Zinc Nanoparticles and their Antifungal Activity against *Alternaria burnsii* Causing Blight of Cumin

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors SBS and RGP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors GBP, MM, KDP and NMG managed the analyses of the study. Author RP managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Zinc nanoparticles (ZnNPs) produced was evaluated against *Alternaria burnsii* to see the inhibitory result of the ZnNPs on the growth of fungal mycelium. Physical characterization of synthesized ZnNPs was 68.04 nm in size, Pdi - 0.263, Keps - 252.4. Among the twelve treatments, the best treatment was synthesized ZnNPs with concentration (750 ppm) evidenced most in effect with 86.17 per cent mycelial growth is inhibited. The next treatment was synthesized ZnNPs with concentration...
concentration (250 ppm) giving 79.26 per cent growth inhibition. Followed by treatment commercial ZnNPs with concentration (1000 ppm) giving 53.46 per cent growth inhibition. The next effective treatment was commercial ZnNPs with concentration (500 ppm) giving 44.69 per cent growth inhibition. Followed by treatment of carbendazim 50 WP with concentration (500 ppm) giving 11.51 per cent growth inhibition. The least effective treatment was commercial ZnNPs with concentration (100 ppm) giving 3.22 per cent growth inhibition. There is directly proportional relationship between increased concentration of the ZnNPs and inhibition of per cent mycelial growth of the pathogen is directly proportional. Further, methylene blue staining was done and the physical changes were studied. The hyphae lost their softness, bulges out followed by reduction in the distance between two hyphae. Branched conidia turned round and conidial development was suppressed. On the basis of this result it can be concluded that ZnNPs inhibited the fungal growth by causing physical damage to the fungal mycelium.

Keywords: Nanoparticles; synthesis; antifungal; keps.

1. INTRODUCTION

Cumin (Cuminum cyminum L.) is the supreme significant spice crop in India and widely known as Jeera or Jiroo. In terms of production, Gujarat stands as the second largest producer contributing to 50-55% of overall production in India, after Rajasthan. Alternaria burnsii commonly known as blight severely affected cumin crop. Its seeds are used for seasoning vegetables, jams, soups, etc. The seeds comprise protein 17.7 per cent, fat 23.8 per cent, carbohydrates with 35.5 per cent and minerals with 7.7 per cent [1]. Cumin grieves from imperative diseases, viz., blight (Alternaria burnsii), wilt (Fusarium oxysporum f.sp. cumini) and powdery mildew (Erysiphe polygoni) due to which significant loss in quality and quantity of cumin yield. Cumin blight (A. burnsii) is of commonly occurred in cumin cultivated parts. The Kaira district of Gujarat having first record of this disease. The [2] recounted its causative agent to be Alternaria burnsii. Afterward, it was conveyed from Rajasthan by Joshi (1955) [3]. The disease usually appears at flowering stage. The infected plants show small, isolated, whitish necrotic areas on the aerial parts, especially on tips of young leaves [4]. Diseased seed are small, deshaped, shriveled, and very light and turn black in colour [5]. Now a days fungicides are having very toxic effect on plants but they are essential to manage this disease. So, eco-friendly products should be find and use to avoid environmental damage. Naturally or green synthesized nanoparticles can develop as impending alternate to toxic fungicides. NPs having various properties such as good surface and size characterization also biological properties, viz., antimicrobial, anticancer and antioxidant etc. The NPs are defined as particles having size less than 100 nm.

Nowadays NPs made from metals such as silver, zinc, iron, copper, sulphur etc. most effectively used. Use of agrochemicals is the best management strategy to control the diseases but it causes development of so many races and biotypes of that pathogen [6]. It also causes residual effect in food chain and harmful for human and microorganisms too [7]. At present, the metallic nanoparticles are potential antimicrobials which are carefully being discovered and widely scrutinised. The nanoparticle comprises of tremendous properties like antimicrobial effect, small in size and high surface to volume ratio. Because of great exterior area of the nanoparticles, its interaction with microbes is increased [8].

