IJCRT.ORG ISSN : 2320-2882
INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

A Review On Combating Black Mold Disease In Onions: A Synergistic Approach To Combat *Aspergillus Niger* For Sustainable Storage

Dr. Shaik Beebi[1a], Dr. Syed Vaziha Tahaseen[1b], Dr. Matala Bhupathi Rayalu [2]
1a. Lecturer in Chemistry, S.R.R. & C.V.R.GDC (A), Vijayawada, Andhra Pradesh, India
1b. Lecturer in Biochemistry, S.R.R. & C.V.R.GDC (A), Vijayawada, Andhra Pradesh, India
2. Lecturer in Botany, Govt. Degree College, Kovvur, East Godavari district, Andhra Pradesh, India

Abstract: Aspergillus niger is a widespread fungal pathogen responsible for black mold on onions and other crops, resulting in significant post-harvest losses, due to reduced shelf life, spoilage, and economic losses to diminished market value and increased waste. The rise in resistance to traditional antifungal treatments has driven the search for alternative, sustainable methods to combat this pathogen. This research review explores the antifungal efficacy of various plant extracts and nanoparticles against A. niger, with a focus on their effectiveness in controlling onion infections. We conducted a comprehensive analysis of existing research on plant extracts from neem *(Azadirachta indica)*, garlic *(Allium sativum)*, clove *(Syzygium aromaticum)*, cinnamon *(Cinnamomum verum)*, and turmeric *(Curcuma longa)* for their ability to inhibit fungal growth. Additionally, recent advancements in the development and application of nanoparticles including silver (AgNPs), zinc oxide (ZnO NPs), copper oxide (CuO NPs), and iron oxide (Fe_3O_4 NPs) were tested for their antifungal properties. These findings suggest that both plant extracts and nanoparticles present promising alternatives for managing A. niger infections in onions, potentially reducing dependence on synthetic fungicides and supporting sustainable agricultural practices. The review underscores the potential of combining biocompatible nanoparticles with plant extracts and offers a cost-effective and sustainable solution for future antifungal agents in food packaging, clinical, and agricultural settings. This analysis highlights the most promising nanoparticle formulations by using fungicidal plant extracts for inhibiting *Aspergillus niger*, thereby enhancing the post-harvest storage of onions.

Keywords: *Aspergillus niger, antifungal properties, plant extracts, Nanoparticles.*

Introduction:

Onions (*Allium cepa* L.) are a staple of Indian cuisine, with Indians consuming around 5 kg per person per month—amounting to approximately 15 million tonnes annually. Onions are the second most consumed vegetable in India, following potatoes. Globally, India is the second-largest producer of onions, accounting for about 14% of the world's onion production, second only to China. Andhra Pradesh is a key onion-growing state, contributing 3.28% to the total onion cultivation area in the country **(APEDA, 2023)**. Onion is a highly perishable vegetable, but with proper care in pre- and post-harvest stages, it can be stored for 8 to 10 months. However, fungal bulb rot can cause a loss of about 15-30% during the storage of various onion varieties. Several fungal pathogens attack onions during the post-harvest storage period, with Aspergillus species (particularly A. niger) being the most aggressive in the field (Pal, 2007) that causes black mold rot in onions, a major disease affecting post-harvest stages (**Wani and Taskeen, 2011**). The pathogen is transmitted through infected soil or seeds, and affected bulbs display neck discoloration, black mycelia, and hidden spores on the outer dry scales (Sumner et al., 1995). The optimal temperature range for A. niger growth is between 28°C and 34°C, with warm and moist conditions further promoting infection (**Tysoni JL,2004**).

