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Research Article

Antivirulent-bacteria and Physical-consequence of Saudi *Nigella sativa* L. Seeds-oil during Corona-pandemic

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Abstract:

Nigella sativa L. named black cumin or black seed, commonly known as "Habbat El Baraka", and its grown in Arab desert, and Asia, the oil treated infectious diseases caused by virulent-bacteria. The aim prompted a trial of use Saudi *Nigella sativa* L. seeds-oil against virulent-bacteria to prove the antivirulent-bacteria and physical properties during the Corona pandemic period. Materials and methods included preparation of Saudi *Nigella sativa* L. Seeds-oil, preparation of virulent-bacteria, procedure steps, turbidity signal determination, biomass wet weight determination, and biomass dry weight determination. The results presented the turbidity were higher with all concentration for Gram-negative than Gram-positive virulent-bacteria. The highest turbidity was with *Klebsiella pneumoniae*, as well *Salmonella typhi*, and *Pseudomonas aeruginosa*. The less turbidity was in *Escherichia coli* and *Proteus* sp. The biomass wet weight was higher with all concentration for Gram-negative than Gram-positive virulent-bacteria. The highest biomass wet weight was in *Klebsiella pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The less was for *Escherichia coli* and *Proteus* sp. The highest biomass dry weight was on *Klebsiella pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Then on *Escherichia coli* and *Proteus* sp. It was concluded that the effective Saudi *Nigella sativa* L. seeds-oil concentrations were 20%. The effects on the Gram-positive more than the Gram-negative virulent-bacteria during Corona-pandemic. It was recommended that the use of Saudi *Nigella sativa* L. seeds-oil as a therapeutic alternative for virulent-bacteria. It is up to the "Pharmacy Department" estimating quantity and concentration for human using doses.

Keywords: *nigella sativa l;, virulent-bacteri;, gram-negative bacteria; gram-positive bacteri;, corona-pandemic*

Introduction:

Nigella sativa L. is an herbaceous plant, family Ranunculaceae, named black cumin or black seed, commonly known as "Habbat El Baraka", and its grown in Arab desert, and Asia, the oil treated infectious diseases caused by virulent-bacteria [1]. The seeds-oil is rich in bio-active contents are fixed oils 32-40%, volatile oil 0.4-0.45%, essential amino acids 8-9 types, some vitamins, and carbohydrates [2], also alkaloids, steroids, saponins, terpenes, monoterpenes and phenolic [3]. Nigellicine, N-oxide, carvone, thymoquinone, thymol nigellicimine, and nigellicimine [4]. The antivirulent-bacteria activities related to (saturated and unsaturated fatty acids), and essential oil [5]. Geographical and agricultural circumstance cause variation in phytoconstituents percent leads to alteration in biological act [6]. The oil applied externally on skin to treat inflammation, rashes, abscesses, boils, and other virulent-bacterial topical infections [7], that for chemical compounds had antivirulent-bacterial drugs [8-9]. Antivirulent-bacterial activities was to *Escherichia coli*, as well *Salmonella* sp., [10], and *Salmonella typhi* [11], also to multiple antibiotics-resistant bacteria *Staphylococcus aureus* [12-14]. The oil used as an antivirulent-bacteria assist in food production to prevent spoiling, it was at 2.0% able to inhibit the growth of 24 virulent-bacteria [15]. It had antivirulent-bacterial effects on *Bacillus* sp., *Escherichia coli*, *Staphylococcus* sp., *Klebsiella pneumoniae* and *Salmonella typhi* [16]. There was antivirulent-bacterial act to *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* sp., the greater against the Gram-positive virulent-bacteria [17]. Thymoquinone oil content displayed act on purchases *Bacillus* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*. It was able to prevent virulent-bacterial biofilm arrangement [2]. The oils in KSA at 2017 showed maximum antivirulent-bacterial activity against *Escherichia coli* related to wide array antivirulent-bacterial compounds. The oils and their components suggested for therapeutic as origin of an alternate medicine and herbal traditional treatment [18]. At 2021, Saudi black cumin oils had antivirulent-bacterial action on *Staphylococcus aureus*, reasoned as achievable alternate to antibiotics for infections care [19]. The aim was depending on the Corona pandemic period, Saudi *Nigella sativa* L. seeds-

oil as herbal treatment was conducted on virulent-bacteria due to the presence of secondary infections. It had been used for its antivirulent-bacteria and physical properties. So, which prompted a trial of use Saudi *Nigella sativa* L. seeds-oil against virulent-bacteria to prove the antivirulent-bacteria and physical properties during Corona-pandemic.

