

## EVALUATION OF ANTIVIRAL AND ANTIBACTERIAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST NEWCASTLE DISEASE VIRUS, *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

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### ABSTRACT:

**Background:** Due to their potential for treating a variety of common illnesses, medicinal plants have recently attracted a lot of attention, also other medicinal assertions are now supported by a wealth of scientific evidence, so nowadays natural products such as essential oils (EOs) and crude extracts provide boundless opportunities for novel drugs. **Objective:** This Study was carried out to investigate the antiviral and antibacterial activity of an essential oils product, Deca-Cel®, against Newcastle disease virus (NDV), Lasota strain, as a viral model and multidrug-resistant *Escherichia coli* and *Salmonella Typhimurium* as bacterial models. **Methodology:** The antiviral activity of these EOs was systematically studied in three experimental protocols viz. virucidal, therapeutic and prophylactic assays employing *in ovo* model. Firstly, toxicity study was estimated for screening the optimal non-toxic concentration of the EOs in the embryonated chicken eggs and then their antiviral efficacy was determined. Embryo survival was observed by candling daily and the survival rates of embryos were recorded on day 4<sup>th</sup> post-inoculation (pi). After the end of experiments, survivors were killed by chilling the eggs in a refrigerator for further examination and allantoic fluid from treated eggs was collected for rapid hemagglutination (HA) test to detect NDV. Minimum inhibitory concentration (MIC) values are used to determine the susceptibilities of some bacterial agents to these EOs to evaluate their antibacterial activity. **Results:** For studying their antiviral activity, it was found that Deca-Cel® oil can completely inhibit NDV growth with a high embryo survival rate reaching 100% with -ve HA activity as a virucidal and prophylactic agent, while 60% embryo survival rate and negative hemagglutination activity had been recorded as a therapeutic agent, comparing to virus control which showed 100% embryo mortality rate within 48:72hrs pi with strong positive HA activity. Regarding the antibacterial activity, it was found that MIC values of these EOs were 0.4 µL/ml and 0.2 µL/ml against *Salmonella Typhimurium* and *E. coli* respectively, which means that these EOs can be used as a good alternative to antibiotics that recently showing resistance. **Conclusion:** The current findings have demonstrated that these EOs have promising antiviral and antibacterial properties against many avian pathogens.

**Keywords:** Deca-Cel®. Essential oils. Newcastle disease virus. Bacterial agents. MIC. *In ovo* assay. Antiviral. Antibacterial. Embryonated chicken eggs.

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## INTRODUCTION:

More than thirty percent of whole animal protein comes from the poultry industry. However, viruses like Newcastle Disease Virus and bacterial agents like *Salmonella* and *E. coli* cause huge financial losses for the poultry sector (Priya, Murthy *et al.*, 2022).

Newcastle Disease caused by Newcastle Disease Virus, poses a serious hazard to the global poultry industry owing to its high mortality rate. NDV belongs to the Paramyxoviridae family and is a negative-sense, single-stranded RNA virus. It is a part of the avian paramyxovirus type 1 (APMV-1). (Taylor, Edbauer *et al.* 1990, De Leeuw and Peeters 1999, Alexander 2000). This virus comprises six major genes in its genome: RNA polymerase, phosphoprotein, Matrix (M), Fusion (F), hemagglutinin-neuraminidase, and Nucleocapsid. (De Leeuw and Peeters 1999, Alexander 2000). Based on their level of pathogenicity, NDV isolates are classified into three pathotypes: Lentogenic, mesogenic and velogenic. (Alexander 2000).

The primary protector against NDV infection is vaccination. Even so, distinct approaches are required to either stop NDV replication or minimize its severe impacts on an infected flock. (Dortmans, Peeters *et al.*, 2012, Miller, Afonso *et al.*, 2013). Investigating the antiviral activity of extracts from medicinal plants against NDV infection is one of those strategies. (Waihenya, Mtambo *et al.*, 2002).

