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Effect of *Nigella sativa* and green synthesized zinc oxide nanoparticles on *Bacillus cereus* isolated from meat and milk products

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ABSTRACT

Bacillus cereus (*B. cereus*) is Gram-positive, spore-forming bacterium not only associated with food-borne outbreaks but also responsible for spoilage of food products. Therefore, the aim of this study is to trying to control of *B. cereus* by safe nanoparticles and studies the antibacterial effects of *Nigella sativa* and green synthesized Zinc Oxide nanoparticles (NPs) on *B. cereus*. The isolated strains of *B. cereus* from meat and milk products with detection of their virulence genes (*nhe*, *cyt*K, *hbl* and ces) by PCR were used to assess the antibacterial activity of these nanoparticles. The nanoparticles were characterized by Scanning Electron Microscope (SEM) and Dynamic Light Scattering (DLS) to investigate their properties which revealed that *Nigella sativa* NPs was 87.5 nm in size and cuboidal in shape, the polydispersity index, zeta potentials, viscosity and conductivity were 0.456, +15.9 mV, 0.877 cp and 58 uS/cm respectively, while Zinc Oxide NPs were 0.2484, -21.8 mV, 0.925 cp and 269 uS/cm respectively and their size was 67.8 nm and rhomboid in shape. Antibacterial activity was determined by using micro wells dilution method to determine Minimum Inhibitory Concentration (MIC). The MIC result for *Nigella sativa* NPs showed slight inhibition with the 30% concentration, while for Zinc Oxide NPs was 1.25 mg/ml. The morphological characters and changes of bacterial cells before and after treatment with nanoparticles were described by SEM. The Results show significant inhibitory effect of Zinc Oxide NPs than *Nigella sativa* NPs on *B. cereus* growth with distinctive destruction in its ultrastructure. SO, applications of nanoparticles in the meat and dairy industry will be a market trend to improve quality by their antibacterial effects and enhancement their shelf life.

Introduction

Bacillus cereus is one of the popular foodborne pathogens in foods (Rahnama *et al*., 2023). Different types of food and foodstuffs can be contaminated by *B. cereus* including rice, dairy products, meat products, pasta, potatoes and vegetable (Aman *et al*., 2016).

The microorganisms constituting the *Bacillus cereus* group are Gram-positive bacteria belonging to the phylum *Firmicutes*. The group of spore-forming, aerobic, facultative anaerobic, rod-shaped bacteria comprises at least eight closely related species: *B. anthracis*, *B. cereus*, *B. thuringiensis, B. mycoides, B. weihenstephanensis, B. pseudomycoides, B. cytotoxicus*, and *B. toyonensis* (Liu *et al*., 2015).

The major toxins of *Bacillus cereus* that have been detected include non-haemotylic enterotoxin (*nhe*), haemolysinbL (*hbl*) and cytotoxin K (*cyt*K) which are the cause of the diarrheal syndrome as well as cereulide (*ces*) that induce the emetic syndrome. The *nhe* is the most virulent enterotoxin of *B. cereus* (JeBberger *et al*., 2014).

Nanotechnology can be applied throughout different aspects of the food chain processing to improve food safety, quality control and increase food shelf life (Baltić *et al*., 2013).

Nanoparticles (NPs) differ in their size range from 1 to 100 nm, smaller particles have bigger surface area, which reflects on functionality and toxicity as well (Lang *et al*., 2021).

Nanoparticles can easily be made from a variety of materials, including flowers, leaves, metals and chemicals (Charitidis *et al*., 2014).

Despite many available synthetic nanoparticles, there is a need for natural nanoparticles with minimal or no side effects. Recent reports showed the health promoting benefits and high nutritional value of Blackseeds (*Nigella sativa*) and its active components (Ahmad *et al*., 2013). *Nigella sativa* is rich in longifolene, thymoquinone, thymohydroquinone, α- thujene, and p-cymene which are known for their strong antibacterial activities (Tariq *et al*., 2019). These components showed complete zone of inhibition against different Gram-negative and Gram-positive bacteria, including *Bacillus cereus* and *Bacillus subtilis* (Hassanien *et al*., 2015).

Nigella sativa seed is also used in the traditional Arabic Islamic medicine and its medicinal uses were also described in the hadith literature attributed to prophet Mohammed that Nigella seed is a medicine for every disease except death (Al-Huqail and Al-saad, 2010).

