ISSN No:-2456-2165

Biopolymer Films Synthesized from Starch-Infused Zinc Oxide Nanoparticle and its Physio-Chemical Characterization.

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Abstract:- Bionanocomposite materials offer many opportunities for a safe and healthy environment by replacing synthetic polymers and materials with organic ones in a variety of applications. Wheat is an excellent source of nutrition, providing essential vitamins and minerals such as iron, zinc, and B vitamins and it is one of the most cultivated food crops on the planet. In the biopolymer sector, fiber, gluten, and wheat starch are essential ingredients. The lack of mechanical properties and hydrophilicity of starch results in a below par ability to absorb water over a long period of time, which is why additives have been added to starch composed biofilms for their efficient bioactive functions. Since zinc-oxide nanoparticles have several properties that make them an excellent additive for the reinforcement of starch-based bioplastics since they are lightweight, biocompatible, nontoxic, and have potent antibacterial properties. The physiological as well as functional characteristics of a starch-based nanocomposite were examined in the present research. The starch-ZnO biofilm outperformed the true starch film in terms of UV absorbance, moisture content, and water solubility. Zinc Oxide nanomaterial showed a synergistic effect on the UV transmission morphology of the wheat starch composed biofilm.

***Keywords**: Bioplastic, Nanoparticle, Zinc-oxide, Starch.*

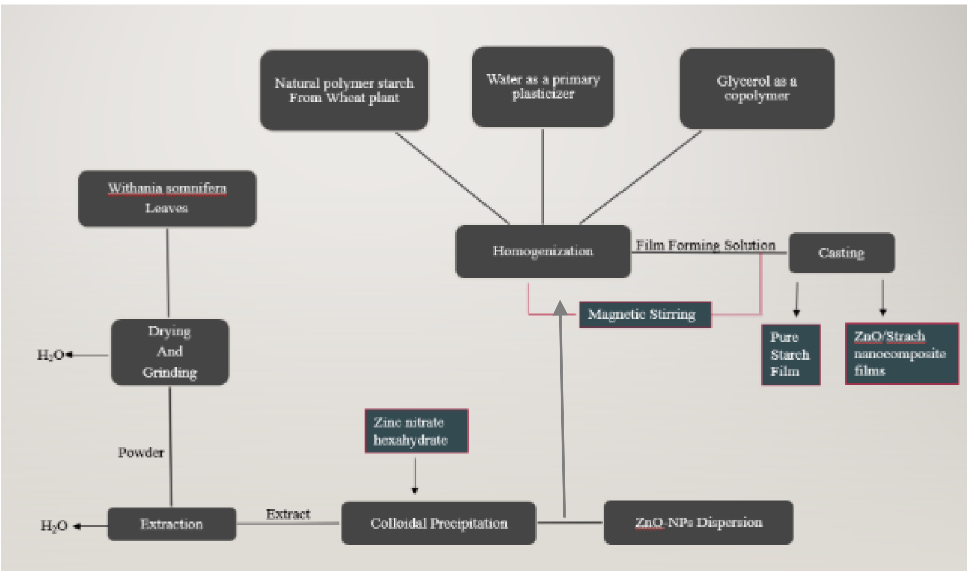

Fig. 1: Biopolymer diagram

I. INTRODUCTION

Bio-based polymers have gained considerable attention recently as a result of the growing concern about environmental issues, which has increased the need for sustainable packaging materials (**Zhang *et al.*, 2016**). Biopolymers have an excellent opportunity to replace synthetic plastics and have a goof film formability (**Vahid *et al.*, 2017, Shaili *et al.*, 2015**). These biomaterials or sustainable packaging materials are anticipated to contribute 5-10% of Europe's present plastic market (approximately 50,000 tonnes) **(Nassiri *et al.*, 2013)**. According to European Bioplastics (EUBP), the organisation that represents the bio-polymers industry, the word "bio-plastic" is used to describe plastics created from renewable resources and biodegradable components. The IUPAC defines bioplastic as a natural outcome of the organic matter or monomeric units of plant sources, at certain stage of processing (**Vert *et al.*, 2012)**

