Antifungal properties of zinc oxide and copper oxide nanoparticles against purple blotch disease in *Allium cepa*: Review

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***Abstract*- Alternaria porri, a notorious fungal pathogen, poses significant threats to various crops, particularly those belonging to the Allium genus. Traditional methods of combating this pathogen often involve the use of chemical fungicides, which can have adverse effects on the environment and human health. In recent years, the development of alternative eco-friendly approaches utilizing nanoparticles has gained considerable attention. Among these, zinc (Zn) and copper (Cu) nanoparticles synthesized through green routes have emerged as promising candidates due to their inherent antifungal properties and environmentally benign synthesis processes. This review aims to provide a comprehensive overview of the effectiveness of green-synthesized Zn and Cu nanoparticles against Alternaria porri. It will discuss the methods employed for the green synthesis of these nanoparticles, their physicochemical properties, mode of action against the fungal pathogen, and their potential applications in agriculture for the management of Alternaria porri. Furthermore, challenges and future prospects associated with the utilization of green-synthesized Zn and Cu nanoparticles in controlling Alternaria porri will be critically evaluated.**

***Index Terms*: *Alternaria porri*, zinc nanoparticles, copper nanoparticles, green synthesis, antifungal activity.**

I. INTRODUCTION

One of the most effective and active avenues for study is nanotechnology, which offers the potential to synthesize nanoparticles using plant leaf extract and boost the biosynthesis of inorganic metallic oxide, such as copper oxide, zinc oxide nanoparticles (NPs). NPs have significantly improved qualities based on particular characteristics including size, delivery and shape[1].

Conventional techniques for creating nanoparticles frequently include the use of dangerous chemicals, high temperatures, and energy-intensive procedures, which raises questions about the environment and possible toxicity. Green synthesis has drawn a lot of attention as a potential solution to these problems. Green synthesis, sometimes referred to as sustainable or environmentally friendly synthesis, is the process of creating nanoparticles by using natural resources, biomolecules, or materials that are not harmful to the environment[2].

The potential of several nanoparticles (NPs) to treat plant diseases has been investigated. It has been stated that copper (Cu) NPs and zinc (Zn) NPs have the potential to be an effective fungicide. To be used in agriculture, the Cu-NPs and Zn-NPs need to be synthesised in an economical manner.According to reports, Cu-NPs function as a fungicide against several plant pathogenic fungal species, including Penicillium italicum, Penicillium digitatum, Fusarium oxysporum, Phoma destructiva, Curvularia lunata, Alternaria alternate, and Fusarium sp[3].

Nanoparticles of copper are incredibly small. Usually, their surface-to-volume ratios are high. They function as antifungal and antibacterial agents. Because nanoparticles oxidise gradually, their near-proximity contact with microbial membranes and the metal ions they release into solutions are what cause their antimicrobial actions. In solutions that have had their cupric ions released. The lipid membrane can produce deadly hydroxyl free radicals when they are in close proximity. Free radicals have the ability to oxidise and break down the lipids in cell membranes, which can cause the membranes to collapse and leak intracellular materials. These cells might not be able to sustain necessary metabolic processes, which could result in cellular modification[4].

Due to the fact that plants contain a vast array of bioactive compounds, numerous plant parts or entire plants have been utilised in the environmentally friendly synthesis of Cu NPs. Plant extracts have been effectively used for this purpose. Extracts from a variety of plant species, such as the peel of Punica granatum, the stem of Zingiber officinale, the juice of Citrus medica Linn.The fruit of Ziziphus spina-christi (L.) Willd, the root and leaf of Asparagus adscendens Roxb, the leaf of Eclipta prostrata, the leaf of Ginkgo biloba Linn, the leaf of Plantago asiatica, Thymus vulgaris L, black tea leaf, Terminalia catappa leaf, and the leaf of Azadirachta indica have all been successfully used in the synthesis of Cu NPs[5].

The synthesis of ZnO-NPs has been reported using a variety of environmentally friendly methods, including microwave- and ultrasonic-assisted synthesis. Microbes or plant extract are used in biological synthesis techniques to aid in the production of nanoparticles. Green practices are more important than chemical processes because of a number of attributes that can be linked to them. The main benefit is that biosynthesized nanoparticles can be used directly in living systems or for biomedical applications. This is because these nanoparticles have a lower level of toxicity than those made using physicochemical techniques. Additional benefits could include the corona formation caused by surface modification of the nanoparticles, which makes them more appropriate in living systems, and the stabilising effects of the biocomponents used in the synthesis process[6].
The commercial routes of ZnNPs preparation are as follows: vapor-phase transport method [7], sol-gel method[8], precipitation method[9], microwave technique[10], aerosol process[11], hydrothermal synthesis[12], electrochemical method, mechano-chemical method, laser ablation, sonochemical, polyol method[13].

