Biogenic Synthesis of Copper oxide and Zinc oxide Nano Particles and their Application as Antifungal Agents

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Abstract

Biogenic methods provide rapid and eco-friendly synthesis of nanoparticles (NPs) which are nontoxic and monodispersed. The copper oxide and zinc oxide NPs were synthesized using food peel extract of Malus domestica as a reducing agent. The synthesized NPs were analyzed byScanning Electron Microscopy (SEM),Fourier Transform Infrared spectroscopy (FT-IR),X-rays Diffraction (XRD) and UV-Visible spectroscopy. The crystallite size of copper oxide NPs was found to be 34nm with definite cubic structure while that of zinc oxide NPs was 12nm with a hexagonal floral pattern. The antifungal potential of synthesized copper oxide and zinc oxide NPs was studied against Aspergillus niger and Lacio diplodia which are common fungal species involved in mango rotting. These NPs have been proved as effective antifungal agents in food industry.

Keywords—*Zinc oxide (ZnO) and Copper oxide (CuO) nanoparticles, Aspergillus niger, Lacio diplodia, Antifungal activity.*

I. INTRODUCTION

Nano-technology kingdom has made our day-to-day existence more gratifying and commodious. One of the flying advancements in nano regime technology is the employment of various biological systems for nano-particle fabrication. The development of eco-friendly, simple, inexpensive, clean and nontoxic synthetic procedures for metal nano-particles (MNPs) has highlighted the importance of nano-biotechnology on global canvas. These bio-nano particles (BNPs) show diverse morphologies, aspect ratio, chemical compositions and biomedical and pharmaceutical claims with no or least hostile effects [1-6]. The attachment of high levels of toxicity and serious hazardous with the MNPs fabricated by non-biological (chemical and physical) routes has kicked them off for medical and biological application. Some of these non-biological routes include thermal evaporation, ultrathin films, lithographic techniques, layer by layer growth, diffusion flame NPs synthesis [7], sol–gel process, electro-deposition, chemical vapor deposition [8,9] chemical solution deposition, hydrolysis [10], catalytic route, wet chemical method [11] and co-precipitation method.

Nano-biotechnology make use of rich biodiversity for NPs fabrication. Natural compounds such as steroids, terpenoids, alkaloids, phenolic acid, saponins and flavonoids which are locked in various plant organs (roots, stem, bark, leaves and flowers) are a potential precursor for NP fabrication. These natural compounds and their metabolites occupy the role of reducing and stabilizing agencies for fabrication of novel BNPs [3]. Flower extracts of Carthamus tinctorius [12], Gnidia glauca [13], Plumeria alba, Nyctanthes arbortristis [14] have been successfully employed for synthesis of gold NPs. On parallel grounds silver NPs are bio fabricated from stem extracts of Ocimum sanctum [15],Coleus aromaticus [16], Boswellia ovalifoliolata [17] and Piper nigrum [18]. Diopyros kaki [19] and Cacumen platycladi [20] leaf and peel extracts were employed for platinum NPs synthesis respectively. NP fabricated from plant extracts depict effective anti-microbial, anti-fungal and medicinal applications. Triangular shaped cadmium oxide NPs obtained from Achillea wilhelmsii leaf extract show enhance anti-microbial activity [21]. Biologically synthesized silver NPs effectively inhibit yeast growth [22]. While platinum NPs efficiently work against cancer cells, anti-fungal and anti-microbial activity [23]. Dong et al. reported flower shaped copper oxide NPs [24]. Ghasemian et al. revealed anti-fungal potential of Cu NPs against Fusarium solani, Alternaria alternata and Pencillium chrysogenum [25]. Rod, spherical and rectangular morphologies of zinc oxide were reported by Sarkar et al. [26]. He et al.studied the anti-fungal prospective for Pencillium expansum and Botrytis cinerea of ZnO NPs [27].

