Determination of Antimicrobial Effect of Nanoparticles in Packaging of Fresh Produce

[1]Arpit R. Badani, [2] Shivamurti Srivastava

[1]Student, [2] Professor and Head
Department of Food Processing Technology,
A. D. Patel Institute of Technology, New V V Nagar, Anand, India, 388121

***Abstract*: India is in second place after China in producing fruits and vegetables in the world. The major wastage of fruits and vegetables in India is due to inappropriate handling during post-harvest practices, improper packaging materials used, and mechanical damage during transportation.**

Apart from this, the temperature is the most important factor for the shelf life of the fresh produce because if the temperature raises, the respiration rates of the fresh produce will also increase. The high respiration rate and moisture level of some fresh produce lead to microbial growth on the packaging. To overcome this problem, the application of Nanocomposite materials has been identified. The major functions of Nanoparticles are to enhance the shelf life of food products. Amongst the various types of nanoparticles, Zinc Oxide Nanoparticles are known for their antimicrobial property.

The purpose of this study is to prevent fungal growth by coating Zinc oxide (ZnO) nanoparticles on 3-ply cardboard boxes that are used for packaging fruits and vegetables. The coating of Zinc oxide (ZnO) nanoparticles with different concentrations was applied on cardboard boxes. The fungal growth was determined by qualitative and quantitative methods and a water absorptivity study (COBB Test). As the concentration of Zinc oxide (ZnO) nanoparticles increases, the fungal growth observed was very less compared to other low concentrations of Zinc oxide (ZnO) nanoparticles. Also, the larger the zone of inhibition found in a higher concentration of Zinc oxide (ZnO) nanoparticles which showed that anti-fungal coating was more effective to inhibit the fungal growth on packaging materials.

Index Terms: Cardboard Box, Fresh Produce, Zinc Oxide Nanoparticles, Fungal Growth, Shelf Life

I. INTRODUCTION

Fruits and vegetables are highly perishable and easily contaminate by climatic factors, which leads to degradation in quality and results in food loss. Microbial food spoilage is a global issue that results in food waste and customer dissatisfaction [1]. According to FAO, a major portion of the total fruits and vegetables produced in the world are spoiled each year [2]. It is required to improve the whole supply chain for the safe and desired quality of fresh produce. It is more difficult to ensure the microbiological spoilage of fruits and vegetables during the whole process from harvesting to the distribution system [3]. The damages were observed during the transportation or distribution system mainly because of vibration, impact, drop and stacking. The major damages were happened during loading and unloading (drop) of CFB boxes which carries fresh produce. Therefore, the mechanical strength of CFB boxes is most important for packaging of horticulture product otherwise it leads to damage the inner product [4]. The post-harvest practices like transportation, storage, packaging, and contact with packaging materials (i.e. boxes, crates, sacks, etc.) may also influence to grow the spoilage microorganisms [5].

Therefore, packaging materials play a significant role to keep fresh produce in vital condition as well as improving its shelf life. Packaging materials also help to protect, preserve, and safe delivery of fruits and vegetables from farm to fork. The major criteria which are used to design and select the packaging materials for fresh produce are the structural properties of packaging materials, product characteristics, and storage and transportation system i.e. loading, filling, and stacking. Poor transportation system is a major problem for fresh produce which may cause soften plant tissue due to high temperature and bruising on fresh produce thereby, increasing microbial contamination. Likewise, to prevent the damages of fresh produce during poor transportation, the study of packaging materials had been performed on various CFB box and wooden peti containing tomatoes as fresh produce and examined several parameters such as drop test, vibration test at frequency and time duration, and compression strength [6]. Performing all these test parameters of packaging materials the appropriate package can be optimized which ensures the safe delivery of the product.

Considering these entire parameters corrugated box is the ideal packaging material for delivering fresh produce in good condition. Paper and CFB box are widely used as bulk packaging or tertiary packaging material for many food products because they are easily recyclable, reusable, renewable, light in weight, provides good protection as well as sustainable in nature. It provides a fresh new box each time that can be used to pack particularly fresh produce that requires strength properties (compression, drop, Impact, etc.), air ventilation, cushioning, resistance to moisture, and protection. A CFB box used for horticulture produce should have enough vent holes to remove surplus heat which is produced by respiration. Therefore, thermal distribution and ventilation requirements need to be determined to keep fresh produce in good condition. In this case, a detailed analysis is required to be done to design a new fresh CFB box for fresh produce [7].