Natural resources such as carbohydrate, proteins and cellulose are extracted straightaway from the plant resources either with or without modification for producing the environmentally friendly biodegradable plastic. Starch, among the various biopolymers, seems to have the potential to be the basic material as it is inexpensive, readily available, and renewable **(Santana *et al.*, 2018)**. It is necessary to alter the fundamental structure of bioplastics composed of starch to overcome their inherent shortcomings, which include hydrophilicity, poor mechanical and barrier properties, and activity against bacteria **(Castillo *et al.*, 2013)**. Numerous experiments have been conducted to see whether adding different additives could boost the biofilm properties. There are several popular strategies employed for the effective biopolymeric film development. Some strategies include the use of composites, multilayer structures, renewable biopolymers with chemical alterations, and polymer blends **(Arrieta *et al.*, 2014)**. It was investigated how glycerol affected the elastic and water absorbing characteristics of bioplastics made from maize starch **(Gujar *et al.*, 2014)**. To get the best results in terms of its water retention %, and the mechanical properties of the bioplastic, particularly the tensile stress, researchers have attempted to fabricate the biofilms with various concentrations of glycerol. Investigators were quite happy with their findings and finally they concluded their work i.e., their biofilm possess the good tensile stress along with good mechanical characteristic **(Marichelvam *et al.*, 2022)**. However, there are several drawbacks to these macroscopic reinforcing elements, such as low interface adhesion that leads to ineffective interaction with the matrix of polymer. Smaller reinforcing components must therefore be added to further increase the characteristics of biofilm developed from the starch as the base material **(Arrieta *et al.*, 2014)**.

A potential discovery in nanotechnology, materials science, and biological sciences was disclosed by the creation of nanostructured materials. They can have a natural composite material with nanoparticle/ nanofillers reinforcement with not less than one nano scale dimension **(Abreu *et al.*, 2015)**. Several types of organic and inorganic nanofillers can be combined with biopolymers to create hybrid materials that improve the mechanical or physical properties of films **(Zubair & Ullah, 2020)**. Zinc oxide nanoparticles (ZnONP) have gained a lot of attention recently as a nanofiller because they exhibit good biocompatibility and are safe and environmentally acceptable **(Abdullah *et al.*, 2022)**. Zinc oxide nanoparticles (ZnONP), a well-known inorganic anti-bacterial agent, are well suited to produce the nano-composite bioactive biofilm through solution casting technique due to its superior thermal characteristics, good tensile strength, and possess an anti-bacterial function. Furthermore, according to the FDA (Food and Drug Administration), zinc oxide nano-particle is toxic less and falls within the category of GRAS (generally recognised as safe) nanomaterials (**Shankar *et al.*, 2015; Xiao *et al.*, 2020).**

The impacts of zinc oxide nano-composite biofilms have been the subject of numerous investigations, and the usefulness of these nanoparticles for their anti-bacterial and functional properties has been documented. **(Babapour *et al.*, 2021)**. The current investigation utilized Zinc oxide to improve the functionality of wheat composed starch bioplastics. Starch bioplastics were examined for their physical as well as UV barrier properties with Zinc-oxide reinforcement.

II. MATERIALS & METHOD

A. Raw material and Chemicals

Zinc nitrate hexahydrate, Sodium Hydroxide, Deionized water, Glycerol and Magnesium Nitrite. In the current study, known concentration chemicals are used, which means they do not require any additional treatment and were obtained from Aldrich chemicals. The wheat plant was procured from the local market of the Agra region and *Withania somnifera* leaves were procured from the school of life sciences, khandari campus, Agra.