Nanomaterials are broadly grouped into organic and inorganic materials, but in both cases, they have different properties than larger particles of the same type (Cushen *et al*., 2012). Nano sized inorganic compounds display strong antibacterial activity at low concentrations and unique chemical and physical properties (Rai *et al*., 2009). Most antibacterial inorganic compounds are metallic nanoparticles and metal oxide nanoparticles such as copper, silver, zinc oxide and titanium oxide (Bradley *et al*., 2011).

Biological synthesis of nanoparticles with the use of microorganisms such as bacteria or fungi and plant extracts is another methodology, which is called green synthesis technology (Thakkar *et al*., 2010). The advantages of green synthesis are due to its simple and cheap methodology to produce nanoparticles, which are less toxic and more environment-friendly in comparison with chemical methods of synthesis (Ijaz *et al*., 2020).

ZnO nanoparticles can be used as an antimicrobial agent to kill pathogenic microorganisms. Depending upon their particle size, shape, concentration and exposure time to the bacterial cell, they first damage the cell wall, penetrate, accumulate in the cell membrane and ultimately cause death by interfering with metabolic functions (Siddiqi *et al*., 2018).

Application of nanoparticles has been commercially approved by the Food and Drug Administration (FDA) but accumulation of nanoparticles

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like heavy metal NPs can lead to mild as well as severe nano-toxicity (Dubey *et al*., 2018).

Materials and methods

Sample collection

A total of 125 random samples of meat products (Luncheon and Sausage) and milk products (Ras or Romy cheese, Karish cheese and Rice pudding) 25 of each, were collected from different shops and markets in El-Gharbia Governorate, Egypt to be examined for isolation, identification and genotypic detection of virulence genes of *Bacillus cereus* and study the effect of *Nigella sativa* and green synthesized Zinc oxide nanoparticles on the *Bacillus cereus* isolate. Each examined sample was taken alone in sterile plastic bags and transferred directly in ice box to the laboratory (Animal health research institute, Tanta branch) for examination.

Isolation and identification of Bacillus cereus

Bacillus cereus was isolated according to APHA (2001) and Tallent *et al*. (2012) using peptone water as enriched and Polymyxin-pyrovate-Egg Yolk-Mannitol-Bromothymol blue Agar base (PEMBA) with Polymyxin B and Egg yolk supplement (Oxoid) as a selective media. The isolates were tested biochemically according to Markey *et al*. (2013).

Genotypic detection of some virulence genes in Bacillus cereus using polymerase chain reaction (PCR)

Multiplex PCR-technique using four sets of primers showed in Table 1, were used for genotypic detection of virulence genes for five isolated *Bacillus cereus* strains. These genes were Diarrheal toxin: haemolysin BL (*hbl*), non-hemolytic enterotoxin (*nhe*) and cytotoxin K (*cyt*K); Emetic toxin: cereulide toxin (*ces*). It was applied on five random isolates of *Bacillus cereus*, following QIAamp® DNA Mini Kit instructions (Catalogue no.51304), Emerald Amp GT PCR master mix (Takara, Japan) with Code No. RR310A and 1.5% agarose gel electrophoreses (Sambrook *et al*., 1989).

Synthesis of Nanoparticles

The nanoparticles (nanoemulsion) were prepared in the Nanomaterials Research and Synthesis Unit, National Research Center (NRC), Dokki, Giza, Egypt.

Black seeds (Nigella sativa) Nanoemulsion preparation

Nigella sativa oil extracts were obtained from Food Science and Technology Research Institute, NRC. Tween (80) was obtained from Sigma-Aldrich Co.. Double-distilled and deionized water was filtered before use. The nanoemulsion was prepared by using *Nigella sativa* (15 ml), Tween 80 (10ml), and distilled deionized water (25 ml) were mixed for half hour in a homogeneous blender 1500 watt, and then distilled water was slowly added to the mixed oil phase according to Rao and McClements (2011).

Green biosynthesis of Zinc oxide nanoparticles

Preparation of Spirulina platensis extract

Typically, 5 g (dry weight) Spirulina platensis powder purchasing from food science and technology research institute, NRC was suspended in 100 ml of double distilled sterile water and boiled for 15 min at 100°C in an Erlenmeyer flask. After boiling, the mixture was cooled and centrifuged at 10,000 rpm for 15 min. Supernatant was collected and was stored at 4 ºC for further analysis (Ali *et al*., 2015).