2. MATERIALS AND METHODS

Synthesized and commercial zinc nanoparticles were procured from Department of Nanotechnology, AAU, Anand for the evaluation of its anti-fungal efficacy against Alternaria burnsii causing blight of cumin under in vitro conditions. Synthesis of ZnNPs was carried out by following method developed at Department of Nanotechnology, AAU, Anand.

2.1 Synthesis of Zinc Nanoparticles

2.1.1 Methodology

This methodology was suggested by Department of Nanotechnology, AAU, Anand for synthesis of 450 ml of liquid solution of Zinc nanoparticle.

Two bottles each of 500 ml were taken and labelled as ZnSO₄ and sodium tripolyphosphate (STPP). 450 ml Milli-q (MQ) water was occupied in a beaker, 450 mg STPP was added and mixed well. Another 450 ml MQ water was taken in another beaker to prepare 0.1
M ZnSO$_4$ and 12.94 g ZnSO$_4$ was added and mixed thoroughly. NaOH 1 N was prepared by adding 4 g NaOH in 100 ml MQ water and added to maintain pH 10.5. ZnSO$_4$ solution was kept for continuous magnetic stirring. Drop by drop addition of NaOH to ZnSO$_4$ solution to fixed the pH 10.5 with the help of pH meter. Solution was filled into 50 ml centrifuge tubes and kept in centrifuge for 6000 rpm for 10 minutes. Pellet was collected after centrifugation, washed with MQ water and then centrifuged it for 6000 rpm for 10 minutes and supernatant was discarded. 50 ml STPP was added to pellet and mixed well. Solution was kept in sonicator for 10 minutes. The tubes were cooled and 1 ml sample was taken in cuvett using micropipette and it was placed into zeta potential meter and physical characterization of synthesized nanoparticles was done. Synthesized nanoparticles were kept for drying at 100 °C in hot air oven till the complete removal of water and dried powder obtained was used without any modification showed in Fig. 1.
2.2 Assessment of Anti-fungal Effectiveness of Zinc Nanoparticles (ZnNPs) Against the Pathogen \textit{In-vitro}

The ZnNPs at different concentrations were executed in poisoned food technique [9] perceive the inhibitory consequence of these ZnNPs on the mycelial growth of \textit{Alternaria burnsii}.

2.3 Experimental Details

\begin{itemize}
\item[a)] Year : 2018-19
\item[b)] Location : Department of Nanotechnology, AAU, Anand
\item[c)] Experimental design : Completely Randomized Design
\item[d)] Treatments : 12
\item[e)] Repetitions : 3
\item[f)] Method : Poisoned food technique
\end{itemize}

The comparative effectiveness of zinc nanoparticles assessed under \textit{in vitro} treatments were described in the table 1 and poisoned food technique is used [9]. Potato dextrose agar medium was prepared followed by sterilization at 15 psi pressure for 20 minutes. In the petri plates, ZnNPs was mixed well with PDA and was allowable to coagulate. Under aseptic conditions in the laminar airflow system, 5 mm sized partition of the test pathogen was placed in the middle of every petri plate. Petri plates having poured PDA and inoculated pathogen served as control. In the poison food technique inhibition study was carried out the plates were incubated at 28±1 °C which is desirable temperature for growth of the pathogen [9]. The ultrastructure changes in the growth of the fungus was observed under microscope by methylene blue staining at the end of the tenth day.

2.4 Observation Recorded

The radial growth (mm) observation was recorded at the end of the tenth day. Plates were incubated at 28±1°C till the control plates are filled with test pathogen. The formula given by [10] was used to calculate the per cent growth inhibition.

\[
PGI = \left(\frac{(DC- DT)}{DC}\right) \times 100
\]

Where,

- PGI = Per cent growth inhibition
- DC = Mean diameter of mycelial colony in control treatment (mm)
- DT = Mean diameter of mycelial colony in treated set (mm)

3. RESULTS AND DISCUSSION

3.1 \textit{In-vitro} Evaluation of Zinc Nanoparticles Against the Pathogen

In the present study, physical characterization of synthesized ZnNPs was 68.04 nm in size, Pdl-0.263, Keps-252.4 Fig. 2 and 3. Evaluation of Zinc nanoparticles (ZnNPs) regarding its inhibitory effect against \textit{Alternaria burnsii} was observed by measuring mycelial growth of pathogen. In Table 2 and Fig. 4, Data concerning to the per cent growth inhibition at varied concentrations were noted and depicted. It is resolved that at diverse concentrations in assessment to the control treatment, ZnNPs ominously inhibited the test pathogen’s mycelial growth.