B. Extraction of starch

Under **Abegunde *et al.* (2013)** alkaline steeping procedure, wheat Bran starch (WBS) was extracted. In this procedure, starch was isolated by immersing the WBS in 1:6 alkali solutions for 24 hours. Three liters of 0.25% NaOH solutions were used to disperse about 500 g of material. The mixture was steeped for 24 hours while being manually stirred occasionally. The slurry underwent wet milling with the addition of de-ionized water which was centrifuged on 5500 rpm aimed at 15 minutes. The liquid portion of the extract was removed and the pellet was filtered. Following a 15-minute centrifugation of the filtrate at 6500 rpm, the supernatant was once more discarded. The purification technique was repeated four times, centrifuging the recovered starch cake after it had been washed with water each time. The isolated starch from wheat bran was then left to dry inside the oven with hot air around 40 degrees Celsius for 6 hours. The extracted starch sample was pulverized and stored at room temperature in plastic bags.

C. Preparation of Zinc oxide nanoparticle

Aqueous extract was made using the procedure below to synthesize the zinc oxide nanoparticle. 10 g of the *W. somnifera* leaves were weighed to create an aqueous leaf extract with a 10% concentration. The leaves were weighed, subsequently chopped up into little fragments and were left to soak over-night with 0.1 liter of deionized H_2O_2. Leaf fragments were grounded inside the mortar pestle to form the thick paste. After which the thick paste of leaf was mixed with 0.1 liter of deionized H_2O_2. To achieve full extraction, the above mixture was left to boil for approximately half an hour at 70-80°C on a heated plate. Once's the complete extraction is done , the above said decoction was filter out using the Whatman paper filter. The aqueous decoction was then appropriately labelled and placed inside the refrigerator at 4°C for further use. First, 50 milliliters of 10 percent aqueous leaf extract were made, followed by a 1 molar (M) solution of zinc nitrate hexahydrate, and both solutions were properly mixed. The mix was subsequently heated for about 30 to 40 minutes on a heated plate with constant stirring keeping the temperature around 75 to 80 °C. Following that, the Whatman's filter paper was employed and the supernatant was discarded & the solid residue was collected and left to dry in hot air oven at 60 to 70 degrees Celsius for two hours. Zinc oxide nanoparticle were then collected and kept in sealed bottles for testing. It was confirmed that nanoparticles had formed by ultraviolet examination **(Din *et al.*, 2021).**

D. Production of nanocomposite biofilm

Starch-ZnO bionanocomposite based biofilms were obtained through a simple hot solvent casting technique. A starch solution of 5% weight was prepared in de-ionized water. To gelatinize starch, the blend was heated to 80 degrees till 60 minutes. In order to produce more flexible films, glycerol with the concentration of 40 percent of dry weight of starch were stirred into the starchy solution. Zinc oxide concentration in the resultant mixture was kept at 1, 3, and 5 percent weight of dry starch. ZnO nanoparticle suspensions were made in water. The suspensions were agitated for 15 minutes, followed by 30 minutes of ultrasonic homogenization. The starch solution and ZnO suspension were progressively combined for 10 minutes. Film-forming mixtures were poured onto 15 cm internal diameter, flat, levelled, nonstick disposable polystyrene plates and left to dry out at the ambient temperature. Remove the dry coatings from the casting surfaces. During each examination, biofilm materials were placed in desiccators filled with concentrated $Mg(NO_2)_2$ to maintain the RH (Relative Humidity) around 50-55% with temperature approximately 25°C for not less than 2 days **(Goudarzi *et al.*, 2016).**

III. CHARACTERIZATION METHODS

A. Determining the biofilm thickness

Thickness of biofilms made from starch were determined with the help of a caliper with a precision around 0.001 millimeters. The average of measurements recorded at three separate places on the films was used in calculations relating to physicochemical property testing.

B. Determining the biofilm for moisture content & water solubility

Moisture percentage of the biofilm was measured by weighing preconditioned biofilms after drying in an oven at 10-32 degree Celsius till they attained a uniform weight. **(Pirsa et al., 2018)**:

$$Moisture\ Content = \frac{MC_1 - MC_2}{MC_1} \times 100 \qquad [i]$$

whereby MC_1 weight of preconditioned biofilm & MC_2 weight of dried biofilms.