A corrugated fibre board box (CFB) is the most hygienic packaging material because the manufacturing of the CFB boxes requires at least 100 °C three times which helps to eliminate microbes or bacteria (www.fefco.org). Also, it is noted that the CFB box keeps fresh produce safer than other common packaging materials like reusable plastic containers, crates, trays, etc. In reusable plastic crates/containers, there might be a chance to leave pathogenic bacteria or microbes in cracks and on the crate's surfaces. Thermal properties of horticulture products, airflow, and heat transfer rate are the major parameters that are being considered while designing the CFB box for fresh produce. These all parameters can easily be controlled by using CFB boxes as a packaging material therefore, the shelf life of fresh produce can be enhanced.

As the paper is hygroscopic and orthotropic in nature, there are several parameters that directly affect the strength properties of the paper or board basically the water absorptivity test (COBB test). Once moisture level or water content increase in the box surface, it directly influences the microbial attack. This phenomenon normally occurs while packing fresh produce in a box or carton. Apart from this, there are several specification criteria that need to be incorporated while designing the CFB box for fresh produce. Some study also performed on long duration transportation of tomatoes packed in wooden peti and observed that, the 3 to 4.5 % damages were found in tomatoes travelled in 120 km and 5.5 to 7 % damages in 270 km of travelling. The damaged was mainly observed in tomatoes such as bruises, skin damage, and bursting of tomatoes which directly cause to microbial or fungal attack [8].

Thus, to overcome the problem of microbial activity, the application of nanocomposite materials has been identified. Nanotechnology used in the combination of nanoparticles is mainly used in food packaging. It helps in improving and extending the shelf life of various food products. Nanocomposite materials have many important properties like UV impermeability, oxygen scavenging, antimicrobial activity, and many other properties. The dissemination of targeted food-borne pathogens is possible by incorporating antimicrobial agents in packaging materials. [9, 10]

There are some nanoparticles which are having properties to inhibit microbial growth. These nanoparticles are titanium oxide (TiO2), copper oxides I (Cu2 O) and II (CuO), silver (AgNP), magnesium oxide (MgO), and zinc oxide (ZnO) [11].

Among these, Zinc oxide (ZnO) nanoparticles have shown significant antimicrobial properties in food packaging because of the increased specific surface area [12]. Also, ZnO nanoparticles help to improve packaging properties like mechanical strength, barrier properties, etc. The US Food and Drug Administration (FDA) has listed ZnO as generally recognized as safe (GRAS) among the five zinc compounds (FDA 2011, 21 CFR 182). therefore, Zinc oxide (ZnO) nanoparticles are majorly used in food packaging as a dynamic antimicrobial substance. ZnO is also used by many industries for important and critical roles in human and animal growth [13]. The paper coated with Zinc oxide (ZnO) nanoparticles has shown improvement in brightness, whiteness, smoothness, print uniformity, UV protection, and other properties also [14]. Zinc oxide (ZnO) nanoparticles have shown antimicrobial activity against E. coli, Staphylococcus aureus, Listeria monocytogens, Salmonella enteritids, and other bacteria by the complex activity of Zn2+ ions and ROS that causes damage to cell destruction and results in cell death [15].

Considering the above parameters, the study was performed on cardboard boxes with a coating of Zinc oxide (ZnO) nanoparticles to determine the effectiveness of the antimicrobial activity. Nanocomposite (NCs) materials have a very limited application on the paper board/CFB box containing fresh produce. The main challenges are the feasibility of Nanocomposite (NCs) materials on the paper structure.

The effectiveness of Zinc oxide (ZnO) nanoparticles on 3-ply cardboard samples was determined by qualitative and quantitative parameters stating visual fungal growth on the cardboard samples and by determining the zone of inhibition of fungal growth [16]. Agar diffusion and contact tests have been used to test the antimicrobial activity of ZnO-containing nanocomposite materials. [17].