Biosynthesis of Zinc oxide nanoparticles

ZnO nanoparticles were synthesized as follows: 0.44 g of Zn $(CH_3COO)_2 \cdot {}_2H_2O$ was dissolved in two mL of distilled H2O; after that, 98 mL of prepared supernatant was added to get a final concentration of 2 mM. The mixture was incubated for 24 h at 30 ◦C ± 2 ◦C and 150 rpm shaking condition. The resultant white precipitate was collected and oven-dried at 200 ◦C for 24 h (Fouda *et al*., 2020) to obtain ZnO-NPs as a powder, which was used after that for further study.

Characterizations of Nanoparticles

Dynamic Light Scattering (DLS)

In the Nanomaterials Research and Synthesis Unit, NRC, Dokki, Giza, Egypt. Using Microtrac FLEX Zeta sizer wave II, USA (12.0.1.0). Instrument to characterize the nanoemulsion and measure electrical conductivity, zeta potential (surface charge) and both size droplet and distribution (polydispersity indexes PDI) of nanoemulsion (Sorour *et al*., 2021).

Scanning Electron Microscope (SEM)

Applied at Center for Entomonematodes (ACE) in the Experimental Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt. The morphology of the produced nanoparticles, including their size and shape were analyzed with SEM. Live specimens were concentrated to pallet affixed to stubs using double-sided sticky tape and sputter coated with gold-palladium microscopy was performed with a JEOL JSM 5200 microscope (Shamseldean and Platzer, 1989).

Determination of the Minimum Inhibitory Concentration (MIC) of Nigella sativa and Zinc Oxide nanoparticles on Bacillus cereus strain

The MIC was defined as the least concentration of NPs that visually inhibited the bacterial growth after 24 h of incubation. MIC was estimated using 96 well disposable sterile micro-titer plates to detect the antibacterial effect of *Nigella sativa* and Zinc oxide nanoparticles against *Bacillus cereus* isolate for each nanoparticles. First, bacterial suspension for *Bacillus cereus* was adjusted to the logarithmic-phase growth to match

Table 1. Oligonucleotide primers sequences.

Primer	Sequence	Amplified product	Reference
hbl	F- GTA AAT TAI GAT GAI CAA TTTC R- AGA ATA GGC ATT CAT AGA TT	1091 bp	Ehling-Schulz *et al*. (2006)
nhe	F- AAG CIG CTC TTC GIA TTC R-ITI GTT GAA ATA AGC TGT GG	766 bp	
*cyt*K	F- ACA GAT ATC GGI CAA AAT GC R-CAA GTI ACT TGA CCI GTT GC	421 bp	
ces	F- GGTGACACATTATCATATAAGGTG R-GTAAGCGAACCTGTCTGTAACAACA	1271 bp	

approximately 10^5 CFU/ml. Two-fold serial dilution of required nanoparticles (*Nigella sativa* and zinc oxide) were prepared. 100 µl of each dilution was added to 100 µl of 10^5 CFU/ ml bacteria and incubated at 37°C for 24 h. After incubation, 10 µl of 5 mg/ml of the 2.5 diphenyl tetrazolium bromide solution was then added and re-incubated for 2-4 h (for the observation of color change each hour) to each well to determine cell viability through the formation of blue/purple color. *Bacillus cereus* isolates (Positive control) and sterile Lysogeny broth (LB) media (negative control) was used. In this way, the MIC values were determined as the lowest concentration of NPs at which no purple colour was observed. The experiment was performed in duplicate (Requena *et al*., 2019).

Scanning Electron Microscope (SEM)

JEOL JSM 5200 microscope was used to examine the morphological characters and changes of bacterial cells before and after treatment with synthesized nanoparticles.

Results

The recovered *Bacillus cereus* isolates as they are mannitol negative and hydrolyze lecithin, they grow well and produce characteristic turquoise to pea cook blue colonies (as the media contain Bromothymol blue indicator) about 5mm in diameter and surrounded by a zone of egg yolk precipitation of the same color on PEMBA agar plate. The result of biochemical reactions showed that all isolates had characteristic biochemical features as that of *B. cereus*.