Because using plants avoids the time-consuming process of cultivating cell cultures and is more beneficial for large-scale nanoparticle synthesis, it is an advantageous process compared to using commercial products. As a result, there is a marked increase in the biogenesis of nanoparticles using plant materials. For example, Acalypa indica and Corriandrum sativum, Aloe barbadensis, Morinda pubescens, Passiflora foetida, Hybanthus enneaspermus, Aloe barbadensis, Parthenium hysterophorus, Moringa oleifera and the milky latex of Calotropis procera are all well-documented plant materials for the synthesis of ZnNPs[14].
Utilising plants to synthesise nanoparticles from leaf extraction is a more straightforward process. There has been no poison released into the environment, and it is not toxic. Additionally, it ought to be substituted with alternative techniques for creating nanoparticles. Due to important information regarding the plant's potential medicinal uses, this study tells a biomimetic method for the environmentally friendly synthesis of zinc oxide nanoparticles from Moringa oleifera and copper nanoparticles from Morinda citrifolia. The copper nanoparticles and zinc oxide nanoparticles functional components, structure, and particle size were studied with UV–vis, FTIR, XRD, SEM, TEM, and DLS analysis. [1, 2].
A significant fungus found all over the world is called Alternaria. The saprophytic, endophytic, and pathogenic species of this hyphomycetous ascomycete with phaeodictyospores can be plant pathogens, post-harvest pathogens, or human pathogens. The majority of Alternaria species with medium-to-large conidia and long beaks are found in the Porri section, which is the largest. Purple blotch, a highly destructive disease that affects onions all over the world, is caused by Alternaria porri, with up to 100% of seeds lost due to the disease, seed and bulb yields are significantly reduced[15].

II. METHODOLOGY

1. Synthesis of zinc nanoparticles by *Abel et al*:

The source of zinc ion was zinc nitrate hydroxide. The solution of (Zn(NO3)2·6H2O) was prepared in deionized water. For the preparation of zinc oxide NPs, the flask containing 50 ml of zinc nitrate hydroxide (0.2 M) was reacted with 10 mm of the aqueous leaf extract of Moringa oleifera and stirred using a magnetic stirrer heated at 70° C and stirring is nonstop until a homogenous mixture of the solution is attained.
The uniform solution is desiccated in a warm air oven at the temperature of 100-110° C for 90 minutes and annealed at 300-350° C for 1 h in a muffle boiler. The particles with yellow color obtained are wrinkled in a metallic mortar and pestle to get green-synthesized zinc oxide nanoparticles.

2. Synthesis of copper nanoparticles

Copper sulphate (CuSO4·5H2O), Hydrochloric acid (HCl) (35%), sodium hydroxide (98%) was used to monitoring the pH. The leaves of *Morinda citrifolia* need to be collected and dried. The dissolution of 2.5 g of CuSO4·5H2O in 100 mL deionized water yielded a 1× 10–2 M stock solution of copper sulphate[2].
Preparation of *Morinda citrifolia* leaf extract:
Morinda citrifolia, a plant of the *Rubiaceae* family. The leaves of *Morinda citrifolia* were weighed and cleaned several times with tap water and deionized water after collecting to get rid of any extra dust or contaminants. After that, slice the leaf in small pieces, add 100 mL of distilled water, and immerse the mixture in a water bath heated to 60 °C for 1 h. The extract was kept at 4 °C for further studies[2].
Synthesis of CuO NPs from *Morinda citrifolia* leaf extract
2.5 g of CuSO4·5H2O was dissolved in 100 mL of Deionized water (DI) to initiate the green synthesis process for CuO NPs. After, 50 mL of *Morinda citrifolia* extract solution to 100 mL of 1× 10–2 M CuSO4·5H2O solution, the pH was kept at 7.0 with NaOH. The solution then underwent to a reflux at a magnetic stirrer. The colour of the solution changed as it was stirring with a from pale-green to a deep-brown while maintaining for 5 h at 70 °C. After centrifuging the solution for 24 h, it was filtered. The solid precipitate was washed three times with deionised water, followed by an 100% ethanol wash for CuO NPs separation, dried at 60 °C for 4 h, and kept at 4 °C for further application[2].

3. Poisoned food technique

Inoculum of A. porri (VPRI 21961, isolated from diseased leeks in Cranbourne, Victoria, in June 1995) and S. vesicarium (VPRI 21964, isolated from diseased garlic in Tenterfield, New South Wales, in October 1994) were prepared from stock cultures stored in sterile deionized water (SDW) at 4°C. A piece of culture medium containing mycelium (approx. 2 × 2 mm) of each fungus was placed centrally on V8 juice agar medium in 5- or 9-cm-diameter disposable Petri dishes and incubated at 25 ± 1°C under a 12-h photoperiod using a near-ultraviolet (NUV, 20 W NEC 10TL) light suspended approximately 20 cm above the cultures. Conidia were dislodged from 5- or 7-day-old cultures by flooding with SDW and brushing gently with a soft bristle paint brush. Conidium concentration was determined using a haemocytometer[16].

III. Results and discussion

Characterization of synthesized Zn NPs:

The crystal phase analysis of the ZnO nanoparticles was characterized by using the Bruker D8 diffract meter with CuKα (1.5406 A°) radiation occupied at 40 mA and 40 kV. The diffractometer was adjusted to scan the nanoparticles at a scanning rate of 0.6 sec-1 and a step size of 0.12, covering a 2θ range of 25-45°. ZnO nanoparticles' optical characteristics were captured using a Shimadzu UV-3600 plus UV-Vis spectrophotometer equipped with a PerkinElmer. Scanning electron microscopy (SEM) combined with an X-ray diffraction (XRD) analyzer was used to examine the films' morphology[1].