The migration limit of nanoparticles to use in food contact plastic materials (FCM) is prescribed in EU regulations 10/2011. The overall migration limit according to regulation is 10 mg/dm2 or 60 mg/kg. The migration study is the very first test to be analysed for safety purpose and if it shows less detectable value then the risk factor will be very less [18], but a detailed toxicity study along with its transformation process like release or diffuse from the material are required when Zinc oxide (ZnO) nanoparticles is directly applied or used in packaging material as an antimicrobial agent [19].

II. MATERIALS & METHODS

The materials used were Zinc Oxide nanoparticles, acrylic resin, water, xylene, 3-ply corrugated board sheets, Aspergillus niger, and czapek yeast extract agar media.

The instruments used were glass apparatus, petri dish, and incubator.

In this study, Zinc oxide (ZnO) nanoparticles were used with some dispersion mediums along with resin material. The packaging material was coated using different concentrations of Zinc oxide (ZnO) nanoparticles along with different dispersion mediums to determine the effectiveness of the coating. The coating with four different concentrations was mentioned below. There are three replicas of each test sample were prepared and analysed.

1. Blank – Non-coating
2. W1 - 2.5 ml Zinc oxide (ZnO) nanoparticles with water base dispersion
3. X1 - 2.5 ml Zinc oxide (ZnO) nanoparticles with Xylene base dispersion
4. Z1 - 10 ml Zinc oxide (ZnO) nanoparticles with Xylene base dispersion

The following methods and procedures were followed during the study:

1. The coating of Zinc oxide (ZnO) nanoparticles was applied on board manually with three different combinations of Zinc oxide (ZnO) nanoparticles to determine the antifungal activity.
2. Water Absorptivity (COBB Test) was performed on the box to determine the amount of water absorbed and to check the adhesion property of the coating. The water absorption test conducted in this study was based on ISO 535 [20] standard for 30 minutes testing duration. Three replicas of each test sample were prepared and average results were reported.
3. A Czapek yeast extract agar media was prepared. After autoclave cool the media and inoculate 5 ml of Aspergillus Niger culture in 120 ml of czapek yeast extract agar. Pour 20 ml of media into a Petri plate. Mark all the plates as per requirement. Prepared a disc from each inoculated cardboard sample. Kept a disc on a czapek yeast extract agar plate with inoculated Aspergillus culture. Incubate all the plates at 25 °C for 5 days.
4. The fungal growth was analyzed qualitatively and quantitatively. Through qualitative analysis, the growth of fungal was observed on the cardboard surface in the Petri dish, and by quantitative analysis, the diameter of the zone of inhibition was measured on the surface of the petri dish. The zone of inhibition on cardboard indicates the effectiveness of the antifungal property of the coating. The higher the zone of inhibition means the coating is more effective and resists fungal growth efficiently.

III. RESULTS AND DISCUSSION:

On day 1 and day 2, there was no growth of fungal observed on the samples as shown in Table 1. On day 3, the growth of fungal was clearly visible (Fig. 1). In the observation on day 4 and day 5, the growth of fungal increased respectively from all four samples. Visual observation from day 1 to day 5 is shown in Table.1.

The fungal growth from day 3 to day 5 in Table 1 showed that on the non-coating sample, the growth of fungal was significantly increased day by day. While on sample Z1 the growth of fungal was very less compared to X1 and W1. Xylene-based Zinc oxide (ZnO) nanoparticles coating was more effective compared to water-based dispersion. On the other hand, the decrease in fungal growth on day 5 with 10 ml Zinc oxide (ZnO) nanoparticles coating on cardboard states that, Zinc oxide (ZnO) nanoparticles have shown significant antimicrobial characteristics on samples.