Over 50 synthetic fungicides are recommended to reduce storage losses in onions by preventing pre- and post-harvest fungal diseases. Common fungicides include carbendazim, Bronopol, Mancozeb, salicylic acid, Bavistin, and maleic hydrazide. However, maleic hydrazide was banned in India in 2009 due to adverse effects. Post-harvest treatments like Falisolan, Mancozeb, and carbendazim reduce fungal pathogens in stored onions. Sulfur dust fumigation also minimizes losses. However, overuse of fungicides increases resistance and poses health and environmental risks, necessitating safer alternatives (**Kumar et al., 2015**). Plant-based antimicrobials hold great promise for controlling pathogenic fungi like Aspergillus niger, offering a sustainable alternative to synthetic fungicides and reducing the associated environmental and health risks.

Research has demonstrated that various plant extracts possess significant antifungal activity against *Aspergillus niger*, a common pathogenic fungus responsible for spoilage and infections. Among the most effective extracts are those from *Syzygium jambolanum* seeds, where methanolic and aqueous extracts show strong efficacy with Minimum Fungicidal Concentrations (MFC) ranging from 500 µg/mL to 125-500 µg/mL, respectively (**Chandrasekaran M, 2004**). Similarly, ethyl alcohol extracts of garlic and onion exhibit notable inhibitory effects against *A. niger*, with Minimum Inhibitory Concentration (MIC) and MFC values of 425 mg/mL and 450 mg/mL, respectively (**Irkin & Korukluoglu, 2007**). *Moringa oleifera* leaf extracts, particularly methanol and ethanol extracts, also display potent antifungal activity, achieving a maximum zone of inhibition of 16 mm at a concentration of 300 mg/mL (Maqsood et al., 2017). Another promising agent is neem leaf aqueous extract, which shows increasing effectiveness against *A. niger*, with growth inhibition rates of 35.22% at a 5% concentration, 49.55% at 10%, 86.22% at 15%, and complete inhibition at 20% concentration (**Mahmoud DA et al., 2011**).

Essential oils (EOs) from clove (*Eugenia caryophyllata*) and pepper (*Piper nigrum*) have been extensively studied for their antifungal properties. These oils effectively inhibited *A. niger* at concentrations of 400 to 500 ppm after 10 days of culturing, with clove essential oil reducing fungal growth by 50% to 70% when applied to tomato fruit samples. Gas chromatography-mass spectrometry (GC-MS) identified eugenol as the main compound in clove oil and limonene, sabinene, and β-caryophyllene in pepper oil, contributing to their antifungal efficacy (**Muñoz Castellanos L et al., 2020**). Among the most potent natural antifungals is cinnamon essential oil, which has a MIC of 25.00 μg/mL against *A. niger* 103. Cinnamon oil disrupts the integrity of the fungal cell wall and membrane, effectively inhibiting growth, with its antifungal activity being concentration-dependent (**Mingcheng Wang et al., 2023**). However, many plant-based extracts, such as polyphenols, flavonoids, and essential oils, often face challenges like poor solubility, low bioavailability, instability, and rapid degradation. Moreover, their direct use in high doses can lead to the development of resistance, limiting their effectiveness over time.

Nanomaterials have emerged as a superior approach to overcoming these limitations. Encapsulating or synthesizing bioactive compounds into nanoparticles significantly enhances their solubility, stability, and bioavailability. Nanoparticles (NPs) possess a high surface area-to-volume ratio, which promotes better interaction with fungal cells and more efficient uptake by the target organism. Additionally, nanomaterials' unique physicochemical properties and enhanced bioactivity allow for a more controlled and sustained release of antifungal agents, minimizing the risk of resistance development. Biocompatible nanoparticles, including metallic, polymeric, lipid-based, and hybrid forms, are being extensively studied for their antifungal activity against A. niger. Their effectiveness is largely influenced by their synthesis methods, surface modifications, and mechanisms of action, which can be precisely tailored to enhance their antimicrobial potency. By leveraging the antifungal properties of plant-based bioactive compounds, nanoparticles can be synthesized to enhance their efficacy and affordability.