The present work is an endeavor for bio fabrication of ZnO and CuO NPs from Malus domestica. To our best knowledge no work has been carried out to record the anti-fungal potential of above cited bio fabricated NPs against two Mango rotting

pathogenic species which are Lacio diplodia and Aspergillus niger.

II. MATERIALS AND METHOD

A. Sample and sample preparation

Malus domestica belongs to the rose family *Rosaceae* known as apple. Its biological components are polyphenols like quercetin, procyanidin, flavonoids and acids like maleic acid.
The apple was bought from the near market of Scheme III Rawalpindi. It was properly washed and then the peels were crushed and weighed. The extract was made of two different concentrations that were 59g and 25g of peel in 1000ml of distilled water. After 5 min boiling, the extract was cooled and filtered.

B. Synthesis of CuO NPs

0.001M salt solution of Cu $(NO_3)_2$ was added in apple extract (25g/L) in a ratio of 1:1, 1:3 and 1:9respectively. The rate of reaction was improved by adding 3M KOH and heating at 30˚C for 4hours at pH 11.Orange colored copper NPs were collected, dried and preserved for further analysis.Similar procedure was followed with 50g/L mass of apple extract.

C. Synthesis of ZnO NPs

0.001M salt solution of Zn $(NO_3)_2$ was added in apple extract (25g/L) in a ratio of 1:1, 1:3 and 1:9respectively. The rate of reaction was improved by adding 3M KOH and heating at 30˚C for 4hours at pH 11. White colored copper NPs were collected, dried and preserved for further analysis.Similar procedure was followed with 50g/L mass of apple extract. The particle appearance was different for the three different ratios of salt solution and extract.
Synthesized NPs were characterized by various standard analytical techniques including; SEM (MIRA3 TESCAN), FTIR (IR Affinity-1S), XRD (MRS-11) and UV-visible spectrophotometer (UV-1800).

III. ANTIFUNGAL ACTIVITY OF CuO AND ZnO NPs

Much concentration has been laid on antifungal and antimicrobial activity of NPs which have been fabricated by physical or chemical courses. The most common fungi intricate in fruit rotting include; *Aspergillus niger* and *Lacio diplodia.* BNPs are more encouraging to use in food products because of their environmental friendship.

A. Media preparation

Potato Dextrose Agar (PDA) was used as the growth media. PDA (30g) was dissolved in 1000ml of sterilized water. After that, media was autoclaved at 121°C for 40minutes. In order to retard the bacterial growth, 30mg/L streptomycin was also added in the media.

B. Plate preparation

After autoclaving, media was allowed to cool at room temperature. When temperature of media was at 60°C then 30mg/L of streptomycin was added to retard bacterial growth. Afterwards the media was poured into petri plates and was left for 24 hours for solidification. After solidification, the plates were kept inverted to avoid any water content. This all work was done in laminar flow to avoid any bacterial growth.

C. Fungi inoculation

At the centre of Petri plates containing PDA media, a small mass of fungi *Aspergillus niger* and *Lacio diplodia* was placed under proper precautionary measures. Then, these plates were incubated for 7 days at 25-30°C temperature for growth of fungal specie. After incubation period, fungal colonies were observed.

D. Analysis of NPs (CuO and ZnO) for antifungal activity

The CuO and ZnO NPs antifungal effect was determined using $0.05mgL^{-1}$ of NPs. After autoclaving the media at 121°C for 30minutes, it was poured in autoclaved and oven dried plates in laminar flow after cooling and 30 mg/L of streptomycin was also added and the plates were solidified. Then, a fungal specie disc was placed at the centre of plates individually and was incubated for 7 days at 25°C. Experiment was conducted in three replicates. The inoculated plates were incubated at 25°C for at least 7 days. The activity of MNPs on the fungal growth was monitored by measuring the diameter of fungal disc. Activity was estimated by measuring growth of fungal isolate in media containing NPs against the controlled media lacking NPs.

III. RESULTS AND DISCUSSION

The different mixing ratios of salt solutions and apple extract (1:9, 1:3 and1:1) give different particle appearance as shown in Table I.

