Current Journal of Applied Science and Technology

39(48): 116-123, 2020; Article no.CJAST.64927
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

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.

Article Information

DOI: 10.9734/CJAST/2020/v39i4831208
Editor(s):
(1) Dr. Ming-Chih Shih, Chinese Culture University, Taiwan.
Reviewers:
(1) Raied K Jamal, Iraq.
(2) Omar Abdulrahman Mohammed, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64927

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

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

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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 $ZnSO_4$ 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

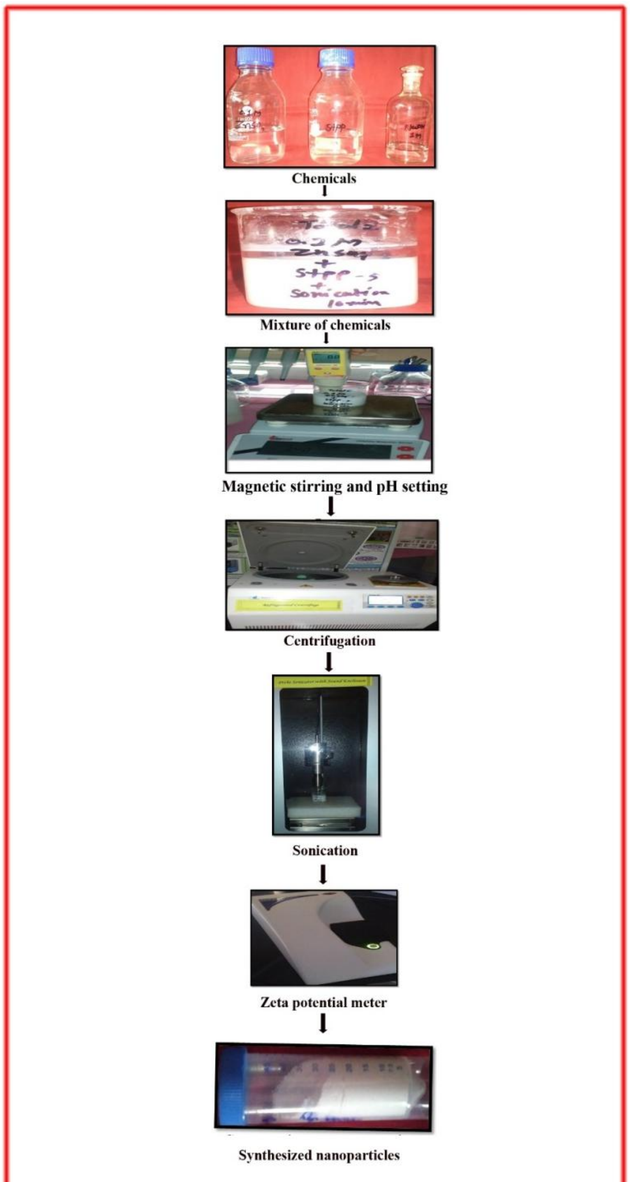


Plate 9: Flow chart of synthesis of Zinc nanoparticles

Fig. 1. Flow chart of synthesis of Zinc nanoparticles

2.2 Assessment of Anti-fungal Effectiveness of Zinc Nanoparticles (ZnNPs) Against the Pathogen *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 *Alternaria burnsii.*

2.3 Experimental Details

a)	Year	:	2018-19
	Location	:	Department of Nanotechnology, AAU, Anand
c)	Experimental design	:	Completely Randomized Design
d)	Treatments	:	12
e)	Repetitions	:	3
f)	Method	:	Poisoned food technique

The comparative effectiveness of zinc nanoparticles assessed under *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 ºC 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.

$$PGI = ((DC-DT) / DC)) \times 100$$

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 *In-vitro* Evaluation of Zinc Nanoparticles Against the Pathogen

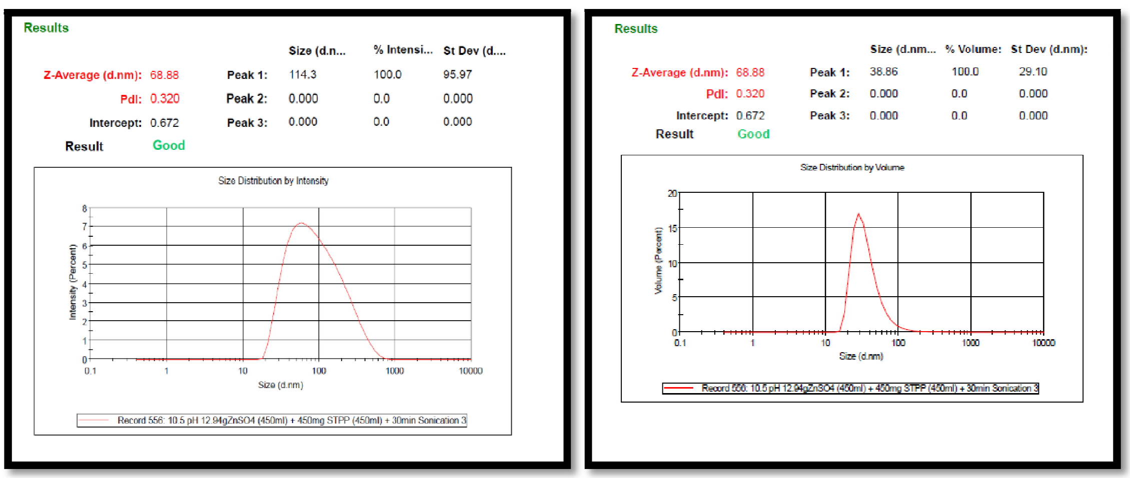
In the present study, physical characterization of synthesized ZnNPs was 68.04 nm in size, Pdi-0.263, Keps-252.4 Fig. 2 and 3. Evaluation of Zinc nanoparticles (ZnNPs) regarding its inhibitory effect against *Alternaria burnsii* was observed by measuring mycelial growth of pathogen. In Table 2 and Fig. 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.