The PCR results for five random studied strains of isolated *Bacillus cereus* (Table 2) showed that (*nhe* and *cyt*K) genes were detected in all five studied strains (100%) while *ces* gene were detected in only three isolates (no. 1, 3, 5) studied strains (60%) but *hbl* gene was not detected in all five studied strains. The *nhe*, *cyt*K and *ces* genes were amplified at 766, 421 and 1271 bp respectively for the five isolates of *Bacillus cereus* (Figures 1-3) but *hbl* gene was not detected at 1091 bp (Figure 4).

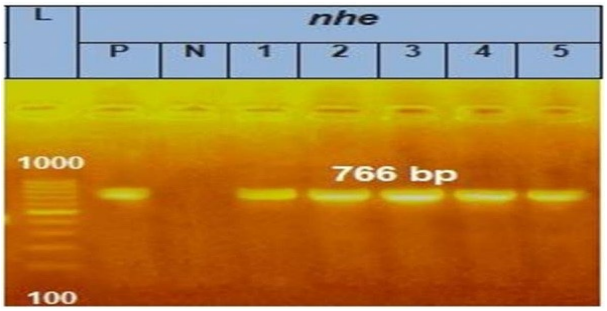

Fig. 1. Electrophoretic pattern of PCR products of positive non-hemolytic enterotoxin (*nhe*) gene amplified by *nhe* primer in 1% gel and stained by ethidium bromide showing product of 766 bp.
Lane (L): 100-1000 bp Ladder; Lane (N): Negative control; Lane (P): Positive control (at 766 bp); Lanes from 1 to 5: *Bacillus cereus* (*nhe*) gene positive.

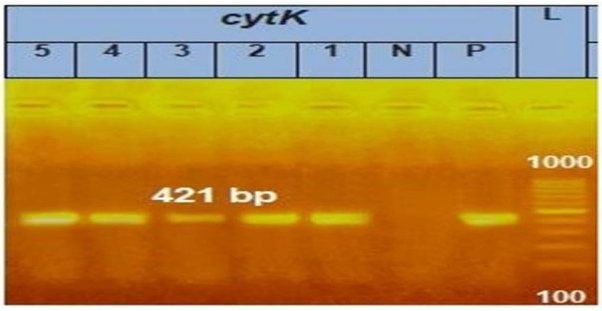

Fig. 2. Electrophoretic pattern of PCR products of positive cytotoxin K (*cyt*K) gene amplified by *cyt*K primer in 1% gel and stained by ethidium bromide showing product of 421 bp.
Lane (L): 100-1000 bp Ladder; Lane (N): Negative control; Lane (P): Positive control (at 421 bp);Lanes from 1 to 5: *Bacillus cereus* (*cyt*K) gene positive.

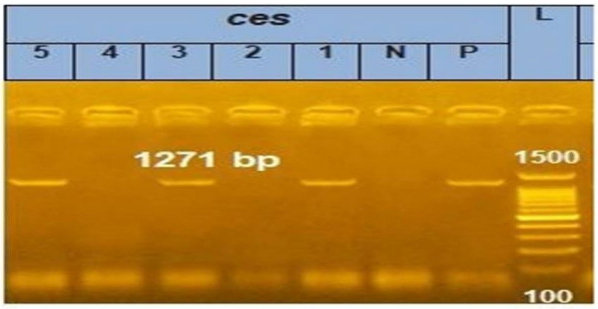

Fig. 3. Electrophoretic pattern of PCR products of positive cereulide toxin (ces) gene amplified by ces primer in 1% gel and stained by ethidium bromide showing product of 1271 bp.
Lane (L): 100-1500 bp Ladder; Lane (N): Negative control; Lane (P): Positive control (at 1271 bp); Lanes 1, 3 and 5: *Bacillus cereus* (ces) gene positive.

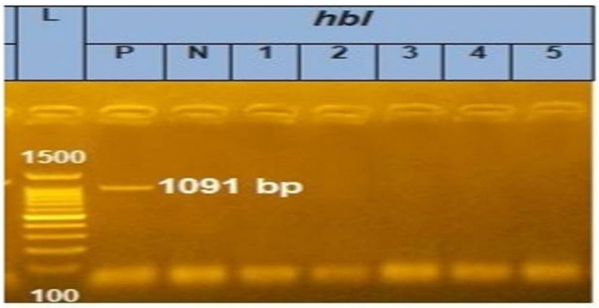

Fig. 4. Electrophoretic pattern of PCR products of positive haemolysin BL (*hbl*) gene amplified by *hbl* primer in 1% gel and stained by ethidium bromide showing product of 1091 bp.
Lane (L): 100-1500 bp Ladder; Lane (N): Negative control; Lane (P): Positive control (at 1091 bp); Lanes from 1 to 5: *Bacillus cereus* (*hbl*) gene negative.