Salt Solution (M)	pH of Salt Solution Settled (by Adding KOH)	Apple Peel Extract (Mass Variation Given in gm)		pH of Extract Settled (by Adding KOH)	Temperature (°C)	Salt Solution : Apple Extract	Formation of NPs
0.001M $Cu(NO_3)_2$	11	50	25	11	30	1:1	No formation
						1:3	Particle appearance
						1:9	Significant particle appearance
0.001M $Zn(NO_3)_2$	11	50	25	11	30	1:1	No formation
						1:3	Particle appearance
						1:9	Significant Particle appearance

Table I Synthesis Parameters Forcuo And Zno Nps

A. *Surface morphology*

SEMmicrograph shows that the synthesized CuO NPs show cubic morphology with size less than 0.80 to 1.47µm. The Fig. 1(a) and 1(b) are the high and low magnification images of CuO NPs respectively. These revealed that the particles are separated and no agglomeration has been observed. Cubic geometry is repeated throughout the agglomerate. CuO NPs were also synthesized by Tamaekong *et al*. by thermal method [28] which had spherical shape and size ranged from 10 to 20nm. Similarly these NPs were also synthesized by Dong *et al.*having flower shaped structures [29].

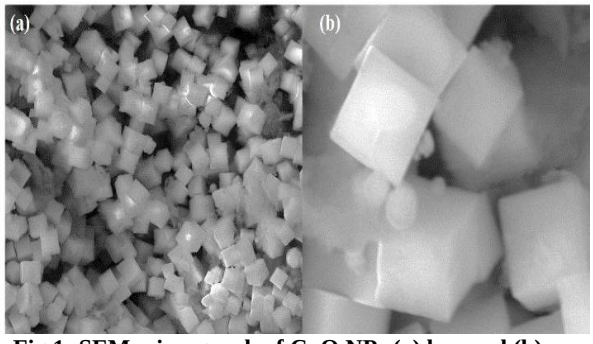

Fig 1: SEM micrograph of CuO NPs (a) low and (b) high magnification

Fig. 2(a) and 2(b) shows definite hexagonal ZnO NPs having size range 1.11 to 2.03µm. These NPs agglomerated in a perfect floral pattern. Synthesis of ZnO NPs with spherical shape have also been reported by biogenic method [30].

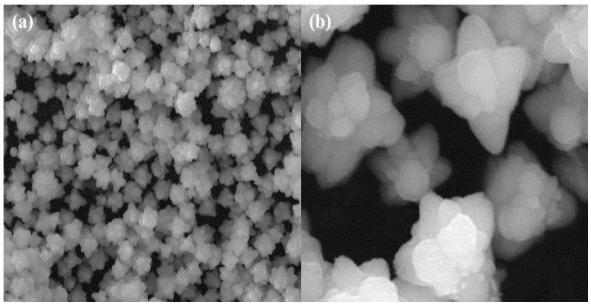

Fig 2: SEM micrograph of ZnO NPs (a) low and (b) high magnification

B. *FTIR studies*

Peaks at 3431, 2230, 1440 cm^{-1} were observed in FTIR spectrum of CuO NPs (Fig. 3). The band at 3431 cm^{-1} is due to –OH stretching. The alkyne bonding is displayed by the peak at 2230 cm^{-1} .This peak is in agreement with Banarjee *et al.* work [31].The peak at 1440 cm^{-1} is due to C-H stretching vibrations of amide linkages. These significant frequencies are highlighted in Table II.