Recent advancements of various nanomaterials as antifungal agents against Aspergillus niger:

Nanotechnology has emerged as a promising field in developing antimicrobial agents for various applications, including treating microbial infections (**Makvandi et al., 2020**). One promising area of interest is the use of metal-based nanomaterials, including metal and metal oxide nanoparticles and transition metal nanosheets, to enhance food storage and packaging due to their potent antimicrobial properties (**Makvandi et al., 2020**). Eco-friendly silver nanoparticles (Ag NPs) were synthesized using *Ocimum sanctum* (Tulasi) leaf extract as a natural reducing agent, highlighting Tulasi's potential as a bioactive compound. The synthesized Ag NPs were characterized by UV-visible spectroscopy, SEM, and XRD, and their antifungal activity against *Aspergillus niger* was evaluated using an agar well diffusion assay. At a concentration of 50 µL, the Ag NPs showed a significant antifungal effect, with a zone of inhibition measuring 12.24 ± 0.03 mm, demonstrating strong efficacy compared to standard antifungal agents Ketoconazole and Itraconazole. This study underscores the potential of medicinal plants like Tulasi in the green synthesis of nanoparticles with potent antifungal properties (**Yogeswari Rout et al**.,**2012).**

Zinc oxide (ZnO) nanoparticles were biosynthesized using *Crataegus oxyacantha* leaf extract, which served as a reducing and stabilizing agent in the synthesis process. The antifungal activity of both the ZnO nanoparticles and the leaf extract was tested against *Aspergillus niger*. While the leaf extract alone exhibited no antifungal effect, the biosynthesized ZnO nanoparticles demonstrated significantly enhanced antifungal potency. The ZnO nanoparticles achieved a minimum inhibitory concentration (MIC) of 500 µg/mL, effectively inhibiting fungal growth, and produced a clear zone of inhibition measuring 18±0.2 mm in the well diffusion assay. These results emphasize that when the bioactive compounds of *Crataegus oxyacantha* are encapsulated in a nanoparticle form, their antifungal properties are significantly amplified. This enhancement can be attributed to the increased surface area, improved bioavailability, and the ability of nanoparticles to penetrate fungal cells more effectively, leading to higher antimicrobial efficacy against pathogens like *A. niger (***Alireza Momeni et al. 2024***)*

Moringa oleifera leaf extracts, particularly methanol and ethanol extracts, have been shown to possess antifungal activity against *Aspergillus niger*, achieving a maximum zone of inhibition of 16 mm at a high concentration of 300 mg/mL. However, encapsulating these bioactive compounds in copper oxide (CuO) nanoparticles significantly enhances their potency. In a study by Pagar et al., CuO nanoparticles synthesized using *Moringa oleifera* leaf extract were characterized by XRD, FESEM, EDX, FT-IR, and UV-DRS, revealing a quasi-spherical morphology with a crystalline size of 35 to 95 nm. These nanoparticles exhibited remarkable antifungal activity, achieving complete inhibition of *A. niger* at just 250 µg/mL in the agar plate method, far outperforming the leaf extracts alone. Compared to the standard antifungal agent Griseofulvin, which required a concentration of 500 µg/mL for similar effects, the CuO nanoparticles demonstrated superior efficacy, underscoring the enhanced potency achieved through nanoparticulation (**Pagar et al., 2020**).

Iron oxide nanoparticles were synthesized using a green approach, with tannic acid (a plant polyphenol) serving as both the reducing and capping agent, resulting in nanoparticles sized between 10–30 nm. The antifungal activity of these nanoparticles was evaluated against *Aspergillus niger* and other fungal species by measuring the zones of inhibition at various concentrations on culture media. The iron oxide nanoparticles effectively inhibited spore germination, showing the largest zone of inhibition against *Penicillium chrysogenum* (28.67 mm), followed by *A. niger* (26.33 mm). The minimum inhibitory concentration (MIC) against *A. niger* was found to be 0.016 mg/mL, compared to the positive control, Hexahit, which had an MIC of 0.004 mg/mL (**Shazia Parveen et al., 2018**).