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Treatment No. & Treatments & Concentrations (ppm) \\
\hline
T1 & Synthesized Zinc nanoparticles & 250 \\
T2 & Synthesized Zinc nanoparticles & 500 \\
T3 & Synthesized Zinc nanoparticles & 550 \\
T4 & Synthesized Zinc nanoparticles & 600 \\
T5 & Synthesized Zinc nanoparticles & 650 \\
T6 & Synthesized Zinc nanoparticles & 700 \\
T7 & Synthesized Zinc nanoparticles & 750 \\
T8 & Commercial Zinc nanoparticles & 100 \\
T9 & Commercial Zinc nanoparticles & 500 \\
T10 & Commercial Zinc nanoparticles & 1000 \\
T11 & Carbendazim 50 WP & 500 \\
T12 & Control (Only pathogen) & -- \\
\hline
\end{tabular}
\caption{Treatment details of ZnNPs used for evaluation of antifungal efficacy against the pathogen}
\end{table}
suggesting that ZnNPs reserved the growth of fungal hyphae followed by decrease in the smoothness of hyphae has been lost and surface is smooth. After treating with ZnNPs, (untreated samples) having typical “net” structure was done and the physical alterations of fungal isolates after ZnNPs action were studied. Fig. 5, 6, 7 showed the images of mycelia in the control (Only pathogen) having 72.33 per cent growth inhibition.

To explore the contrivance by which ZnNPs affect mycelial growth of A. burnsii, methylene blue staining with concentration (250 ppm) giving 79.26 per cent growth inhibition, respectively. The next treatment was synthesized ZnNPs with concentration (700 ppm) giving 83.40 per cent growth inhibition, respectively. The next effective treatment was synthesized ZnNPs with concentration 650 ppm with 82.49 per cent and treatments of synthesized ZnNPs with concentration 600 ppm, 550 ppm, 500 ppm giving 82.02 per cent growth inhibition which was at par with the next treatment was synthesized ZnNPs with concentration 700 ppm with 83.40 per cent growth inhibition which was at par with commercial Zinc nanoparticles giving 79.26 per cent growth inhibition.

Among the twelve treatments, the best treatment was synthesized ZnNPs with concentration (750 ppm) evidenced most effective and inhibited mycelial growth of A. burnsii with 86.17 per cent. To explore the contrivance by which ZnNPs affect the growth of A. burnsii, methylene blue staining was done and the physical alterations of fungal isolates after ZnNPs action were studied. Fig. 5, 6, 7 showed the images of mycelia in the control (untreated samples) having typical “net” structure and surface is smooth. After treating with ZnNPs, the smoothness of hyphae has been lost and uncommon bulges were formed on the external of fungal hyphae followed by decrease in the distance between two hyphae. Figs. 5, 6, 7 suggesting that ZnNPs reserved the growth of A. burnsii causing distortion of the fungal hyphae structure and branching of conidia which became round- and chain-like structure and their development was also repressed. These results suggest that ZnNPs not only affected and dented the conidia but also inhibited fungal growth greatly.

The next effective treatment was synthesized ZnNPs with concentration (700 ppm) giving 83.40 per cent growth inhibition which was at par with treatments of synthesized ZnNPs with concentration 650 ppm with 82.49 per cent and 600 ppm, 550 ppm, 500 ppm giving 82.02 per cent growth inhibition, respectively. The next treatment was synthesized ZnNPs with concentration (250 ppm) giving 79.26 per cent growth inhibition, respectively. The next treatment was synthesized ZnNPs with concentration (700 ppm) giving 83.40 per cent growth inhibition which was at par with the next treatment was synthesized ZnNPs with concentration 650 ppm with 82.49 per cent and treatments of synthesized ZnNPs with concentration 600 ppm, 550 ppm, 500 ppm giving 82.02 per cent growth inhibition, respectively.