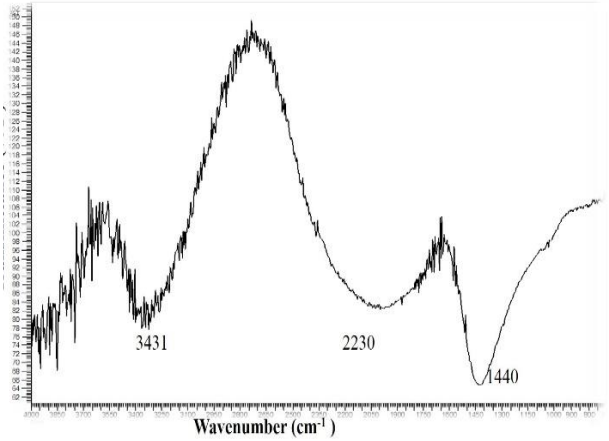

Fig 3: FTIR spectrum of CuO NPs synthesized biogenically

Table II Ftir Peak Assignment For Cuo Nps Synthesized Biogenically

Functionalities	Peaks for CuO NPs (cm^{-1})	Literature Cited
-OH of -COOH	3431	(Daniel et al; 2012)
-C-H stretching vibrations of amide linkages	1440	(Awwad, Salem et al; 2013)

Fig 4 shows FTIR analysis of ZnO NPs. Peaks at 3492, 1627, 1406, 913, 538 cm^{-1} were detected. The peak of –COOH group for –OH stretching appeared at 3492 cm^{-1} .The absorption at 1627 cm^{-1} is assigned to the stretching vibration of aromatic carbonyl (C=O) group. The spectrum also shows band at 1406 cm^{-1} for amide group which arises due to amino stretching vibrations in amide linkages of the protein. The Table III is illustrating the peak values obtained from the spectrum.

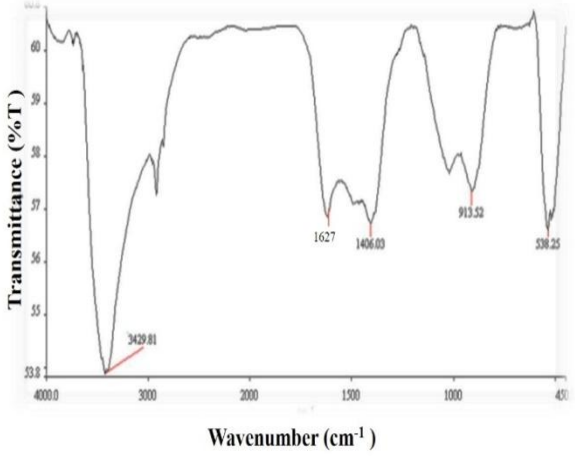

Fig 4: FTIR spectrum of ZnO NPs synthesized biogenically

Table III Ftir Peak Assignment For Zno Nps Synthesized Biogenically

Functionality	Peaks for ZnO NPs (cm^{-1})	Literature Cited
-OH of -COOH	3429	(Balashanmuga & Santhosh et al; 2014)
C=O stretching vibrations	1627	(Zahir et al; 2013)
-C-H stretching vibrations of amide linkages	1406	(Awwad, Salem et al; 2013)

As –OH peak is present in spectra of both NPs synthesized by using fruit peel of *Malus domestica* which confirms the bonding of phenols to metal species. The stretching vibrations of amide linkages are also detected which assures the bonding of metal particles with protein molecules in *Malus domestica* matrix.

C. XRD measurements

The crystalline nature of synthesized NPs was confirmed by XRDanalysis. The peaks at 2Θ values of 29.647, 36.521, 42.423, 52.607, 73.793 corresponds to (110), (111), (200), (211) and (311) Bragg's reflection planes of CuO NPs as shown in Fig. 5. The crystallite size was obtained byDebye-Scherer's formula given as:

$$D = K\frac{\lambda}{\beta}(cos\ \theta)$$

Where D is crystal size; K is Bragg's constant having value 0.9; λ corresponds to the wavelength of CuK_{α} radiations(λ = 0.15406nm); β is line width at half maximum heightand ɵ is diffraction angle. For CuO NPsthe particles or crystallite size is 34nm.