Silver nanoparticles (AgNPs) were biosynthesized by Wan Nur Atiqah Wan Shamsudin, using neem leaf aqueous extract (*Azadirachta Indica*), and their antifungal properties were tested against *Aspergillus niger*. The AgNPs displayed a UV-vis absorption peak around 421 nm, confirming their crystalline structure. These particles exhibited a spherical morphology with an average diameter of 20.13 ± 3.3 nm. Key phytochemicals in neem leaf extract, including 8-Debenzoylpaeoniflorin, Icariside B4, Picrasinoside A, 4,8,12-Trimethyltridecanoic acid, 11-O-p-Coumarylnepeticin, and Ganoderic acid S, served as reducing and capping agents in the nanoparticle synthesis process. The biosynthesized AgNPs demonstrated strong antifungal activity against *Aspergillus* spp., particularly in the agar well diffusion assay, where they achieved a maximum inhibition zone of 26.54 ± 1.23 mm. FESEM analysis revealed significant hyphal damage and deformation in the fungus when exposed to AgNPs, resulting in inhibited fungal growth and reproduction. The inhibition zones ranged from 26.54 ± 1.23 mm at 100 µg/mL to 17.76 ± 0.54 mm at 10 µg/mL, highlighting the dose-dependent antifungal effects of AgNPs against *Aspergillus* species (**Wan Nur Atiqah, W. S, 2023**).

Among the nanoparticles discussed, Copper Oxide Nanoparticles (CuO NPs) and Zinc Oxide Nanoparticles (ZnO NPs) are the most cost-effective options. They provide a good balance of antifungal activity and affordability, making them suitable for practical applications in the storage of onions. CuO NPs, in particular, offer a high level of efficacy at lower concentrations and are cost-effective, making them a preferred choice for large-scale applications.

Mechanism: How NPs disrupt fungal growth?

Momeni et al. reported that the antimicrobial activity of ZnO nanoparticles is primarily due to their interaction with the microbial membrane, leading to its rupture and causing leakage of the fungal cytoplasm. The nanoscale ZnO particles exhibit antimicrobial properties by inducing oxidative stress through the generation of reactive oxygen species (ROS) on their surfaces. These ROS, which includes molecules such as oxygen, perhydroxyl radicals, superoxide anions, and hydroxyl radicals, have the ability to damage DNA, RNA, and oxidize proteins and lipids, ultimately resulting in the disintegration of fungal cells (**Momeni et al, 2024**).

Shazia Parveen et al. explained that the antimicrobial activity of nanoparticles is largely attributed to their small size and high surface area-to-volume ratio, allowing them to effectively envelop microorganisms and restrict oxygen supply for respiration (Shazia Parveen et al., 2018). Additionally, the inhibitory effect of nanoparticles arises from oxidative stress induction, metal ion release, and non-oxidative mechanisms. In the case of iron oxide nanoparticles, their antimicrobial activity is primarily due to the formation of reactive oxygen species (ROS), which cause oxidative stress and damage proteins and DNA in microorganisms. Iron nanoparticles generate ROS and induce the Fenton reaction, as iron is a strong reducing agent that promotes the breakdown of functional groups in membrane proteins and lipopolysaccharides. This process leads to oxidative damage through intracellular oxygen, further exacerbating the Fenton reaction. The nanoparticles penetrate disrupted membranes, causing additional cellular damage and ultimately leading to cell death (**Changha Lee et al., 2008**)

Wan Nur Atiqah Wan Shamsudin explained the antifungal mechanism of AgNPs synthesized using neem extract by targeting the hyphae, the tubular structures in filamentous fungi responsible for nutrient absorption and maintaining cellular functions. Disruption of hyphal morphology impairs vital cellular processes, leading to growth inhibition and reduced fungal reproduction. FESEM analysis provided direct visualization of these morphological changes in *Aspergillus niger* hyphae treated with AgNPs. In the control group, hyphae appeared smooth, linear, and intact, while those exposed to AgNPs exhibited noticeable shrinkage, severe malformation, and significant cell damage. The biosynthesized AgNPs caused structural changes in the fungal hyphae, leading to drying and extreme shrinkage of the cell walls. This damage was primarily due to the release of silver ions from the AgNPs, which adhered to the fungal cell walls and membranes, penetrating the cells and causing various physiological disruptions. These intracellular changes ultimately led to fungal cell death, highlighting the potent antifungal action of AgNPs (**Wan Nur Atiqah, W. S, 2023**).

