

First report in Sudan: Detection of Antibacterial Activity of the Black Cumin (*Nigella sativa*) Seed Extract against *Mycoplasma mycoides* subsp *mycoides* (*Mmm*)

ABSTRACT:

Aims: This study was focused on the effect of *Nigella sativa* seed oil on the *in vitro* growth of *Mycoplasma mycoides* subsp *mycoides* (*Mmm*).

Study design: Three strains of (*Mmm*) were subjected to different dilutions of extracted *Nigella sativa* seed oil and the inhibitory zones were recorded. Type of effect (bactericidal or bacteriostatic) was studied.

Place and duration of study: The study was carried out at Mycoplasma and biochemistry departments Central Veterinary Research Laboratory (CVRL) - 2018.

Methodology: The tested strains were: Reference strain (T1/44), recent local strain (RH strain) and the last one was an old local strain (F strain). Different dilutions were used from *N. sativa* (25%, 50%, 75% and 100%) respectively.

Result: The *N. sativa* revealed different inhibition zones on the growth of the three mentioned cultures. In addition to that bactericidal effect on (*Mmm*) growth was observed.

Conclusion: The result of this study suggests the potential use of Cumin seed (*N. sativa*) against (*Mmm*) *in vitro*, and this result contributes in CBPP disease control using this type of natural seeds.

Key words: *Nigella sativa*, Cumin, *Mycoplasma mycoides* subsp *mycoides*, CBPP, *In vitro*, Bactericidal

1. INTRODUCTION:

The *Mycoplasmas* (*Mollicutes*), formerly called PPLO (pleuropneumonia-like organisms), are non-sporulating, Gram-negative, and non-motile bacteria. They are the smallest of the free-living prokaryotes, and have no cell wall [1].

Mycoplasma mycoides subsp *mycoides* (*Mmm*) is responsible for Contagious Bovine Pleuropneumonia (CBPP) in *bovidae*. It is a notifiable disease to the Office International des Epizootics (OIE) [2] and highly contagious disease [3, 4]. The disease is manifested by anorexia, fever and respiratory signs such as dyspnoea, polypnoea, cough and nasal discharges in cattle [2, 5]. The disease is endemic in many African countries, and the Sahara region [2]. In Sudan recent study which directed to estimate the actual prevalence of CBPP in IGAD countries (Surveillance of trade Sensitive diseases project STSD, 2016 revealed 8.7% prevalence using ELISA test (708 positive samples out of 8121 serum samples), the

disease is concentrated in Central Darfur (26.7%), North Darfur (26%) and Al Gadaref states (12%) (In press).

A large amount of antibiotics have been introduced and can be used effectively to treat major infectious diseases [6], however, there is a continuing quest for safe and effective antimicrobial agents against them. This need has been heightened recently due to the antimicrobial resistance. The usage of antibiotics and antibacterial chemotherapeutics is becoming more and more restricted because the bacteria are capable of developing resistance to antibiotics soon after their introduction [7], also most antibiotics have side effects. Therefore, it becomes essential to search for newer drugs with a lesser rate of resistance development and lesser toxicity [7, 8]. On the other hand, the treatment is largely ineffective and even counter-productive because of the risk of creating subclinical carriers [9].

Since ancient civilization, natural sources especially plants are used as medicinal therapy because they contain several components which are believed to cure various infectious diseases. The biodiversity of plants provides an important source of chemical compounds, which have many therapeutic applications such as antiviral, antibacterial, antifungal and anticancer activities [10].

Nigella sativa is an herbal plant belonging to family Ranunculaceae. It is popularly called black cumin, black seed and the seed of blessing (Habatul-barakah in Arabic countries). It has been used for humans for decades for both culinary and medicinal purposes. It is also used as a natural remedy for asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, and influenza [11, 12]. *N. sativa* oil, extracts were found to have inhibitory activity against gram positive and gram-negative bacteria [13, 14]. Modern studies have been done to investigate immuno-modulatory, immunosuppressive and anticancer properties of these black seeds [15, 16]. The present paper deals with the *in vitro* antimicrobial behavior of *N. sativa* seeds against *Mycoplasma mycoides* subsp *mycoides* strains.

2. Material And Method

2.1 Extraction of *Nigella sativa*:

Black Cumin (*N. sativa*) was collected from a local market. The plant material was taxonomically identified and Authenticated by taxonomy expert at Herbarium of Medicinal and Aromatic plant Research

Institute (MAPRI). National Center for Research (NCR) Khartoum, Sudan where the voucher specimen has been deposited. Ethanolic Extraction was carried out according to the method described by Sukhdeev *et,al* (2008) [17]. Specific weight of each sample was grounded using mortar and pestle and extracted by soaking 80% ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness and the yield percentages were calculated as followed:

$$\text{Weight of extract / weight of sample} * 100.$$

2.2 Mycoplasma strains:

Three strains were used: one of them was reference strain (T1/44) which obtained from PANVAC reference laboratory (Ethiopia) as a vaccine strain. The second one was recent local strain (RH) which was recovered from a dairy farm at Khartoum state-Sudan. The animals showed typical acute clinical signs including nasal discharge, shallow and rapid respiration and cough. The postmortem findings were adhesions to the chest and lung lobules showing red color and hepitzation. The strain was confirmed using conventional and Molecular tests. The last one was an old Sudanese local strain (F strain) which was isolated from lung showed typical signs of CBPP and confirmed.