Solubility of H_2O_2 was defined to be the percentage of the film's dry material that was soluble after 6 hours in pure water **(Maizura *et al.* 2007)**. Three replications were carried out. The aforementioned formula was adopted to determine the water solubility of the specimen.

$$water\ solubility = \frac{M_1 - M_2}{M_1} \times 100 \qquad [ii]$$

C. Determining the ultraviolet visible spectroscopy.

To test the accuracy of ultraviolet light transmission of nanocomposite biofilms spectrophotometer was used. Spectrophotometer measured wavelengths spanning between 200 to 800 nanometers. In this examination, wavelengths 200-400 nm were associated with UV, and wavelengths 400-700 nm were associated with visible light **(Akbariazam et al., 2016)**.

D. Statistical Evaluation

The findings of all studies were analyzed using Excel utilizing one-way ANOVA method. At P 0.05, student t-tests were employed to evaluate the variations in mean values of film specimen attributes. Each test has been carried out three times, and the findings were expressed using deviation from the mean.

IV. RESULT & DISCUSSION

A. Structural characterization of ZnONP

The current study's main focus was to produce the zinc oxide nanoparticles by reducing zinc nitrate hexahydrate with polar extracts of *W. somnifera*. Using UV-Vis, produced ZnO nanoparticles were characterized. The wavelength peak around 350-400 nanometer was seen using ultraviolet-visible (UV-Vis) spectroscopy for confirming the stability & formation of ZnONPs in aqueous extracts of *W somnifera* leaves as shown in Figure 1. The technique of UV-Visible spectroscopy, according to one paper, can be used to determine the shape as well as the dimensions of nanomaterials. **(Panda *et al.*, 2018).**

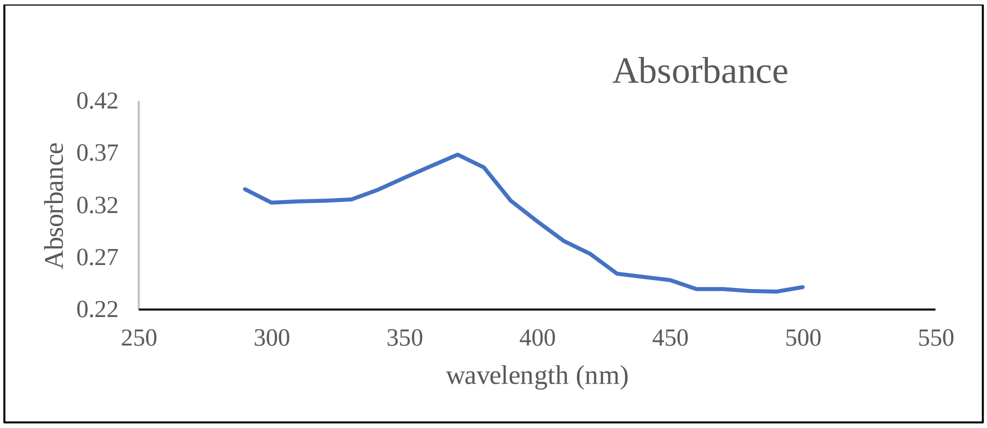

Fig. 1 : Spectra of nano-ZnO (*Withania. Somnifera* aqueous extract)

B. The impact of the presence of ZnO nanomaterial on the thickness

Figure 2. compares the thickness value of nanocomposite films including a combination of ZnO-NPs. The overall average thickness of starch film specimens did not differ statistically significantly. The normal film thickness was between 0.12-0.15 mm. There was no discernible effect on the thickness of polylactic acid biofilm (**Heydari-Majd *et al.*, 2020**).

C. The impact of the presence of ZnO nanomaterial upon the moisture content (MC)

Moisture sensitivity remains a critical factor for the success of a biopolymer in a variety of applications. It depends on the type of nanofiller used and the polymer that is employed. Water absorption can loosen the barrier structure of the biofilm through plasticization method and making it easier to travel across the biofilm more easily. As a result, it is necessary to reduce the film's susceptibility to moisture **(Bourtoom and Chinnan, 2008)**. Figure number 3 depicts the moisture level of the nanocomposite film which significantly decreased at higher ZnO nanoparticle concentrations in "comparison to the standard biofilm. The MC of the starch biofilms that contained ZnONPs decreased the most. While the MC of the control starch biofilm remained at 13.28%; the zinc oxide nano-particle were decreased in the 2% film to 9.63%. The symmetrical structure characterized by excellent cohesiveness, a smaller surface area, and a less hydrophobic properties of zinc oxide nanomaterial than a starchy based polymers is the cause of the lower moisture level of starch biofilms incorporating zinc oxide nanoparticle as compared with control **(Alboofetileh *et al.*, 2013 Tamimi *et al.*, 2021**).

