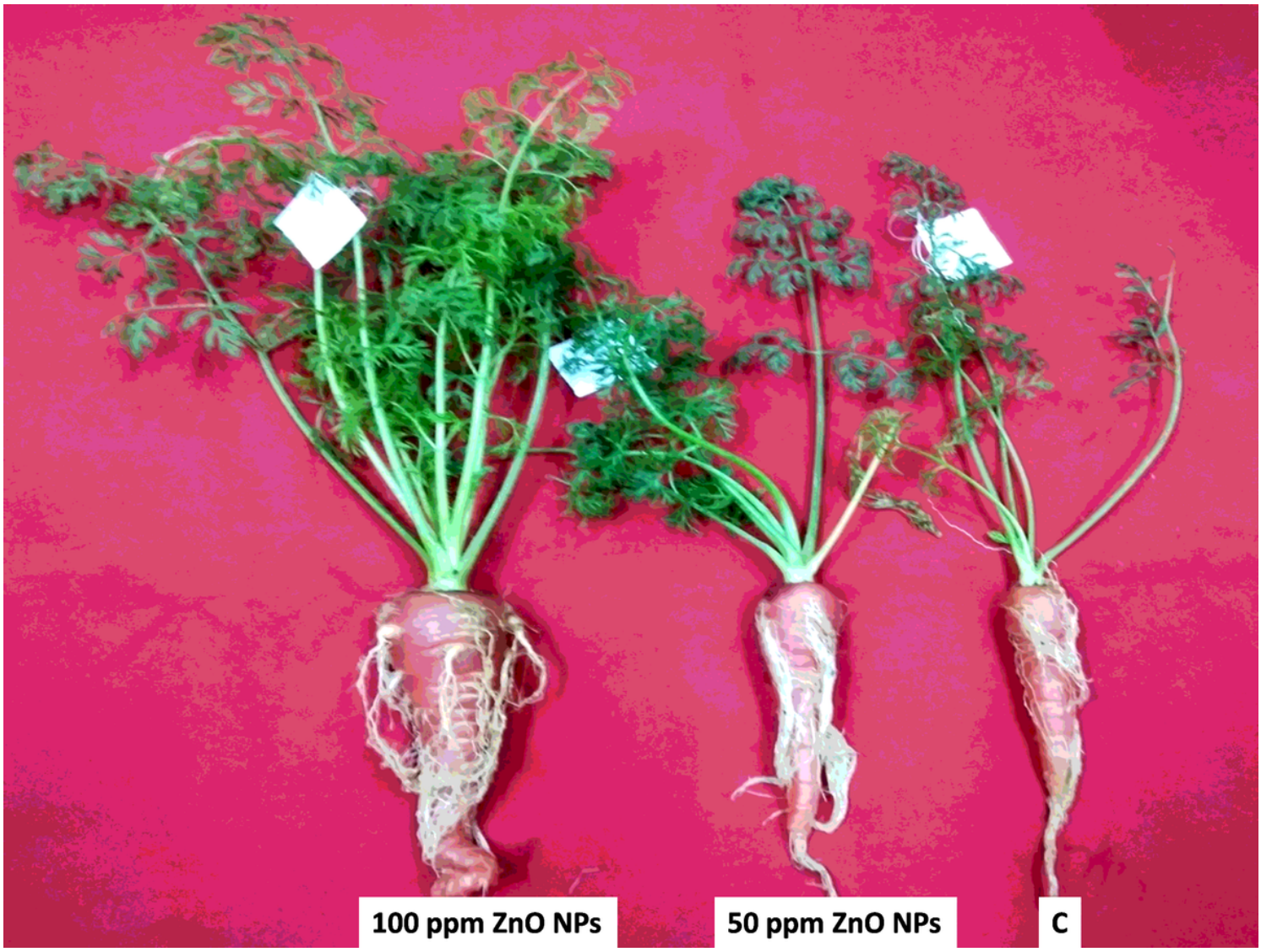

Figure 6

Carrot plants sprayed with 100 ppm and 50 ppm ZnO NPs and one control (without spraying of ZnO NPs).