Materials and Methods:

- **Preparation of Saudi *Nigella sativa* L. Seeds-oil:** The locally manufactured authorized Saudi was purchased from the "Approved Al-Atara Market Stores". It was chosen supported on use conventional medicine, the quality was much than 98%. It was used "The Tube Dilution Method" to determine the concentration oil inhibit virulent-bacteria. The oil was diluted by Ethanol 99.9% and DMSO(di-methyl-sulphate) obtained the used dilutions (5, 10, 15 and 20%). They were stored in tight capped glass bottles [20].
- **Preparation of Virulent-bacteria:** The identified virulence-bacteria were from the "Bacterial Laboratory". They were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Bacillus* sp., *Listeria monocytogenes*, *Escherichia coli*, *Proteus* sp., *Klebsiella pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. They were cultured on Mueller Hinton Agar (Lab M Ltd., UK) for 24 hours. The colonies were grown in Peptone Water (Oxoid, Basingstoke, UK), for suspension matched the 0.5 McFarland turbidity standard equal to ($1.5X10^8$ CFU / mL) [21].
- **Procedure Steps:** Five ml of the virulent-bacterial suspension was added, then one ml of diluted Saudi *Nigella sativa* L. seeds-oil. Then tighten the cap and were incubated overnight at 37°C [22].
- **Turbidity Signal Determination:** The first test tubes were determined the degree of turbidity based on an adjusted against "McFarland Standard" [23].
- **Biomass Wet Weight Determination:** The second test tubes culture was centrifuged to pellets the cells and were weight the wet pellets [24].
- **Biomass Dry Weight Determination:** The third test tubes cells were centrifuged to pellets the cells. Then the pellets were dried and were weight the dry pellets [24].
- **Statistical Management of Results:** The results data were treated by applied statistics using the "IBM. Statistics, Software V. 28, RAM, N. Windows"; (IBM, Armonk, NY, USA) [25].

Results and Discussions:

- **Turbidity signal of virulent-bacteria:** The results showed the turbidity were higher with all concentration for Gram-negative than Gram-positive virulent-bacteria. The highest turbidity was with *Klebsiella pneumoniae*, *Salmonella typhi*, as well *Pseudomonas aeruginosa*. The less turbidity was in *Escherichia coli* and *Proteus* sp. The effects were less on Gram-negative virulent-bacteria. This indicated the effectiveness on the Gram-positive were more than on the Gram-negative. That showed all concentration gave the most effect by turbidity, and it was less owing to the effect of the oil on all virulent-bacteria (Table 1). The oil chemical compounds had antivirulent-bacterial drugs [8-9], to *Escherichia coli*, as well *Salmonella* sp., [10], and *Salmonella typhi* [11]. Also to multiple antibiotics-resistant bacteria *Staphylococcus aureus* [12-14]. It had on *Bacillus* sp., *Escherichia coli*, *Staphylococcus* sp., *Klebsiella pneumoniae* and *Salmonella typhi* [2, 16]. *Pseudomonas aeruginosa*, and *Proteus* sp. [17]. The oils in KSA at 2017 antivirulent-bacterial activity against *Escherichia coli* [18]. At 2021, Saudi black oils had act happening for *Staphylococcus aureus*, [19].

Virulent- bacteria	*Nigella sativa L. seeds-oil concentration*			
	5%	*10%*	*15%*	*20%*
Staphylococcus aureus	++++	+++	++	+
Streptococcus pyogenes	++++	+++	++	+
Streptococcus pneumoniae	++++	+++	++	+
Bacillus sp.	++++	+++	++	+
Listeria monocytogenes	++++	+++	++	+
Escherichia coli	++++	++++	+++	++
Proteus sp.	++++	++++	+++	++
Klebsiella pneumoniae	++++	++++	++++	+++
Salmonella typhi	++++	++++	++++	+++
Pseudomonas aeruginosa	++++	++++	++++	+++