The usage of natural antibacterial agents and the combination of essential oils as a multiple pathogen control strategy have garnered more attention recently. There are known to be around 3,000 EOs, about 300 of which are applied in the flavor and perfume industries and have significant commercial value. (Baghban-Kanani, Hosseintabar-Ghasemabad *et al.*, 2019)

Essential oils are plant secondary metabolites that are extracted by mechanical or distillation methods. The activity of EOs differ based on the component, which may be antibacterial (Qorbanpour, Fahim *et al.*, 2018), antiviral (Gado, Ellakany *et al.*, 2019), anticoccidial (Mohiti-Asli and Ghanaatparast-Rashti 2015) or antifungal activity (Sadiek, Abd El Motelib *et al.*, 2019).

Even though the market is currently flooded with several antimicrobial agents, there is a constant need for novel drugs.

Deca-Cel® is one of the commercial products accessible in the Egyptian market for chicken farms. It consists of a number of essential oils such as Origanum, Galectin and Eucalyptus. These essential oils contain bioactive compounds that have antibacterial, anti-inflammatory, insecticidal, antioxidative and antiradical activity (Cheng, Huang *et al.*, 2009, Silva, Oliveira *et al.* 2010, Huang, Ho *et al.*, 2015, Song, Li *et al.*, 2017, Alagawany, Abd El-Hack *et al.*, 2018).

Compared to human medicine, there is a lack of comprehensive data in veterinary clinical practice about the *in vivo* application of essential oils and their susceptibility testing *in vitro*. So, this study aimed to assess the antiviral and antibacterial activity of some essential oils against NDV, *Salmonella Typhimurium* and *Escherichia coli*.

## MATERIAL AND METHODS:

### Materials:

- Deca-Cel®, a commercial product accessible in the Egyptian markets for chicken farms consists of a group of different essential oils, each 0.5 liter of the oil contains, Origanum (90 g), Galectin (90 g), Ochrophyta (90 g), Eucalyptus (45 g), Yucca (25 g),

Sodium butyrate (20 g), Mint (4 g) and Potassium iodide (1 g).

- A stock of live Lentogenic Newcastle disease virus LaSota strain (obtained from Mevac vaccine, manufacture date 5/2023 and expire date 5/2025) containing  $10^{9.5}$  egg infective dose 50 % (EID<sub>50</sub>) per ml was used in the study.
- *Salmonella* *Typhimurium* ATCC®14028
- Multidrug-resistant APEC® avian pathogenic *E. coli* (Abd El Aziz, Shehata et al. 2022)

#### **Embryonated chicken eggs (ECEs):**

Fertile eggs of the breed of hens (from the farm of the faculty of agriculture, Assiut University, Egypt) were obtained on the day of laying. The eggs were incubated horizontally under forced air circulation at 37 °C and 50–60 % relative humidity. The eggs were automatically turned 180 degrees on their axis every 4 hours starting from day 3 of incubation. Before inoculation on day 10, the eggs were candled to eliminate dead embryos and infertile eggs.

#### **Preparation of ND virus titer:**

For the experiments in embryonated chicken eggs, ND virus stock suspension was diluted serially tenfold with sterile phosphate-buffered saline (PBS) to achieve a serial dilution containing  $10^{4.5}$  EID<sub>50</sub>/0.1ml. This virus concentration was selected as it was the lowest virus concentration resulting in 100 % embryo mortality.

#### **Toxicity study of Deca-Cel®:**

To estimate the maximum non-toxic concentration (MNTC) of Deca-Cel® on ECEs, ten-fold serial dilution of Deca-Cel® ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) were prepared and 100µL from each dilution was inoculated into the allantoic cavity of 4 ten-day-old ECEs. Another group of ECEs was inoculated with 100 µL of PBS alone that served as vehicle control (diluent control). All the inoculated ECEs were incubated at

37 °C till hatching and were candled daily to check embryo survival.

#### **Assessment of the activity of Deca-Cel® against ND virus using an *in-ovo* model**

Three distinct approaches were used to evaluate the antiviral efficacy of Deca-Cel® in ECEs; virucidal, therapeutic and prophylactic.