Nigella sativa nanoemulsion size was 87.5 nm with a narrow size distribution (polydispersity index: 0.456), indicating greater homogeneity in nanodroplet size. The zeta potential indicates stable suspensions, generally taken by using DLS (Figure 5a), which had a +15.9 mV, viscosity 0.877 (cp) and conductivity 58 uS /cm. While ZnO nanoparticles size was 67.8 nm with a narrow size distribution (polydispersity index: 0.2484), indicating greater homogeneity in nanodroplet size. The zeta potential indicates stable suspensions, generally taken by using DLS (Figure 5b), which had a -21.8 mV, viscosity 0.925 cp and conductivity 269 uS/cm.

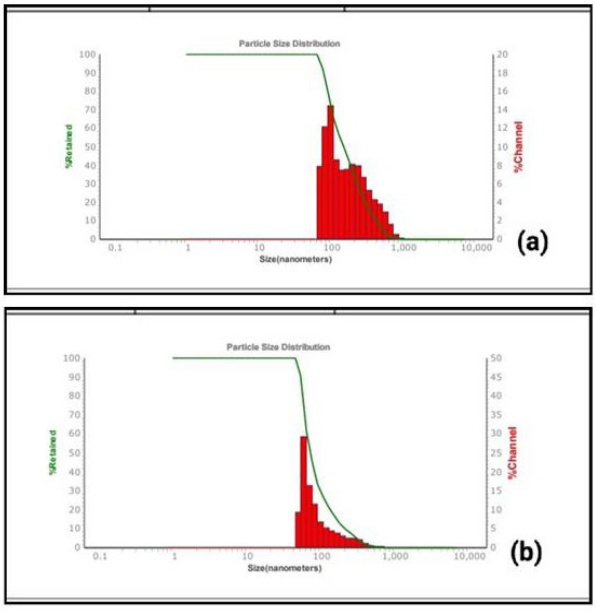

Fig. 5. Particle size analysis of Blackseeds (*Nigella sativa*) nanoemulsion (a) and Zinc Oxide nanoparticles (b) by Dynamic Light Scattering.

The image of SEM of *Nigella sativa* nanoemulsions (Figure 6a) showed that, NPs was cuboidal in shape and there is no aggregation. And the SEM image of ZnO nanoparticles (Figure 6b) showed that, NPs was rhomboid in shape and there is no aggregation.

Minimum Inhibitory Concentration (MIC) was described as the lowest

nanoparticles concentration that prevented *Bacillus cereus* growth; hence the MIC for Blackseeds (*Nigella sativa*) nanoemulsion showed slight inhibition with the 30% concentration. However, ZnO nanoparticles were 1.25 mg/ml (Figure 7).

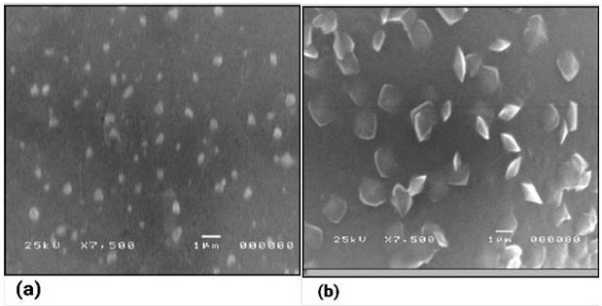

Fig. 6. SEM image of nanoparticles at magnification power of x7.500: (a) Blackseeds (*Nigella sativa*) nanoemulsion. (b) Zinc Oxide nanoparticles.