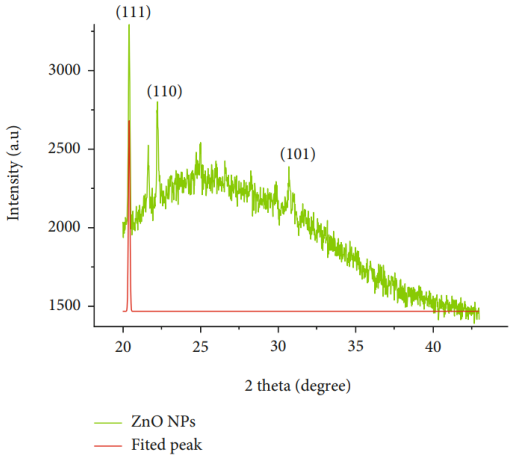

Fig. 1 X-ray diffraction patterns of ZnO NPs[1].

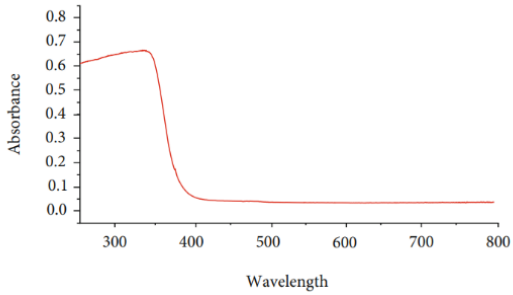

Fig. 2 UV-visible analysis of ZnO NPs[1].

Table 1: Comparison of evaluated crystal size D (nm) by using XRD results[1].

No.	***2Theta (degree)***	***FWHM (radians)***	***D(nm)***
1	20.41	0.095	90.51
2	23.42	7.85	1.10
3	30.72	0.154	55.21
4	30.81	8.62	0.90

Characterization of synthesized Cu NPs:

The UV–Visible spectrum of effectively obtained CuO NPs was collected with an spectrophotometer. The UV spectrum of copper oxide nanoparticle synthesis in colloidal solution was observed at wavelengths ranging from 200 to 800 nm. The FTIR spectrometer with KBr pellet was used for collecting functional group data in the region of 4000–400 cm−1. The FTIR spectrum of obtained CuO NPs was examined. Different modes of vibration in the CuO NPs have been identifed and assigned to evaluate the presence of diferent functional groups that aid the extract of the Morinda citrifolia plant. XRD measurement of the CuO NPs, where only 5.0 ml of the extract was added, was done on a Shimadzu XRD-6000 difractometer operating at a voltage of 40 kV and current of 20 mA with Cu-Kα radiation (λ= 1.54 Å). Te XRD spectrum has been examined and acquired with scanning range values of 20° and 80°.SEM study of the surface morphology of CuO NPs was performed (CARL ZEISS, Jena, Germany). Te inner morphology of the CuO nanoparticles

was studied with Morinda citrifolia extract, and images were captured using a TEM (JEOL, JEM-2100, Japan). For descriptive purposes, a 5.0 ml of the materials were sonicated in ethanol, and a drop of it was cast in a copper grid with a 300-mesh carbon layer by layer for magnetic measurements. Te particle size distribution (PSD) of the synthesized CuO NPs have been measured with the Dynamic Light Scattering (DLS) measurements instrument's standard operating procedure[2].

Assay for antifungal activity by poisoned food technique:
The poisoned food methodology was used to examine the impact of Zn and Cu NPs on the growth of A. porri mycelium. The target organism is cultivated on a culture medium containing the test chemical as part of the procedure. The first medium selected was PDA. As the medium is being poured into Petri plates, the necessary quantity of Zn and Cu NPs has been aseptically added to 100 ml of PDA in sterile flasks from the Zn and Cu NPs stock solution. N treatments, including the control, were prepared.
To guarantee uniform chemical dispersion, the medium was gently shaken; however, bubble formation was prevented. Subsequently, a 20 ml aseptic medium was transferred into each petri plate individually and allowed to solidify. A 4 mm-diameter mycelial disc was cut from the edge of fungus cultures that were 10 days old in order to inoculate the Petri plates. After being inoculated, the mycelia disc was raised for an additional 10–12 days at 27 ± 1 °C and flipped towards the centre of the Petri plates to allow direct contact with the toxic medium. By cultivating the A. Porri on free media containing Zn and Cu NPs, appropriate control was also preserved at the same time. When the fungus reached maximum development in the control petri dish, observations of linear growth were taken. The percentage inhibition of fungal development in the case of respective treatment was assessed using the formula.
Inhibition percentage (I) = (C-T)/C × 100
where.
I= Percent inhibition,
C = Colony diameter in control (mm),
T = Colony diameter in respective treatment (mm).

The data on percent inhibition were transformed in arcs and statistically examined using a completely randomized design (CRD)[17].

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