#### **Virucidal activity:**

A virucidal assay was carried out to check the activity of Deca-Cel® in embryonated chicken eggs against NDV. For this purpose, ten-day-old embryonated chicken eggs (n=30) were designated into three groups (G1:G3) consisting of 10 eggs per group corresponding to the virucidal group, virus control and negative control group respectively. 0.1 ml of ten-fold dilution of pre-calculated MNTC of the oil  $10^{-4}$  was incubated with  $10^{4.5}$  EID<sub>50</sub>/0.1 ml of ND virus separately at 37 °C for 2 h and the virus control group included  $10^{4.5}$  EID<sub>50</sub>/0.1 ml of ND virus incubated with 0.1 ml PBS for 2 h at 37 °C. After the eggshell was cleaned and disinfected using a gauze pad moistened with 70 % ethyl alcohol, 0.2 ml from each treatment group was inoculated into the allantoic cavity of 10-day-old ECEs separately and incubated at 37 °C, while ECEs of G3 were left uninoculated as a negative control.

#### **Therapeutic activity:**

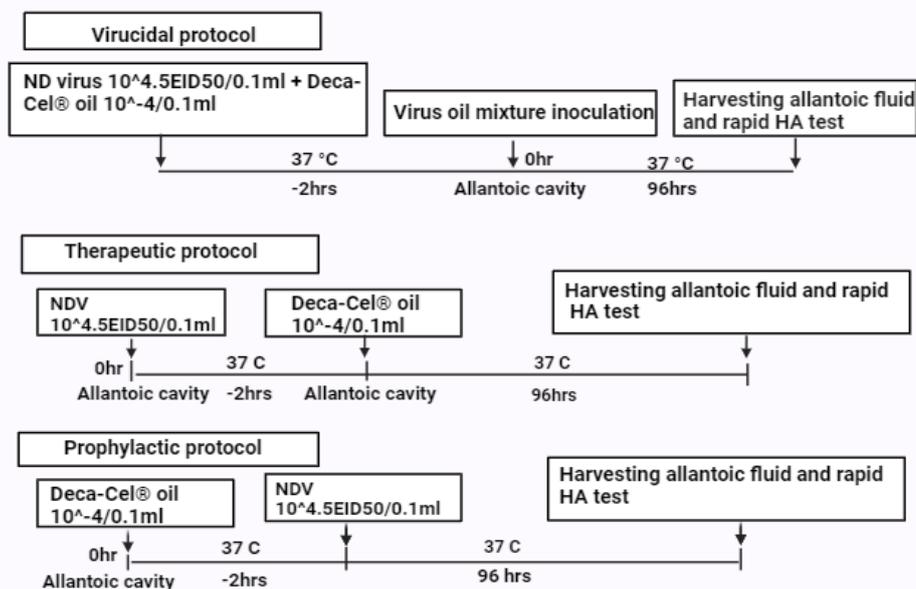
Two treatment groups G1 and G2 (except the negative control group) as formulated for testing of virucidal activity were made for testing the therapeutic efficacy corresponding to the therapeutic group and virus control group respectively. In each of the treatment groups,  $10^{4.5}$  EID<sub>50</sub> /0.1 ml of ND virus was inoculated into the allantoic cavity of 10-day-old ECEs (10 eggs for each group) and incubated at 37 °C for 2 h. Then, 0.1 ml of the MNTC dose of the oil  $10^{-4}$  and 0.1 ml of PBS were injected into the allantoic cavity of ECEs of group 1 and group 2 respectively and incubated at 37 °C.

### Prophylactic activity:

Also for testing of prophylactic efficacy, two treatment groups G1 and G2 as formulated for testing of therapeutic activity were made corresponding to the prophylactic group and virus control group respectively. 0.1 ml of MNTC of the oil  $10^{-4}$  and 0.1 ml of PBS were inoculated into the allantoic cavity of ECEs of G1 and G2 respectively and incubated at 37 °C for 2 h. Then,  $10^{4.5}$  EID<sub>50</sub> /0.1 ml of ND virus was inoculated into the allantoic cavity of ECEs of the two groups and incubated at 37 °C till the end of the experiment.

All ECEs were inoculated via the allantoic sac route according to the standard egg inoculation procedure (OIE 2021) by using a tuberculin syringe equipped with a 27-gauge needle. Following inoculation, the holes in the eggshells were sealed, and the eggs were incubated horizontally without

turning under previously described standard conditions. Daily egg candling was carried out to assess the chick embryos' survival. The criterion of embryo death was recorded as being the absence of movement of the embryo, breakdown of the visible blood vessel, blood leaking into the yolk or allantoic fluid areas, appearance of disseminated coagulation and widespread autolysis. Embryos that were found dead throughout the first 24 h post-inoculation were considered as non-related unspecific death and were removed. Eggs containing dead embryos were discarded from the experiment. The survival rates of the embryos were recorded on day 4<sup>th</sup> post-inoculation. After the end of the experiments, survivors were killed by chilling the eggs in a refrigerator. Allantoic fluids were harvested and subjected to rapid Haemagglutination test by using 5 % (v/v) washed chicken red blood cells (RBCs).