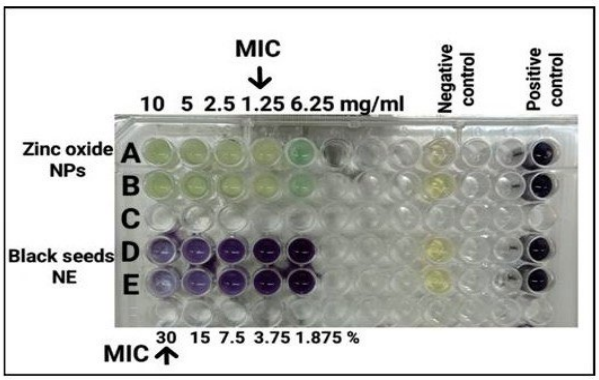

Fig. 7. MIC of ZnO NPs and *Nigella sativa* NE on *Bacillus cereus* strain: Raw A-B served as Zinc Oxide NPs on *Bacillus cereus* isolate and raw D-E served as Black seeds (*Nigella sativa*) NE on *Bacillus cereus* isolate; Column 1-5 two-fold serial diluted nanoparticles, column 9 LB media (Negative control) and column 12 *Bacillus cereus* isolate (Positive control).

SEM was used to examine the morphological characters of control untreated *Bacillus cereus* (Figure 8a) showed that the bacterial cells were in normal sizes with smooth cell line (rod shaped bacilli with square ends).

SEM was used to assess the morphological changes of *Bacillus cereus* cells after treatment with *Nigella sativa* nanoemulsion (Figure 8b) showed that membranes of the bacterial cells were deformed and shrinks. And the morphological changes of *Bacillus cereus* cells after treatment with ZnO nanoparticles (Figure 8c) showed that membranes of the bacterial cells were rupture and intracellular contents had leaked from cells due to damage of cell membrane, the sizes of ZnO nanoparticles attached to bacterial cells appeared larger and clustered as compared to those shown in Figure 6b.

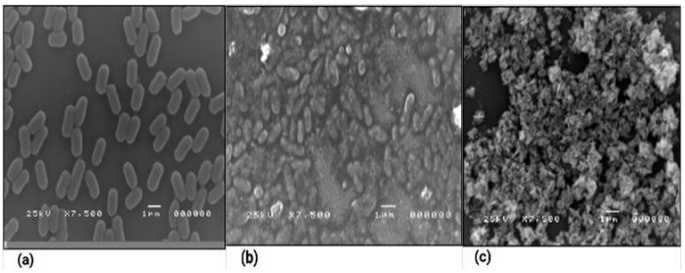

Fig. 8. SEM analyses of the morphological characters and changes of bacterial cells before and after treatment with synthesized nanoparticles at magnification power of x7.500:(a) Control untreated *Bacillus cereus*. (b) Blackseeds (*Nigella sativa*) nanoemulsion on *Bacillus cereus*. (c) Zinc Oxide nanoparticles on *Bacillus cereus*.

Table 2. The results of PCR amplification of different used genes of *Bacillus cereus* strains.

Sample	*hbl*	*nhe*	*ces*	*cyt*K
1	-	+	+	+
2	-	+	-	+
3	-	+	+	+
4	-	+	-	+
5	-	+	+	+
Total	0	5	3	5

hbl (haemolysin BL): Diarrheal toxin; *nhe* (non-hemolytic enterotoxin): Diarrheal toxin; *cyt*K (cytotoxin K): Diarrheal toxin; *ces* (cereulide toxin): Emetic toxin

Discussion

Bacillus cereus is counted among food pathogens. It causes outbreaks of food poisoning with vomiting or diarrhoea (Ehling-Schulz *et al*., 2015).

Regarding to the colonial appearance and biochemical profile of *Bacillus cereus*, it was similar to those previously reported by Abd El-Tawab *et al*. (2015) and Savić *et al*. (2015).

The PCR results for five studied strains of isolated *Bacillus cereus* (Table 2) showed that all five studied strains were virulent strains.

Regarding to Diarrheal toxin: *nhe*, *hbl* and *cyt*K: The results of PCR for amplification of non-hemolytic enterotoxin (*nhe*) gene in *Bacillus cereus* isolates (Figure 1) showed that, the *nhe* gene was amplified in all five studied strains (100.0%) giving product of 766 bp. Similar findings were recorded by Rather *et al*. (2011); Jung *et al*. (2017); Özdemir and Arslan (2018) and Zeighami *et al*. (2020). The results of PCR for amplification of cytotoxin K (*cyt*K) gene in *Bacillus cereus* isolates (Figure 2) showed that, the *cyt*K gene was amplified in all five studied strains (100.0%) giving product of 421 bp. The results came in harmony with those recorded by Zhang *et al*., (2016); Jung *et al*. (2017) and Yu *et al*. (2020), Meanwhile Özdemir and Arslan (2018) failed to detect *cyt*K gene in *Bacillus cereus* strains. The result of PCR for amplification of haemolysin BL (*hbl*) gene in *Bacillus cereus* isolates (Figure 4) showed that, it was not detected in all five studied strains of *Bacillus cereus* isolates in the current study. Meanwhile Zhang *et al*. (2016); Jung *et al*. (2017) and Özdemir and Arslan (2018) who detected (*hbl*) in *Bacillus cereus* isolates.