D. The impact of the presence of ZnO nanomaterial upon the water solubility

There are several key factors that need to be consider when specifying the biofilms for packaging of the food items, including the moisture content of the films, the water solubility of the biofilms, and the absorption capacity of the films. The packaging materials must be capable of keeping the levels of moisture in the packaged food product at the required level **(Tamimi *et al.*, 2021)**. Figure 4 depicts the water-soluble percentage of biofilms incorporating various concentrations of Zinc oxide nanomaterial. The control exhibited the highest water solubility (28.29%) and the solubility percentage decline with the addition of the zinc oxide nanomaterial in the biofilm i.e., from 25.32-19.16 percentage. There is a decline in the water solubility because there is a reduction in the free water molecules in the biofilms which remain unbonded earlier in the control sample of starch-based biofilm. The hydrophilicity of films made from starch is reduced as a result of ZnO nanoparticles reducing the OH group in the polymer base that are accessible to H_2O_2 molecules **(Tunç & Duman, 2010)**.

According to **Jafarzadeh *et al.* (2017)**, the nano-rods of zinc oxide in the semolina composite decreased the water solubility in the biofilms. A number of research have noted that nanoparticles have an improved effect upon the long-term resistance of water in the biofilms **(Ahmed *et al.*, 2016)**. According to these investigations, adding nano zinc oxide caused the development of additional number of hydrogen-bonds in between the biofilm matrix and nanoparticles **(Tamimi *et al.*, 2021).** The inclusion of zinc oxide nanomaterial reduces the water-soluble percentage of pectin/alginate polymer film **(Ngo *et al.*, 2018; Tamimi *et al.*, 2021)**