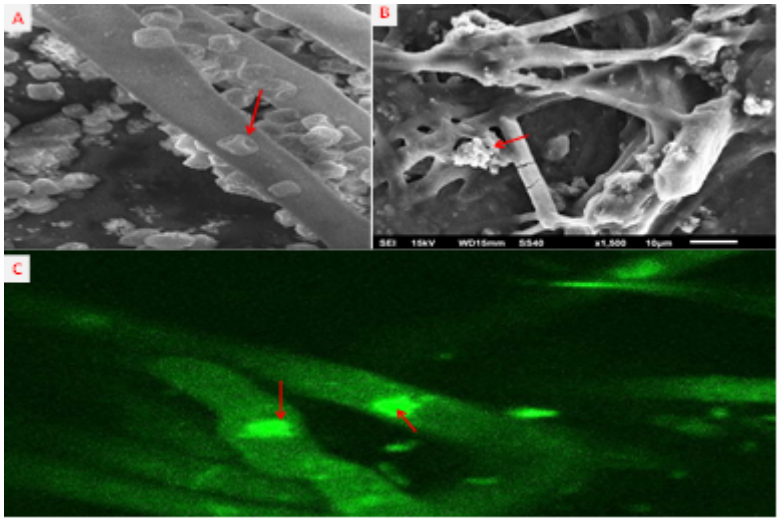

Figure 7

A- SEM image showing distorted mycelium treated with synthesized nanocomposite. 7B- Confocal image showing nanocomposite on fungal mycelium surface and 7C- Confocal image showing penetration of nanoparticles in fungal mycelium

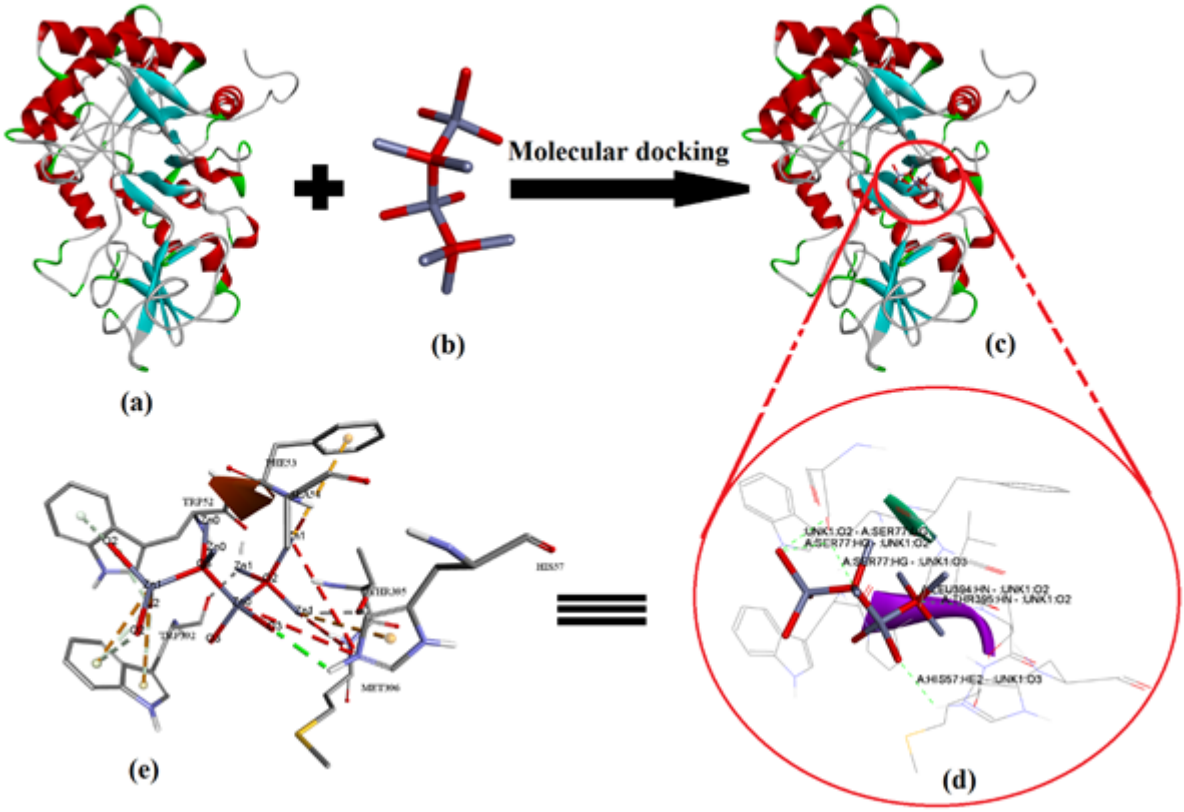

Figure 8

Figure showing molecular docking