Regarding to Emetic toxin: cereulide synthetase toxin (*ces*): The results of PCR for amplification of cereulide synthetase toxin (*ces*) gene in *Bacillus cereus* isolates (Figure 3) showed that, it was amplified in only three (no. 1,3,5) studied strains isolates (60.0%) giving product of 1271 bp. Similar findings were recorded by Savić *et al*. (2015); Jung *et al*. (2017) and Yu *et al*. (2020). Meanwhile Ankolekar *et al*. (2009) and Ahaotu *et al*. (2013) failed to detect *ces* gene in *Bacillus cereus* strains.

Nanoemulsion (NEs) typically have widths between 20 and 200 nm and can be translucent or milky white depending on the size of their droplets, Because of their tiny droplet size and large surface area, they can increase the bioavailability of the bioactive substances (Acevedo *et al*., 2017).

In general, bacterial cell size is in the microm¬eter range, while its outer cellular membranes have pores in the nanometer range. Since nanoparticles can be smaller in size than bacterial pores, they will have a unique ability of crossing the cell membrane. Therefore, nanoparticles have received great attention due to their unique chemical, physical and effective biological properties in various fields, including medicine.

Due to the development of several microorganisms that are antibiotic-resistant. Therefore, it became vital to look for alternatives, such as oily plant extracts and nanomaterials made from them that safeguard humans and preserve food against harmful microorganisms (AL Siraj *et al*., 2023).

The well-defined nano-formulations with therapeutic significance require proper NP characterization through determination of particle size and surface charge. The use of dynamic light scattering and zeta potential techniques to determine particle size and surface charge has grown in favor (Bhattacharjee, 2016).

Nigella sativa nanoemulsion size was 87.5 nm with a narrow size distribution (polydispersity index: 0.456), indicating greater homogeneity in nanodroplet size. The zeta potential indicates stable suspensions, generally taken by using DLS (Figure 5a), which had a +15.9 mV, viscosity 0.877 (cp) and conductivity 58 uS /cm. While ZnO nanoparticles size was 67.8 nm with a narrow size distribution (polydispersity index: 0.2484), indicating greater homogeneity in nanodroplet size. The zeta potential indicates stable suspensions, generally taken by using DLS (Figure 5b), which had a -21.8 mV, viscosity 0.925 cp and conductivity 269 uS/cm.

The size droplets distribution width is represented in the PDI, in which a 0.1-0.25 PDI value means a narrow size distribution according

to Patravale *et al*. (2004), while a value > 0.5 shows a particle size broad distribution according to Gibis *et al*. (2013).

A high value of zeta potential theoretically shows high stability, while low value of zeta potential means a low stability as repulsive force between the droplets that is low prevents them from flocculate and close contact (Bouyer *et al*., 2012) the adequate droplet electrostatic repulsion for nanoemulsion to prevent coalescence should be maintained at zeta potential value of +/- 30 mV (Yin *et al*., 2009).

The SEM image of *Nigella sativa* nanoemulsions (Figure 6a) showed that, NEs was cuboidal in shape and there is no aggregation.

Scanning Electron Microscopy (SEM), this approach is frequently used for high resolution nanomaterials and is effective in determining ZnO nanoparticles crystallinity, as well as their structural surface morphology, shape, size, size distribution and dispersion (Liou *et al*., 2022).

The image of SEM of ZnO nanoparticles (Figure 6b) showed that, NPs was rhomboid in shape and there is no aggregation. Contradictory, other studies as Divya *et al*. (2013) reported spherical and hexagonal shape of zinc oxide nanoparticles synthesized from *H. rosa-sinensis*. In another report, Salam *et al*. (2014) synthesized zinc oxide nanoparticles from *O. basilicum L. var. purpurascens Benth.-Lamiaceae* leaf extract and observed hexagonal (wurtzite) shape with the size than 50 nm. Anand-Raj and Jayalakshmy (2015) reported spherical shape of zinc oxide nanoparticles synthesized from *Zingiber officinale* with the average size from 30 to 50 nm.