Table 1 Visible observation of fungal growth

Sr. No.	Variants	Fungal Growth Observed				
		Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*
1	Non-coating (Blank)	--	--	++++4	++++	++++
2	W1 - 2.5 ml Nano - ZnO with water base dispersion	--	--	+++[3]	+++	++++
3	X1 - 2.5 ml Nano - ZnO with Xylene base dispersion	--	--	++[2]	++	++
4	Z1 -10 ml Nano - ZnO with Xylene base dispersion	--	--	+[1]	+	+

(--) no fungal growth

[1] (+) mild fungal growth

[2] (++) moderate fungal growth

[3] (+++) high fungal growth

4 (++++) very high fungal growth

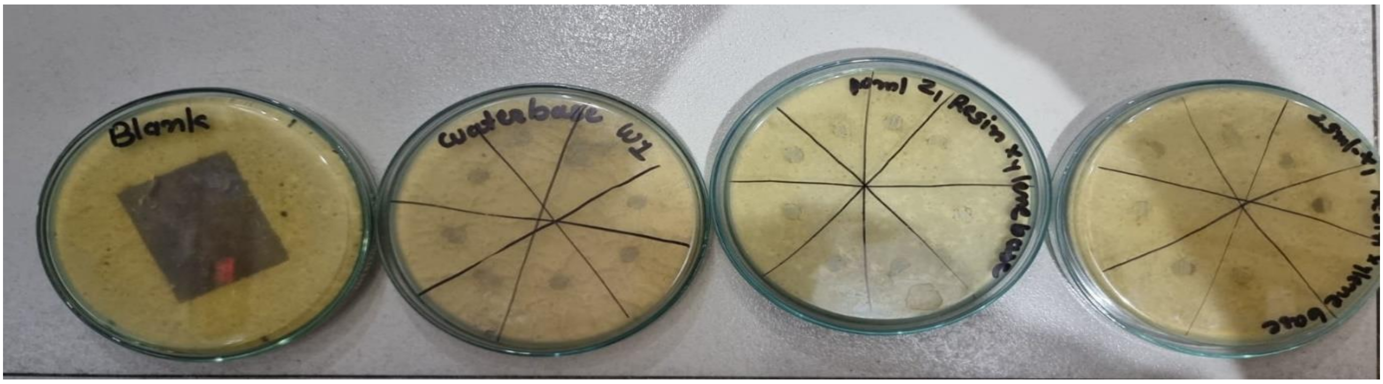

Fig. 1: Antifungal effect on uncoated and coated corrugated sheets on day 3 of observation.

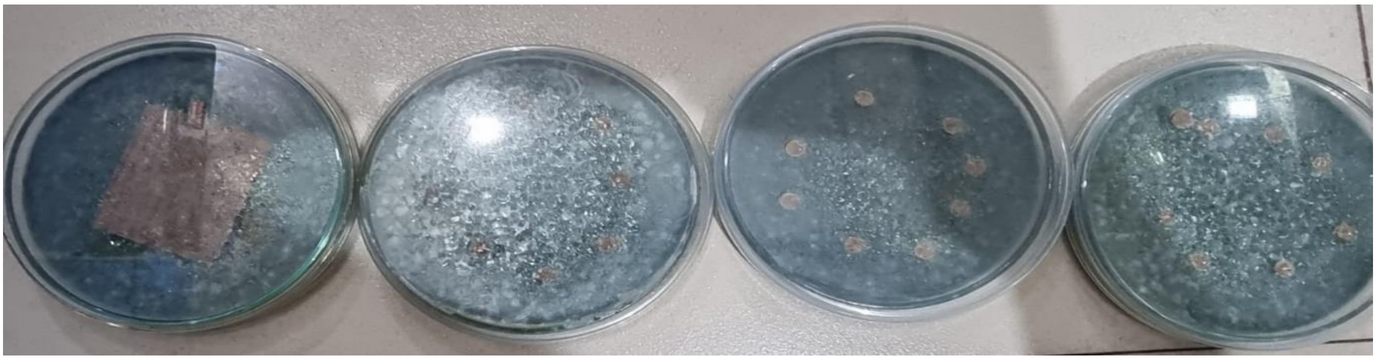

Fig. 2: Antifungal effect on uncoated and coated corrugated sheets on day 5 of observation.