Biocompatibility of antifungal nature of plant extracts and Nanoparticles for Onion storage and packaging:

Plant extracts, such as neem, garlic, and clove, offer a natural alternative to synthetic fungicides. Their biocompatibility and effectiveness make them suitable candidates for use in food packaging. Neem extract, with its azadirachtin, and garlic extract, rich in allicin, both show strong antifungal properties and have been demonstrated to effectively reduce fungal contamination on onions. Clove extract, containing eugenol, also provides robust antifungal protection. The integration of these extracts into packaging materials helps to maintain onion quality by inhibiting fungal growth without introducing harmful residues into the food.

To further enhance the antifungal efficacy of food packing material nanoparticles such as silver (AgNPs), zinc oxide (ZnO NPs), and copper (CuNPs) have emerged as powerful tools.AgNPs, ZnO NPs, and CuNPs effectively disrupt fungal cell membranes and generate reactive oxygen species that lead to fungal cell death. These nanoparticles can offer a high degree of efficacy in controlling fungal contamination on onions due to their large surface area and ability to interact closely with fungal cells. However, their biocompatibility is a critical concern. Proper formulation and encapsulation are necessary to prevent any potential leaching of nanoparticles into the food. Studies have shown that well-formulated nanoparticles can be used in packaging materials with minimal risk of harmful leaching, making them a viable option for food storage.

The application of a combination of antifungal plant extracts and nanoparticles in food packaging presents an effective solution for controlling fungal contamination in onions. Plant extracts offer a natural and biocompatible approach, while nanoparticles provide potent antifungal action with minimal safety concerns. Ongoing research and development are crucial to optimizing these materials for safe and effective use in food packaging, enhancing the preservation and quality of onions.

Conclusion:

While both plant extracts and nanoparticles show promise, some challenges need addressing. For plant extracts, variability in natural sources and the potential for inconsistent antifungal activity can be issues. For nanoparticles, potential concerns include environmental impact and long-term safety. Future research should focus on optimizing the formulation and application methods to maximize efficacy while ensuring safety. Additionally, exploring combinations of plant extracts and nanoparticles could enhance antifungal performance and reduce reliance on single agents. In conclusion, antifungal plant extracts and nanoparticles offer innovative and effective solutions for controlling fungal contamination in onions. Their successful application in food packaging hinges on ensuring their biocompatibility and safety.

Reference :

Agricultural and Processed Food Products Export Development Authority (APEDA) (2023).

Pal M. Veterinary and Medical Mycology. First Edition. Indian Council of Agricultural Research, New Delhi, India; c2007.

Wani AH, Taskeen UN. Management of black mold rot of onion. Journal of Mycology and Plant Pathology. 2011;9:43-49.

Tysoni JL, Fullerton RA. ,2004, Effect of soil born inoculums on incidence of onion black mold (Aspergillus niger).NewZealandPlantProtection; 57: 138-141.

Kumar, Vinay & ., Neeraj & Sharma, Sunil & Sagar, Narashans. (2015). Post Harvest Management of Fungal Diseases in Onion - A Review. International Journal of Current Microbiology and Applied Sciences. 4. 737-752.

Chandrasekaran, M., & Venkatesalu, V. (2004). Antibacterial and antifungal activity of Syzygium jambolanum seeds. Journal of Ethnopharmacology, 91(1), 105–108.

Irkin, Reyhan & Korukluoglu, Mihriban. (2006). Control of Aspergillus niger with garlic, onion and leek extracts. African Journal of Biotechnology (ISSN: 1684-5315) Vol 6 Num 4. 6.