Table 2. Assessment of zinc nanoparticles at different concentrations against the mycelial growth of A. burnsii, in-vitro

| Tr. No. | Treatments                        | Concentrations (ppm) | Average mycelial growth (mm) | Percent growth inhibition (%) |
|---------|-----------------------------------|----------------------|------------------------------|-----------------------------|
| T1      | Synthesized Zinc nanoparticles    | 250                  | 15.00                        | 79.26                       |
| T2      | Synthesized Zinc nanoparticles    | 500                  | 14.00                        | 82.02                       |
| T3      | Synthesized Zinc nanoparticles    | 550                  | 14.00                        | 82.02                       |
| T4      | Synthesized Zinc nanoparticles    | 600                  | 13.00                        | 82.02                       |
| T5      | Synthesized Zinc nanoparticles    | 650                  | 12.66                        | 82.49                       |
| T6      | Synthesized Zinc nanoparticles    | 700                  | 12.00                        | 83.40                       |
| T7      | Synthesized Zinc nanoparticles    | 750                  | 10.00                        | 86.17                       |
| T8      | Commercial Zinc nanoparticles     | 100                  | 70.00                        | 3.22                        |
| T9      | Commercial Zinc nanoparticles     | 500                  | 40.00                        | 44.69                       |
| T10     | Commercial Zinc nanoparticles     | 1000                 | 33.66                        | 53.46                       |
| T11     | Carbendazim 50 WP                 | 500                  | 64.00                        | 11.51                       |
| T12     | Control (Only pathogen)           | --                   | 72.33                        | 0                           |

S. Em. ± 0.782  
C. D. at 5% 2.282  
C. V. % 4.383

Figs. 2 and 3. Size distribution of zinc nanoparticles by intensity and volume
growth inhibition. Followed by treatment commercial ZnNPs with concentration (1000 ppm) giving 53.46 per cent growth inhibition.

The next effective treatment was commercial ZnNPs with concentration (500 ppm) giving 44.69 per cent growth inhibition. Followed by treatment of carbendazim 50 WP with concentration (500 ppm) giving 11.51 per cent growth inhibition. The least effective treatment was commercial ZnNPs with concentration (100 ppm) giving 3.22 per cent growth inhibition.

Fig. 4. *In-vitro* efficacy of zinc nanoparticles against *A. burnsii* pathogen

Figs. 5, 6 and 7. Methylene blue staining of mycelium of *A. burnsii*
It was also observed that concentration of the ZnNPs and per cent mycelial growth inhibition of the pathogen is directly proportional to each other. The Poisoned food technique has been used by several workers to estimate zinc nanoparticles effectiveness against different pathogen [11-24] but this nanoparticle is first time used against the Alternaria burnsii of cumin blight in this study.

4. CONCLUSION

We assessed the antifungal nature of these nanoparticles against the Alternaria burnsii. The data evidently confirmed that the zinc nanoparticles sturdily reduced fungal growth and caused damage to the development of cell walls. These findings recommend the opportunity of using zinc nanoparticles to exterminate Alternaria burnsii.