Table 1: *Turbidity signal of virulent-bacteria*

- **Biomass wet weight by gram of virulent-bacteria:** The results showed the biomass wet weight were higher with all concentration for Gram-negative than Gram-positive virulent-bacteria. The highest biomass wet weight was in *Klebsiella pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The less was for *Escherichia coli* and *Proteus* sp. So, the effects were less on Gram-negative virulent-bacteria. The Gram-positive were more effected than the Gram-negative. That showed all concentration gave the most biomass wet weight effect and was less due to the effect of the oil on all virulent-bacteria (Table 2). The oil had antivirulent-bacterial drugs [8-9], was to *Escherichia coli*, as well *Salmonella* sp., [10], and *Salmonella typhi* [11], also to multiple antibiotics-resistant bacteria *Staphylococcus aureus* [12-14]. It had antivirulent-bacterial effects on *Bacillus* sp., *Escherichia coli*, *Staphylococcus* sp., *Klebsiella pneumoniae* and *Salmonella typhi* [16]. Also, to *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* sp., the greater against the Gram-positive virulent-bacteria [17]. As well on *Bacillus* sp. *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*. It was able to prevent virulent-bacterial biofilm arrangement [2]. The oils in KSA at 2017 showed activity against *Escherichia coli* an alternate medicine and herbal traditional treatment [18]. At 2021, Saudi black cumin oils had antivirulent-bacterial activity on *Staphylococcus aureus*, alternate to antibiotics for infections care [19].

Virulent-bacteria	*Nigella sativa L. seeds-oil concentration*			
	5%	*10%*	*15%*	*20%*
Staphylococcus aureus	*1.03*	*0.72*	*0.40*	*0.17*
Streptococcus pyogenes	*1.04*	*0.68*	*0.45*	*0.20*
Streptococcus pneumoniae	*1.06*	*0.76*	*0.52*	*0.23*
Bacillus sp.	*1.71*	*1.31*	*1.02*	*0.51*
Listeria monocytogenes	*1.13*	*0.78*	*0.53*	*0.23*
Escherichia coli	*1.12*	*1.11*	*0.82*	*0.53*
Proteus sp.	*1.64*	*1.63*	*1.31*	*1.05*
Klebsiella pneumoniae	*1.68*	*1.66*	*1.61*	*1.32*
Salmonella typhi	*1.58*	*1.52*	*1.45*	*1.21*
Pseudomonas aeruginosa	*1.66*	*1.58*	*1.53*	*1.28*

Table 2: *Biomass wet weight by gram of virulent-bacteria*

- **Biomass dry weight by gram of virulent-bacteria:** The results showed the biomass dry weight were higher with all concentration for virulent-bacteria. The highest biomass dry weight was on *Klebsiella pneumoniae*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Then on *Escherichia coli* and *Proteus* sp. So, the effects were less on Gram-negative virulent-bacteria. This indicated the effectiveness on the Gram-positive were more. That showed all concentration provided the most biomass dry weight effect, that was less owing to the oil effect on all virulent-bacteria (Table 3). The oil had antivirulent-bacterial drugs [8-9], to *Escherichia coli*, as well *Salmonella* sp., [10], and *Salmonella typhi* [11], and *Staphylococcus aureus* [12-14]. It had antivirulent-bacterial effects on *Bacillus* sp., *Escherichia coli*, *Staphylococcus* sp., *Klebsiella pneumoniae* and *Salmonella typhi* [16-17]. Thymoquinone oil content displayed act on virulent-bacteria [2]. The oils in KSA at 2017 showed maximum antivirulent-bacterial activity against *Escherichia coli* [18]. At 2021, Saudi black cumin oils had antivirulent-bacterial activity on *Staphylococcus aureus*, reasoned [19].

Virulent-bacteria	*Nigella sativa L. seeds-oil concentration*			
	5%	*10%*	*15%*	*20%*
Staphylococcus aureus	*0.61*	*0.42*	*0.23*	*0.10*
Streptococcus pyogenes	*0.60*	*0.41*	*0.27*	*0.12*
Streptococcus pneumoniae	*0.62*	*0.45*	*0.30*	*0.13*
Bacillus sp.	*0.98*	*0.76*	*0.59*	*0.29*
Listeria monocytogenes	*0.67*	*0.46*	*0.31*	*0.14*
Escherichia coli	*0.66*	*0.65*	*0.49*	*0.32*
Proteus sp.	*0.96*	*0.95*	*0.78*	*0.61*
Klebsiella pneumoniae	*0.98*	*0.97*	*0.93*	*0.79*
Salmonella typhi	*0.92*	*0.89*	*0.86*	*0.71*
Pseudomonas aeruginosa	*0.97*	*0.92*	*0.90*	*0.76*

Table 3: *Biomass dry weight by gram of virulent-bacteria*

Conclusions:

It remained that from the experiment results was found the effective Saudi *Nigella sativa* L. seeds-oil concentrations were 20%. It remained the effects on the Gram-positive more than the Gram-negative virulent-bacteria during Corona-pandemic.

Recommendations:

It was optional the Saudi *Nigella sativa* L. seeds-oil usage as a therapeutic alternative for virulent-bacteria. It is up to the "Pharmacy Department" to estimate the quantity and concentration for human using doses.

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