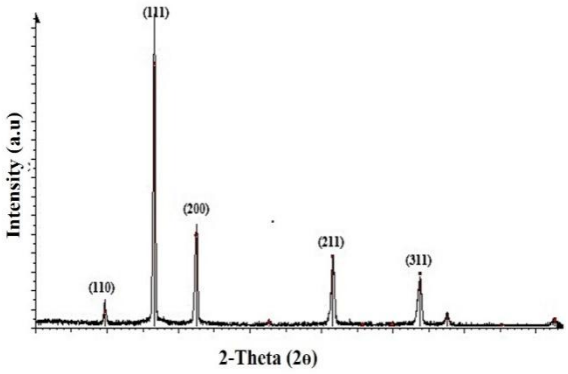

Fig 5: XRD pattern for CuO NPs

Similarly the peaks at 2Θ = 31.773, 34.441, 36.262, 47.558, 56.604, 62.888 and 67.968 corresponding to (100), (002), (101), (102), (110),(103), and (112) planesof ZnO NPs respectively as shown in Fig. 6. Exactly same results were reported by Muthuvinayagam *et al.*[32].The size of ZnO NPs comes out to be 12nm by Debye-Scherer's formula.

These results confirm the presence of ZnO and CuO NPs and assure that the sample is 100% pure. So the procedure adopted for the synthesis of NPs in this work holds very good control over particle size.

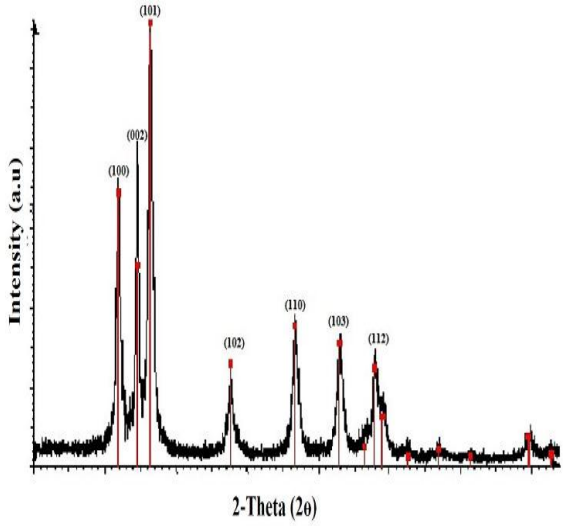

Fig 6: XRD pattern for ZnO NPs

D. UV-Visible analysis

The optical properties of samples were examined by UV-visible spectroscopy. For CuO NPs, a band exactly at 380nm was observed (Fig. 7). This band corresponds to the characteristic surface

Plasmon resonance of the valence electrons of CuO NPsand ascertains the presence of CuO NPs. These values counterpartthe band value reported by Karnith and Geetha [33].

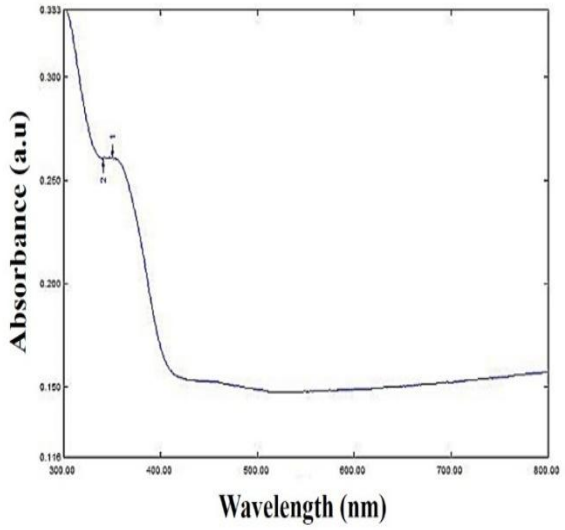

Fig 7: UV-Visible spectrum for CuO NPs

For ZnO NPs, an absorption band at 430 nm was spottedas shown in Fig. 8. The same peak was observed by Kunda et al.[34].