Morphology of zinc oxide nanoparticles depends on the process of synthesis. They may be nanospheres, nanorods, nanoplates, nanoboxes, nanocages, hexagonal, nanowires, tripods, tetrapods, nanotubes, nanorings and nanoflowers (Siddiqi *et al*., 2018).

The lowest concentration of an antimicrobial agent that can inhibit microbial growth after 24 h of incubation is known as the minimum inhibitory concentration (MIC) (Salem *et al*., 2015).

Black seeds (*Nigella sativa*) nanoemulsion showed slight inhibition with the 30% concentration (figure 7).

Abd-El Aal *et al*. (2021) reported that *Nigella sativa* nanoemulsion reduced *B. cereus*-produced biofilm in a concentration-dependent manner.

Mineral nanoparticles synthesized using a plant extract have also been shown to exhibit greater biocidal activity than nanoparticles prepared chemically (Amini and Akbari, 2019). The biological method, which involves the use of plants and non-pathogenic microorganisms, is cost-effective, energy effective, and environment-friendly (Ndikau *et al*., 2017). Spirulina is a Cyanobacterium rich in carotenoid, chlorophyll, phycocyanin, amino acids, minerals, and other useful chemicals (Singh *et al*., 2005).

The result of MIC for ZnO nanoparticles was 1.25 mg/ml against *Bacillus cereus* (figure 7). This result was nearly similar to that recorded by Krzepiłko *et al*. (2023) who reported that the MIC value for ZnO nanoparticles against *Bacillus cereus* was 1.6 mg/ml, and Mostafa (2015) who reported that ZnO nanoparticles exhibited remarkable antibacterial activity and demonstrated a lethal effect against *Bacillus cereus*. The MIC was < 10 µg/mL for *Bacillus cereus*.

Ali *et al*. (2015) reported a direct, simple, efficient and environmental benign synthesis method for ZnO nanoparticles aqueous extract of Spirulina platensis as a reducing and capping agent. The study revealed that ZnO nanoparticles had shown inhibitory effects against *Bacillus cereus* (25.3±0.48) and concluded that the ZnO nanoparticles can be used as an effective biocidal agent for Gram-positive and Gram-negative bacteria.

It is necessary to understand the mechanism of action of ZnO nanoparticles against bacteria to make better use of ZnO nanoparticles in food products and contribution in the development of powerful, non-toxic, antimicrobial derivatives. Few studies have suggested that antibacterial activity may be from the disruption of cell membrane permeability (Brayner *et al*., 2006). ZnO nanoparticles alone induce generation of reactive oxygen species (ROS) and oxidative stress. They cause lipid peroxidation, oxidative modifications of proteins, organelle dysfunction, inflammation, and DNA damage (Premanathan *et al*., 2011).

The morphological change of *Bacillus cereus* cells after treatment with *Nigella sativa* nanoemulsion (figure 8b) showed that membranes of the bacterial cells were deformed and shrinks, may be the cause of incomplete destruction.

The morphological change of *Bacillus cereus* cells after treatment of ZnO nanoparticles (figure 8c) showed that membranes of the bacterial cells were rupture and intracellular contents had leaked from cells due to damage of cell membrane. This result was nearly similar to that recorded by Mostafa (2015) who reported that *Bacillus cereus* cells growing in the nutrient broth media containing 10 µg/mL of ZnO-NPs were clearly damaged by ZnO nanoparticles. The membranes of the bacterial cells were deformed and intracellular structures were disorganized. Many null cells were found in the bacterial samples treated with ZnO nanoparticles, indicating that the intracellular contents had leaked out of the cells owing to the damage and disorganization of the cell membrane.

Conclusion

Nigella sativa nanoemulsion and Zinc oxide nanoparticles have antibacterial activity against *Bacillus cereus* strain. The results show a significant inhibitory effect of Zinc oxide nanopartricles than *Nigella sativa* nanoemulsion on *Bacillus cereus* growth with distinctive destruction in its ultrastructure. This study suggests the use of these nanoparticles as antibacterial agent instead of traditional antibiotics to avoid the development of drug resistance. Applications of Nps in the meat and dairy industry will be a market trend to improve quality by their antibacterial properties and enhancement their shelf life.

Conflict of interest

The authors declare that they have no conflict of interest.

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