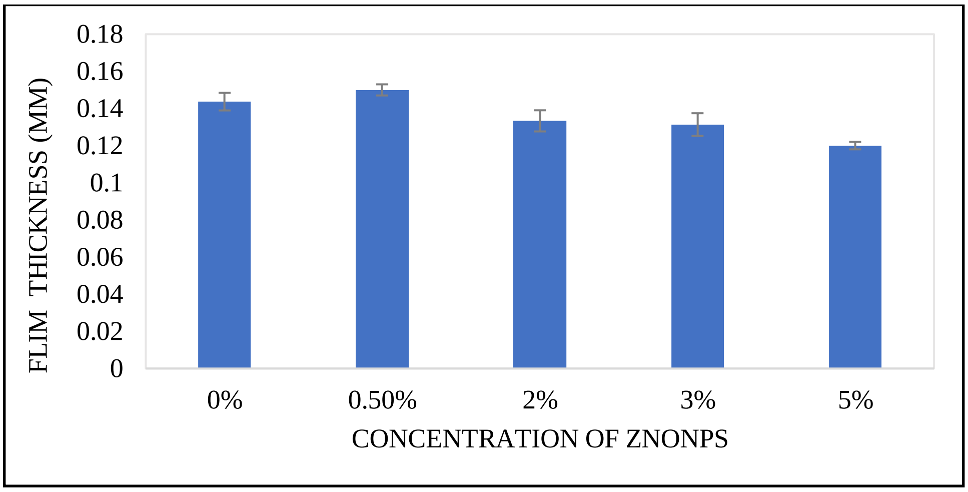

Fig. 2: Effects of ZnO nanomaterial on thickness (mm) of starch films

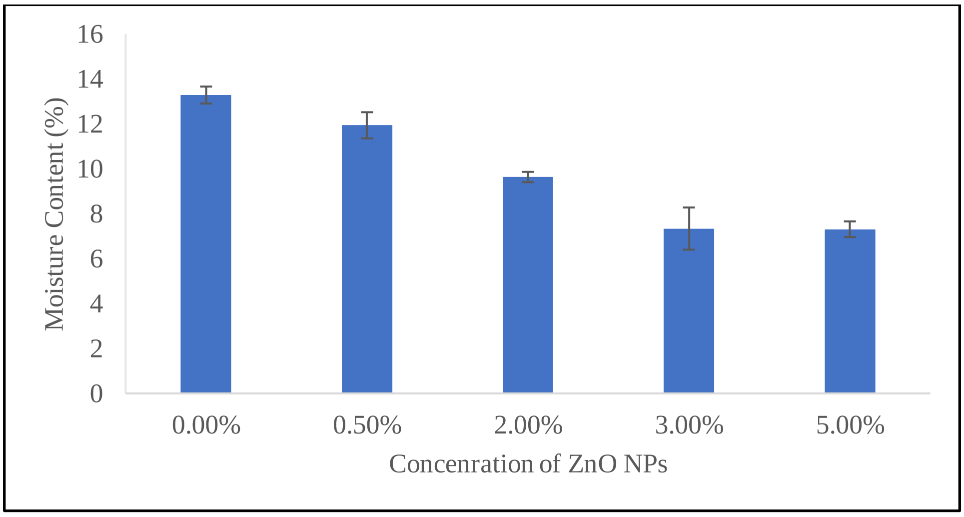

Fig. 3 : Effects of ZnO nanomaterial upon the moisture content of the biofilm

ISSN No:-2456-2165

E. The impact of the ZnO nanomaterial on the UV- vis transmission of light on biofilm

One of the key characteristics that impacts how it is used and reveals if a material can be mixed without compromising its attributes of film transparency **(Balasubramanian *et al.*, 2019)**. On absorption of the ultraviolet rays, the photochemical reaction generates the reactive nitrogen and oxygen species that causes a negative/deteriorating effect on food products. The ultraviolet rays degrade the efficiency and the effectiveness of the food product by destroying their active properties such as nutrients, colour, aroma, production of off-flavours components and so on **(Vahid *et al.*, 2016; Balasubramanian *et al.*, 2019).**

A presence of zinc-oxide nanomaterial in the biofilm made up of starch confirmed the reflecting characteristics. Surprisingly, every single biofilm absorbed UV radiation at spectra of 200-300 nanometer as shown in the Figure number 5, while the reinforcements increased the barrier characteristic ranges from 300-400 nanometer, which is favourable for food preservation.

The results reveal that the inclusion of ZnO in the material reduces the material's average penetration percentage from 80 percent to thirty percent, indicating that less ultraviolet (UV) rays can travel through the material due to ZnO's high ability to absorb or scatter qualities of UV light. The control sample devoid of ZnO particles showed a significant ultraviolet transmittance.