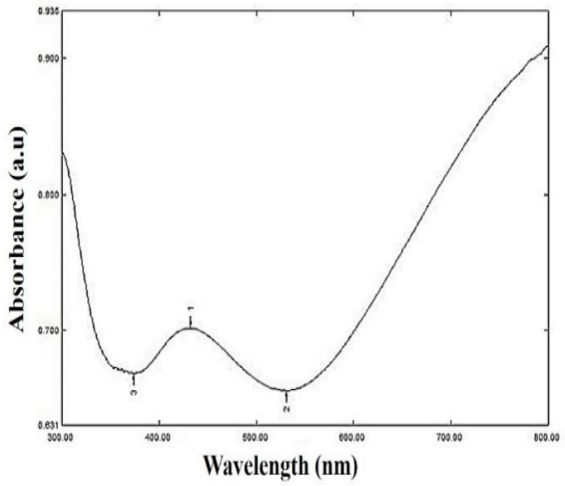

Fig 8: UV-Visible spectrum for ZnO NPs

E.Antifungal activity

The antifungal potential of synthesized NPs was evaluated against two common notorious fungi, responsible for mango rotting, Lacio diplodia andAspergillus nigerZnO NPs showed enhanced activity as compared to CuO NPs against Lacio diplodia. Similar results were witnessed by both MNPs against Aspergillus niger. The antifungal influence of CuO and ZnO NPs clearly shows thatLacio diplodia is more sensitive to the effect of ZnO NPs compared to CuONPs with 0.05g/L dose, as evident from petri dishes (Fig. 9(a), 9(b), 10 (a) and 10 (b)).

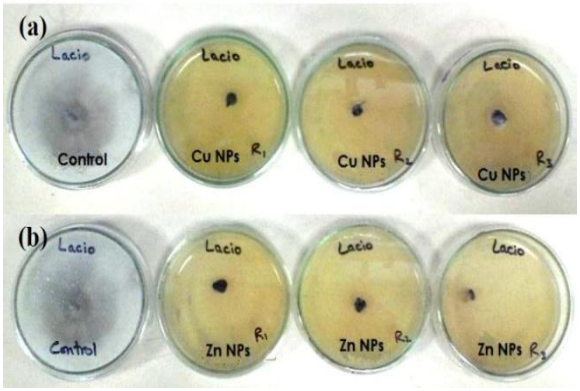

Fig 9:Antifungal potential of (a) CuO NPs and (b) ZnO NPs against *Lacio diplodia*

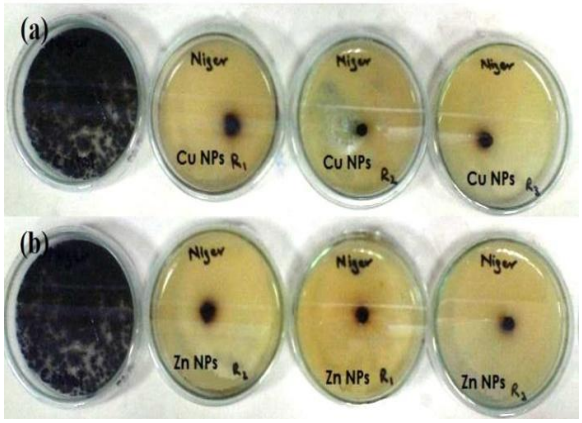

Fig 10:Antifungal potential of (a) CuO NPs and (b) ZnO NPs against Aspergillus niger

The results were evaluated by taking the average of three replicas as shown in Table IV.

TABLE IV Controlled And Radial Growth Of*lacio Diplodia*and*aspergillus Niger*in The Presence Ofcuo And Zno Nps

	Lacio diplodia				*Aspergillus niger*			
	R1	R2	R3	Av	R1	R2	R3	Av
Radial Growth in Presence of CuO NPs	0.56	0.5	0.74	0.6	0.77	0.60	0.87	0.7
Radial Growth in Presence of ZnO NPs	0.67	0.53	0.23	0.48	0.78	0.64	0.53	0.65
Control	5.16	5.16	5.16	5.16	9	9	9	9

The percentage growth inhibition was calculated by using the formula given below:

$$I = \frac{C - T}{T} 100$$

Where I is inhibited growth, C is control and T is treatment sample.