The quantitative test was performed to measure the diameters of the zone of inhibition and observe the growth of the sample on incubated petri dish. The effectiveness of Zinc oxide (ZnO) nanoparticles was determined by the inhibition zone that appeared on cardboard samples.

Table 2 Zone of inhibition

Sr. No.	Variants	Diameter in mm		
		Day 1	*Day 3*	*Day 5*
1	Non-coating	No zone of inhibition	No zone of inhibition	No zone of inhibition
2	W1 – 2.5 ml Nano - ZnO with water base dispersion	No zone of inhibition	1.0 mm	1.0 mm
3	X1 - 2.5 ml Nano - ZnO with Xylene base dispersion	No zone of inhibition	3.9 mm	3.5 mm
4	Z1 – 10 ml Nano - ZnO with Xylene base dispersion	No zone of inhibition	20.4 mm	16.5 mm

In Table 2, the zone of inhibition was shown on day 1, day 3, and day 5. The higher the zone of inhibition means the effectiveness of Zinc oxide (ZnO) nanoparticles is more compelling in inhibiting the fungal growth on cardboard samples. It is observed that the higher concentration of Zinc oxide (ZnO) nanoparticles (Z1) showed very strong inhibition compare to the low concentration of Zinc oxide (ZnO) nanoparticles. The sample without coating had no zone of inhibition observed.

The water absorptivity study (COBB test) represents the amount of water absorbed by the cardboard box. A higher COBB value indicates the water absorbed by the box is higher and it directly relates to the moisture level of the packaging material and affects the strength properties of the corrugated boxes and it also influences the fungal growth. The cardboard boxes were coated with different concentrations of Zinc oxide (ZnO) nanoparticles are mentioned in Table 3.

Table 3 COBB test value

Sr. No.	Variants	COBB Test (g/m^2)
1	Non-coating	119.00
2	W1 – 2.5 ml Nano - ZnO with water base dispersion	108.23
3	X1 – 2.5 ml Nano - ZnO with Xylene base dispersion	41.07
4	Z1 – 10 ml Nano - ZnO with Xylene base dispersion	24.45

The COBB test results observed on the non-coating sample was 119.00, while after coating with different variants of Zinc oxide (ZnO) nanoparticles, the results found were gradually decreased. The COBB test values were 108.23, 41.07 & 24.45 at different combinations of Zinc oxide (ZnO) nanoparticles W1, X1, and Z1 respectively. The results showed that, after coating with a higher concentration of Zinc oxide (ZnO) nanoparticles, the water absorption property of the box was significantly improved. The coating of Zinc oxide (ZnO) nanoparticles helped to inhibit the fungal growth by reducing the water content on packaging material.

IV. CONCLUSION:

The zone of inhibition of 10 ml Zinc oxide (ZnO) nanoparticles (Z1) coating was observed 16.5 mm which is significantly higher compared to X1 and W1. As the amount of Zinc oxide (ZnO) nanoparticles increased in the coating, then the zone of inhibition observed was higher and showed strong antifungal activity. Hence, it is concluded that Zinc oxide (ZnO) nanoparticles have strong antifungal characteristics on cardboard samples.

The application of Zinc oxide (ZnO) nanoparticles on the packaging of fresh produce can inhibit the growth of fungal and the shelf life of the fresh produce during the post-harvest process can be improved. The commercial application of Zinc oxide (ZnO) nanoparticles during the manufacturing of corrugated boxes may be further developed to reduce the microbial spoilage of fruits and vegetables.

References

[1] A. B. Snyder, R. W. Worobo, "Fungal Spoilage in Food Processing," *Journal of Food Protection,* pp. Vol. 81, No. 6,Pages 1035–1040, 2018.

[2] H. K. Jeswani, G. F.-Torres and A. Azapagic, "The extent of food waste generation in the UK and its environmental," *Sustainable Production and Consumption,* vol. 26, no. 2021, pp. 532-547, 2021.

[3] O. Alegbeleye, O. A. Odeyemi, M. Strateva and D. Stratev, "Microbial spoilage of vegetables, fruits and cereals," *Applied Food Research,* vol. 2, no. 2022, p. 100122, 2022.