Maqsood, Muhammad & Qureshi, Rahmatullah & Arshad, Muhammad & Ahmad, Muhammad & Ikram, Masroor. (2017). Preliminary phytochemical screening, antifungal and cytotoxic activities of leaves extract of Moringa oleifera lam. From salt range, Pakistan. Pakistan Journal of Botany. 49. 353-359.

Mahmoud DA, Hassanein NM, Youssef KA, Abou Zeid MA. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Braz J Microbiol. 2011 Jul;42(3):1007-16.

Muñoz Castellanos L, Amaya Olivas N, Ayala-Soto J, De La O Contreras CM, Zermeño Ortega M, Sandoval Salas F, Hernández-Ochoa L. *In Vitro* and *In Vivo* Antifungal Activity of Clove (*Eugenia caryophyllata*) and Pepper (*Piper nigrum* L.) Essential Oils and Functional Extracts Against *Fusarium oxysporum* and *Aspergillus niger* in Tomato (*Solanum lycopersicum* L.). Int J Microbiol. 2020 Apr 30.

Wang, Mingcheng, Liu, Huiyuan, Dang, Yuanyuan, Li, Dahong, Qiao, Zhu, Wang, Gailing, Liu, Guo, Xu, Jin, Li, Enzhong, Antifungal Mechanism of Cinnamon Essential Oil against Chinese Yam-Derived *Aspergillus niger*, *Journal of Food Processing and Preservation*, 2023, 5777460, 9 pages, 2023.

Pooyan Makvandi, Chenyu Wang, Ehsan Nazarzadeh Zare, Assunta Borzacchiello, Li-na Niu, Franklin R. Tay, Metal-Based Nanomaterials in Biomedical Applications: Antimicrobial Activity and Cytotoxicity Aspects. *Advanced Functional Materials*, 2020. https://doi.org/10.1002/adfm.201910021.

Rout, Yogeswari et al. “Green synthesis of silver nanoparticles using Ocimum sanctum (Tulashi) and study of their antibacterial and antifungal activities.” Journal of Microbiology and Antimicrobials Vol. 4(6), pp. 103-109, November 2012.

Momeni, A., Meshkatalsadat, M.H. & Pouramiri, B. Catalytic, Antioxidant, and Antifungal Applications of ZnO Nanoparticles: Facile Green Synthesis by *Crataegus oxyacantha* Leaf Aqueous Extract. *BioNanoSci.* **14**, 457–473 (2024). https://doi.org/10.1007/s12668-023-01238-3

Khanderao Pagar; Suresh Ghotekar; Trupti Pagar; Amol Nikam; Shreyas Pansambal; Rajeshwari Oza; Dnyaneshwar Sanap; Harshal Dabhane. "Antifungal activity of biosynthesized CuO nanoparticles using leaves extract of Moringa oleifera and their structural characterizations", *Asian Journal of Nanoscience and Materials*, 3, 1, 2020, 15-23. doi: 10.26655/AJNANOMAT.2020.1.2

Shazia Parveen, Abdul Hamid Wani, Mohammad Ashraf Shah, Henam Sylvia Devi, Mohd Yaqub Bhat, Jahangir Abdullah Koka, Preparation, characterization and antifungal activity of iron oxide nanoparticles, Microbial Pathogenesis, Volume 115, 2018, Pages 287-292, ISSN 0882-4010, https://doi.org/10.1016/j.micpath.2017.12.068.

Wan Nur Atiqah, W. S., Che Ku Mohammad Faizal, C. K. Y., & Siti Hajar Noor, S. (2023). *Antifungal activity of biosynthesized silver nanoparticles mediated by neem leaf extract against aspergillus sp*. AMG Transcend Association. Volume 13, Issue 6, 2023, 583.

Changha Lee, Jee Yeon Kim, Won Il Lee, Kara L. Nelson, Jeyong Yoon, and David L. Sedlak. Bactericidal Effect of Zero-Valent Iron Nanoparticles on Escherichia coli. Environmental Science & Technology, 2008 *42* (13), 4927-4933 DOI: 10.1021/es800408u