The percentage inhibition ofLacio diplodia by CuO and ZnO NPs is 88% and 90% respectively, while that for Aspergillus nigeris 92% for both MNPs.

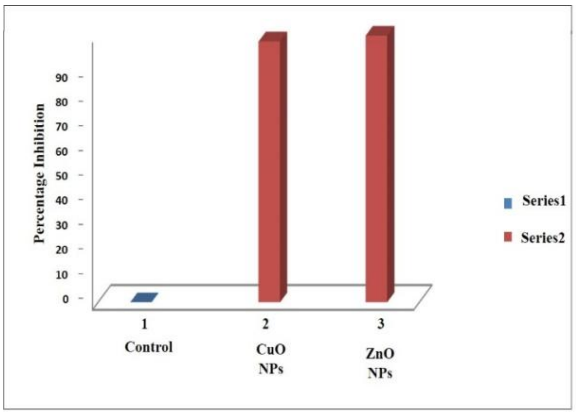

Fig11: Percentage inhibition plots of *Lacio diplodia* by CuO and ZnO NPs

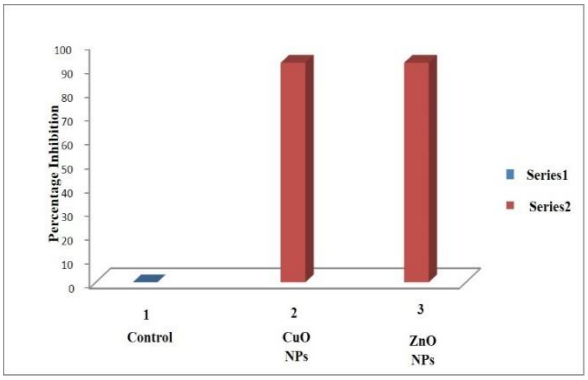

Fig 12: Percentage inhibition plots of*Aspergillus niger*by CuO and ZnO NPs

IV. CONCLUSIONS

The method adopted provides proficient and easy route for the synthesis of different MNPs from plants extract. The method is economically favorable without reducing the efficiency of NPs. It leads to green and eco-friendly approach reducing metals from non-toxic reducing agents. The reducing agent opted in the present work was *Malus domestica* (apple) peel extract. The salts of metals selected were copper nitrate (Cu $(NO_3)_2$) andzinc nitrate (Zn $(NO_3)_2$) and 0.001 M solution of each was prepared. The fruit peel extract was prepared by taking the fruit and washed thoroughly, weighed, crushed and then boiled for 5min. Two concentrations of fruit peel extract were made to optimize the parameters. The fruit peel extract and the salt solution of each metal was mixed individually in a definite ratio.

KOH was added to accelerate the rate of reaction. Metal oxide NPs were appeared in the reaction mixture after 4hours. The CuO particles were red and those of ZnO were white in coloration. The morphology and crystallite size was described by using SEM, XRD, UV and FTIR. CuO NPs were of cubic morphology with the size of 34nm and ZnO NPs were of hexagonal floral geometry with 12nm size. The FTIR spectra confirmed that phenols and proteins were involved in the reduction of metal ions. The antifungal activity of both the metal oxide NPs was studied *against Lacio* diplodia and *Aspergillus niger* which are common rotting fungi of fruits. The potential of ZnO NPs was more for *Lacio diplodia* than the CuO NPs. While *Aspergillus niger* was equally inhibited by both of MNPs. Thus the biogenically synthesized metal oxide NPs possess novel features including shape, size and effective potential towards fungal growth retardation. The particles are green, safe and effective to be employed in food business.

ACKNOWLEDGMENT

The authors are pleased to acknowledge Dr. Aliya Fazal, National Center for Physics (NCP), Islamabad, Pakistan, for granting permission to use lab facilities for the completion of this research work.

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