[4] G. Sharan, S. Srivastava and M. Khandelwal, "Mechanical Properties of wood carton used for long distance transport of tomatoes," *Packaging India,* vol. 31 (5), pp. 7-15, 1998.

[5] V. H. Tournas, "Spoilage of Vegetable Crops by Bacteria and Fungi," *Critical Reviews in Microbiology,* no. 2005, pp. 31-44, 2005.

[6] G. Sharan, U. Dave, S. Srivastava and Sreenivas, "Performance of Vastrapur carton in laboratory," *Packaging India,* vol. 32(2), pp. 39-44, 1999.

[7] G. Sharan, S. Srivastava, "Temperature distribution analysis in tomato carton by finite difference approximation method," *Processed Food Industry, New Delhi,* vol. 11(8), pp. 34-37, 2008.

[8] G. Sharan, S. Srivastav, K. P. Rawale and U. Dave, "Development of Corrugated Fiber Board Cartons for Long Distance Transport of Tomatoes in India," *International Journal for Service Learning in Engineering,* Vols. 4, 1, no. Spring 2009, pp. 31-43, 2009.

[9] A. Ashfaq, N. Khursheed, S. Fatima, Z. Anjum, and K. Younis, "Application of nanotechnology in food packaging: Pros and Cons," *Journal of Agriculture and Food Research,* vol. 7, 2022.

[10] K. K. Gaikwad, S. Singh and Y. S. Lee, "Functional Corrugated Board with Organic and Inorganic Materials in Food Packaging Applications: A Review," *KOREAN JOURNAL OF PACKAGING SCIENCE & TECHNOLOGY,* Vols. 22, no. 3, no. 2016, pp. 49-48, 2016.

[11] E. L. Bradley, L. Castle, and Q. Chaudhry, "Applications of nanomaterials in food packaging with a consideration of opportunities for developing countries," *Trends in Food Science & Technology,* vol. 22, no. 2011, pp. 604-610, 2011.

[12] H. R. GHORBANI, "Coating of ZnO Nanoparticles om Cardboard," *ACADEMIA ROMÂNĂ,* vol. 63 (2), no. 2018, pp. 103-106, 2018.

[13] L. Shi, J. Zhou, and S. Gunasekaran, "Low temperature fabrication of ZnO–whey protein isolate nanocomposite," *Materials Letters,* vol. 62, no. 2008, pp. 4383-4385, 2008.

[14] V. Prasad, A.J. Shaikh, A.A. Kathe, D.K. Bisoyi, A.K. Verma, and N. Vigneshwaran, "Functional behaviour of paper coated with zinc oxide–soluble starch nanocomposites," *Journal of Materials Processing Technology,* vol. 210, no. 2010, pp. 1962-1967, 2010.

[15] I. Kim, K. Viswanathan, G. Kasi, S. Thanakkasaranee, K. Sadeghi & J. Seo, "ZnO Nanostructures in Active Antibacterial Food Packaging: Preparation Methods, Antimicrobial Mechanisms, Safety Issues, Future Prospects, and Challenges," *Food Reviews International,* vol. 38:4, no. 2022, pp. 537-565, 2022.

[16] Muryeti, K. Alimuddin, "The Inhibitory Effect of Coating in Controlling Fungal Growth in Packaging," *Journal of Physics,* no. 2019, p. 1364, 2018.

[17] P. Espitia, N. F. F. Soares, J. S. R. Coimbra, N. J. de Andrade, E. A. A. Medeiros and R. S. Cruz, "Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications," *Springer,* vol. 5, pp. 1447-1464, 2012.

[18] J. Sarfraz, T. G. Sarfraz, J. N. Nygaard, and M. K. Pettersen, "Nanocomposites for Food Packaging Applications: An Overview," *MDPI,* 2021.

[19] P.J.P. Espitia, C.G. Otoni, and N.F.F. Soares, "Zinc Oxide Nanoparticles for Food Packaging Applications," *Antimicrobial Food Packaging,* 2016.

[20] ISO 535, Paper and board - Determination of water absorptiveness - Cobb method.