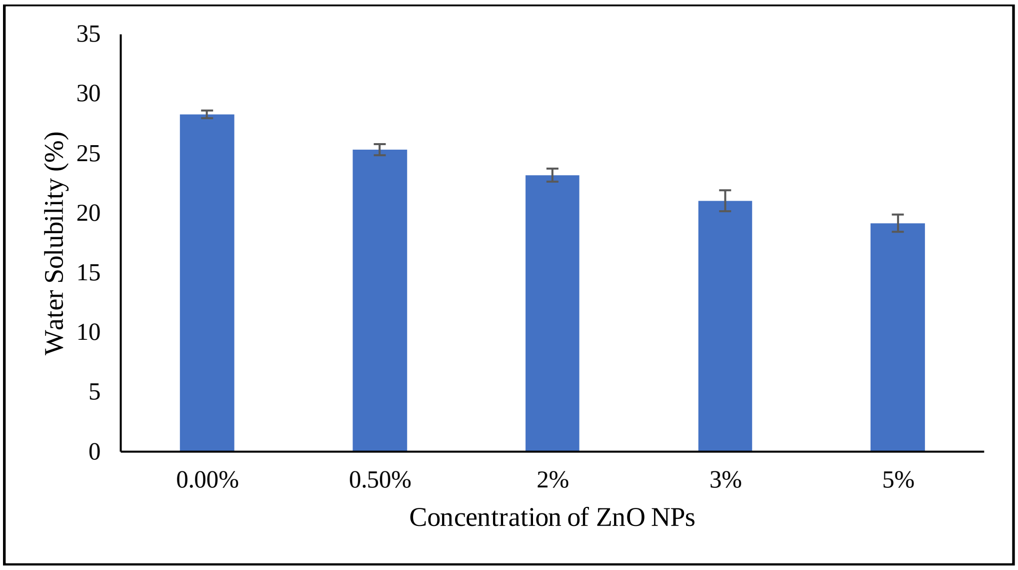

Fig. 4: Effects of ZnO nanomaterial upon the water solubility of the biofilms.

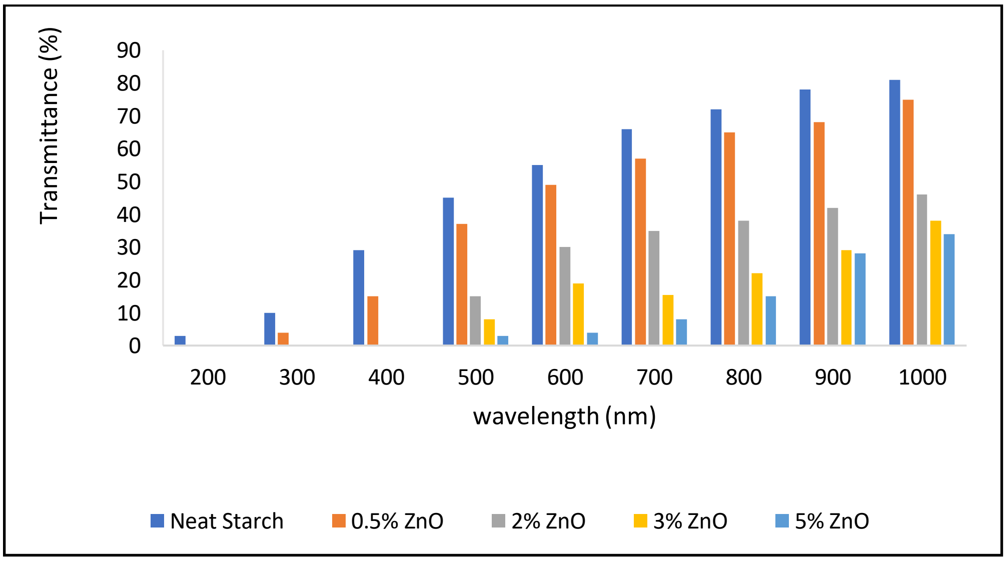

Fig. 5 : Effects of ZnO nanomaterial upon the ultraviolet-visible light transmittance of the biofilms

The figure depicts the ultraviolet light percentage penetrating across the starch infused zinc oxide nanomaterial. It can be concluded from the figure that the percentage of the ultraviolet light reduced dramatically as we increase the zinc oxide nanomaterial concentration in the biofilm. The control sample without the incorporation of the zinc oxide possess the highest ultraviolet transmittance percentage. On the other hand, the sample containing the highest amount of the nanomaterial possess the least transmittance value. **Nafchi *et al.* (2014)** demonstrated how Zinc oxide nanoparticles may significantly reduce the amount of UV radiation that could flow through starch-based nanocomposite films**.**

V. CONCLUSION

According to this study, numerous analyses have successfully identified starch-based bioplastics and bio-nanocomposite bioplastics. The spectral, chemical, and physical characteristics associated with the nano infused starch biofilm showed the considerable results that can be used for packaging the food items for a longer duration of time. In the starch matrix, ZnO nanoparticles were evenly distributed, and starch and ZnO interacted via hydrogen bonds. Bio nanocomposite films outperformed control films in terms of moisture resistance. The resulting nanocomposite starch films were effective UV light barriers. As a result of these discoveries, ZnO may be effective as a bioplastic reinforcing ingredient to increase the functionality of wheat starch composed biofilm.

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