2.3 Antimicrobial Investigations:

The extract of *Nigella sativa* was tested against the three types of (*Mmm*) isolates. Four dilutions were used; 25%, 50%, 75% and 100% using ethanol as a diluent. As described by Wallace [18], running drop technique was used to show the inhibitory zone clearly. A sterile filter paper discs (6mm) (Whatman No. 1) was saturated with *Nigella sativa* oil and placed carefully on the middle of the running drop of Mycoplasma culture (10^7 CCU /ml) on Brucella agar media using sterile forceps. The plates were incubated aerobically in a humid chambers at 37 °C for 7 days and examined using stereomicroscope on low magnification for evidence of a zone without colonies encircling of the disk and the diameters of these zones were measured in millimeters. The control plate without the addition of essential oil was also maintained under the same conditions.

2.4 Bactericidal and bacteriostatic effect of *Nigella sativa*

The effect of *Nigella sativa* on the growth of (*Mmm*) was investigated by re-isolation trial of the organism. Swab samples were taken from the surrounded inhibitory zone and cultured on Brucella broth and agar media then incubated at 37 °C for 10 days.

3. Result and Discussion

Fig 1, Fig 2 and Fig 3 show the inhibitory zones of reference and two local strains of (*Mmm*) respectively. Each strain and dilutions showed different inhibitory zones as shown in table 1.

Table 1: Zones of inhibition using different dilutions and strains

Strain	Zone of inhibition in mm \ Dilution			
	25%	50%	75%	100%
Reference strain (T1/44)	16	17	20	21
Recent local strain (RH)	14	15	17	25
Old local strain (F)	13	14	15	15

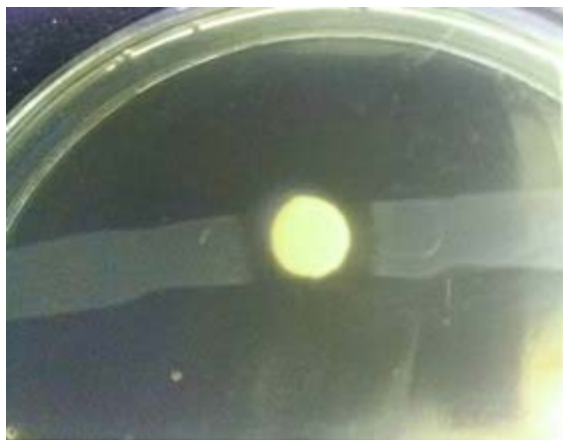


Fig 1: sensitivity of *M.mycoides* sub sp *mycoides* (reference strain) to *N.Sativa*



Fig 2: sensitivity of *M. mycoides* sub sp *mycoides* (Local strain) to *N. Sativa*



Fig 3: sensitivity of *M.mycoides* sub sp *mycoides* (old strain) to *Nigella Sativa*.



Nigella sativa seeds

Nigella sativa has a bactericidal effect on the growth of (*Mmm*). All the Swab samples revealed no growth of mycoplasma.

The antibacterial and antifungal activity of black seed and its crude extracts have been demonstrated by several research groups [19-21]. The results obtained in antimicrobial investigations of black cumin oil were in good agreement with the previous reported work [22].

The Thymoquinone, pcymene (monoterpene), longifolene (sesquiterpene), and thymohydroquinone were responsible for strong antimicrobial activity of *N. sativa* oil [23].

The Inhibition zone of the mentioned strains was obviously very clear although Gilles [24] and Salman [21] mentioned that Gram-negative bacteria were generally less susceptible than Gram-positive bacteria.

The difference in the susceptibility of the bacteria arises as a result of differences in their cell membrane structure which is more complex in case of Gram-negative bacteria.

On the basis of the above results, it showed that the extract of *N. sativa* exhibited a graduated inhibition zones according to the different concentrations of *N. sativa*, this is in agreement with Nor' Aishah Hasan [25] who studied the correlation between the concentration of *Nigella sativa* extract and inhibition zones against different types of bacteria. The Reference strain exhibited widest inhibitory zone at (25%) dilution, and this may be due to the attenuation of this strain; this makes it weak and susceptible to low concentration. The two local strains (Recent and old) showed close and wide inhibitory zones; using (25%) dilution. This has a valuable indicator about the effect of *N. sativa* against (*Mmm*) even in low concentration. The Recent local strain reported the biggest zone at 100% among the three strains, this encourages using *N. sativa* for controlling mycoplasma infections when used in concentrated form. *Nigella sativa* has a bactericidal effect on the growth of (*Mmm*) and this result is in agreement of Eman (14); who stated that it has inhibitory and lethal effects against both gram-negative and gram-positive bacteria.

This is the first report directed to study the effectiveness of the Black cumon on *Mycoplasma mycoides* subsp *mycoides* strains.

4. CONCLUSION

It concluded from this study that *N. sativa* seed extract possesses antibacterial activity towards (*Mmm*). Thus, it has a great potential as an effective antibacterial agent for CBPP treatment; this encourages producing products for pharmaceutical applications after *in vivo* application. The knowledge about the cell damage and inhibition of growth of various pathogens -beside Mycoplasma- extract of cumin can be extended for future investigation and application into the field of pharmacology for the development of better medicinal preparations. Further studies are required to confirm these results and advocate its systemic use in infectious diseases.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent: NA

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Ethical Clearance:



To: International Journal of Pathogen Research

To whom it may concern

Subject: Ethical Clearance

This is to certify that the following authors: Raghad Ahmed, Muna Ahmed and Samia Abdelwahab were veterinary researchers and they did this work in the Central Veterinary Research Laboratory, Khartoum, Sudan. I can assure that they legally sampled the animal following all the ethical procedures and they have run the requested laboratory test perfectly. The center administration and the authors agree to publish this article after submitting to your journal.

With best regard

Dr. Magdi Badawi Abdelrahman
Director / Central Veterinary Research Laboratory

Date: 30/12/2018