ACKNOWLEDGEMENT

I am very grateful to major advisor and member of advisory committee who always backed me and guided in carrying out this research work. I am also thankful to the department of plant pathology and Department of Nanotechnology, Anand Agriculture University, Anand, Gujarat for providing research facility and support staff.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chadha KL. Handbook of Horticulture (New delhi); 2014,2006.
2. BN, Desai MK, Kamat MK. Alternaria blight of cumin. Indian Journal of Agricultural Science. 1936;7:49-62.
3. Joshi NC. Note on two diseases of Cuminum cyminum L. hitherto unreported from Ajmer State. Science and Culture. 1955;21:101-102.
4. Sharma S. Studies on cumin blight incited by Alternaria burnsii (Uppal, Patel and Kamat) and its management. Master’s thesis, Anand Agricultural University, Anand; 2010.
5. Gemawat PD Prasad. Efficacy of different fungicide for the control of Alternaria blight of cuminum cyminum. L. Indian Phytopathology. 1972;22(1):49-52.
6. Lamsal K, Kim SW, Jung JH, Kim YS, Kim KS, Lee YS. Application of silver nanoparticles for the control of Colletotrichum species in-vitro and pepper anthracnose disease in field. Mycobiology. 2011;39:194-199.
7. Bartlett DW, Clough JM, Godwin JR, Hall AA, Hamer M, Parr-Dobrzanski B. The strobilurin fungicides. Pest Management Science. 2002;58:649-662.
8. Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N. Synthesis, characterization and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. Nanotechnology. 2010;6:681-688.
9. Groover RK, Moore JD. Toximetric studies of fungicides against the brown rot organisms, sclerotia fruticola and S. laxa. Phytopathology. 1962;52:876-879.
10. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159:850-850.
11. Grijalba A, Portela M, Sanchez L, Vergas G, Rodriguez J. ZnO nanoparticles and their antifungal activity against coffee fungus Erythricium salmonicolor. Applied Nanoscience. 2017;7(5):225-241.
12. Jasim N. Antifungal activity of zinc oxide nanoparticles on Aspergillus fumigatus and Candida albicans yeast. Journal of National Sciences Research. 2015; 5(4):2015.
13. Raveendran P, Fu J, Wallen SL. A simple and green method for the synthesis of Au, Ag and Au-Ag alloy nanoparticles. Green Chemistry. 2206;8:34-38.
14. Singhal, G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research. 2011;13(7):2981-2988.
15. He L, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles agstructureagainst Botrytis cinerea and Penicillium expansum. Microbiological research. 2010;166(3):207-215.
16. Kaur P, Thakur R, Choudhary A. An in-vitro study of the antifungal activity of silver/chitosan nano-formulations against important seed borne pathogens. International Journal of Scientific and Technology Research. 2012;1(6):83-86.
17. Krishnaraj C, Ramachandran R, Mohan K, Kalaichelvan PT. Optimization for rapid
synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Molecular and Biomolecular Spectroscopy. 2012; 93:95-99.

18. Ouda SM. Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternata* and *Botrytis cinerea*. Research Journal of Microbiology. 2014;9(1):34-42.

19. Negi M. Bio-efficacy of silver nanoparticles of botanicals against *Alternaria zinniae* causing leaf spot disease in marigold. M.Sc. Thesis, Dr. Y. S. Parmar University of Horticulture and Forestry, Solan, HP; 2016. Available: krishikosh.egranth.ac.in/handle/1/92938

20. Abbas A, Sohaila NS, Syed SA. Antimicrobial activity of silver nanoparticles (Ag NPs) against *Erwinia carotovora* and *Alternaria solani*. International Journal of Biosciences. 2015;6(10):9-14.

21. Ismail A, Nagwa M, Rawhia A, Rasha M, Ahmed I. Evaluation of in-vitro antifungal activity of silver and selenium nanoparticles against *Alternaria solani* caused early blight disease on potato. British Biotechnology Journal. 2016;12(3):1-11.

22. Sukhwal A, Joshi A, Rawal P, Jain D. Assessment of antimicrobial activity of biogenic silver nanoparticles against plant pathogens. Journal of Pure and Applied Microbiology. 2014;8(6):4593-4600.

23. Kim SW, Jung JH, Lamsal K, Kim YS, Min JS, Lee YS. Antifungal effects of silver nanoparticles against various plant pathogenic fungi. Mycobiology. 2012;40(1):53-58.

24. Mohammad Ateeq Aldosari, Sawsan S Darwish, Mahmoud A Adam, Nagib A Elmarzugi, Sayed M. Ahmed. Using ZnO nanoparticles in fungal inhibition and self-protection of exposed marble columns in historic sites. Archaeological and Anthropological Sciences. 2019;(11):3407–3422.