Table 1. Treatment details of ZnNPs used for evaluation of antifungal efficacy against the pathogen

Treatment No.	Treatments	Concentrations (ppm)
T_1	Synthesized Zinc nanoparticles	250
T_2	Synthesized Zinc nanoparticles	500
T_3	Synthesized Zinc nanoparticles	550
T_4	Synthesized Zinc nanoparticles	600
T_5	Synthesized Zinc nanoparticles	650
T_6	Synthesized Zinc nanoparticles	700
T_7	Synthesized Zinc nanoparticles	750
T_8	Commercial Zinc nanoparticles	100
T_9	Commercial Zinc nanoparticles	500
T_{10}	Commercial Zinc nanoparticles	1000
T_{11}	Carbendazim 50 WP	500
T_{12}	Control (Only pathogen)	--

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 (%)
T_1	Synthesized Zinc nanoparticles	250	15.00	79.26
T_2	Synthesized Zinc nanoparticles	500	14.00	82.02
T_3	Synthesized Zinc nanoparticles	550	14.00	82.02
T_4	Synthesized Zinc nanoparticles	600	13.00	82.02
T_5	Synthesized Zinc nanoparticles	650	12.66	82.49
T_6	Synthesized Zinc nanoparticles	700	12.00	83.40
T_7	Synthesized Zinc nanoparticles	750	10.00	86.17
T_8	Commercial Zinc nanoparticles	100	70.00	3.22
T_9	Commercial Zinc nanoparticles	500	40.00	44.69
T_{10}	Commercial Zinc nanoparticles	1000	33.66	53.46
T_{11}	Carbendazim 50 WP	500	64.00	11.51
T_{12}	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

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. 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.

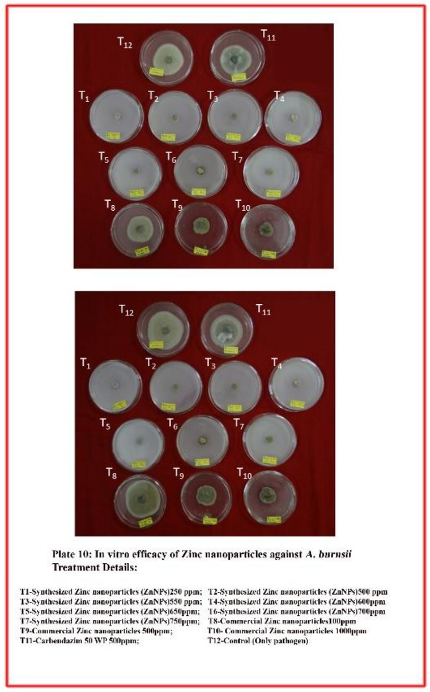
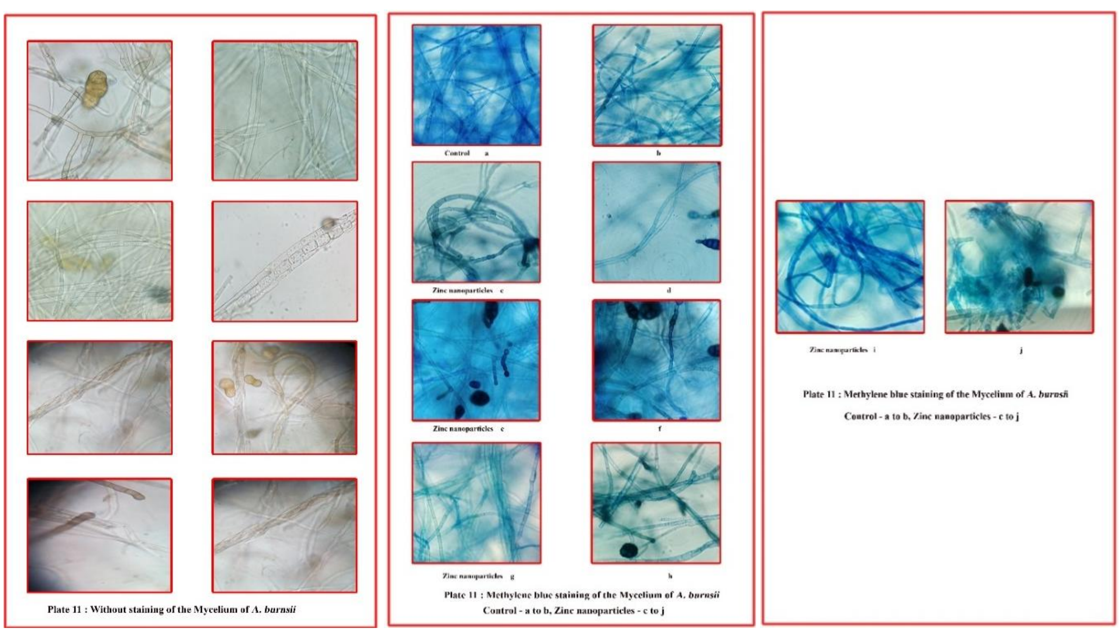


Fig. 4. *In-vitro* efficacy of zinc nanoparticles against *A. burnsii* pathogen



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.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64927