**Fig. 1:** Experimental protocols for assessing the antiviral activity of Deca-Cel® oil against NDV using *in ovo* model.

### Rapid haemagglutination test:

This was done to confirm the propagation of the virus in the allantoic fluid. A drop of the allantoic fluid was mixed thoroughly with a drop of 5 % chicken red blood cell on a clean slide for observation of agglutination (Young 2002). This was done for each egg

during the experiment and the findings were recorded.

### Antibacterial assay:

MICs of Deca-Cel® oil and some antibiotics commonly used in poultry farms were evaluated using the broth microdilution method in Mueller-Hinton broth (MHB) with

an initial inoculum of  $5 \times 10^5$  cells in non-treated polystyrene microtiter plates CC7672-7596; (CytoOne) according to the Clinical and Laboratory Standards Institute (CLSI, 2007). Bacteria were prepared in phosphate-buffered saline until a McFarland standard of 0.5 was achieved. The solution was subsequently diluted 1:300 in Mueller Hinton broth (MHB) to reach a starting inoculum of  $5 \times 10^5$  colony-forming units (CFU/mL). Bacteria were then transferred to a 96-well microtiter plate. The oil and antibiotics were added (in triplicate) to wells in the first row of the microtiter plate and then serially diluted along the vertical axis. The plate was incubated at 37 °C for 22–24 hours before the MIC was determined. MIC was defined as the lowest concentration which inhibited the visible bacterial growth.

## RESULTS:

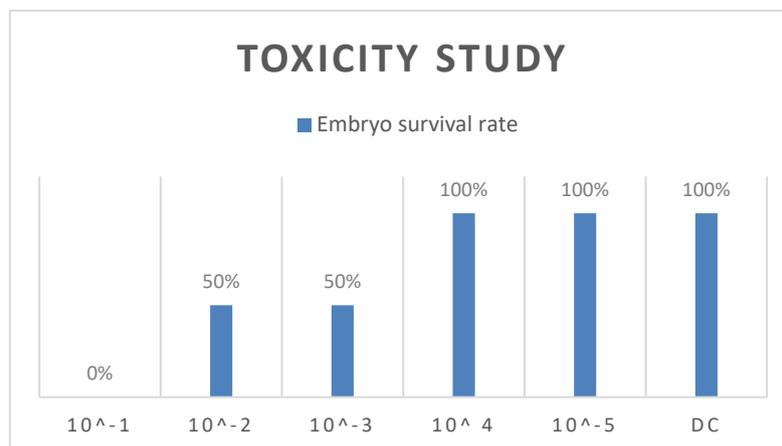
### Toxicity study of Deca-Cel® in ECEs:

In the first set of experiments, the MNTC of Deca-Cel® in ECEs was calculated. The criterion of non-toxicity was recognized as the absence of death of embryos till hatching, all the ECEs that had received  $10^{-1}$  of the oil showed 100% embryo mortality. A mortality of 50% was observed in ECEs given  $10^{-2}$  and  $10^{-3}$  of the oil, while those received  $10^{-4}$  or less showed no evidence of mortality till hatching. Also, the same survival rate was recorded in the diluent control group (**Table 1**), (**Fig. 2**).

**Table 1:** Results of toxicity study of Deca-Cel® in ECEs.

| Deca-Cel® dilution | Embryo survival |       |
|--------------------|-----------------|-------|
|                    | N               | %     |
| $10^{-1}$          | 0/4             | 0 %   |
| $10^{-2}$          | 2/4             | 50 %  |
| $10^{-3}$          | 2/4             | 50 %  |
| $10^{-4}$          | 4/4             | 100 % |
| $10^{-5}$          | 4/4             | 100 % |
| Dc                 | 4/4             | 100 % |

Key: Dc: diluent control, ECEs: embryonated chicken eggs



**Fig. 2:** Embryo survival rate by different Deca-Cel® concentrations.

### Virucidal assay:

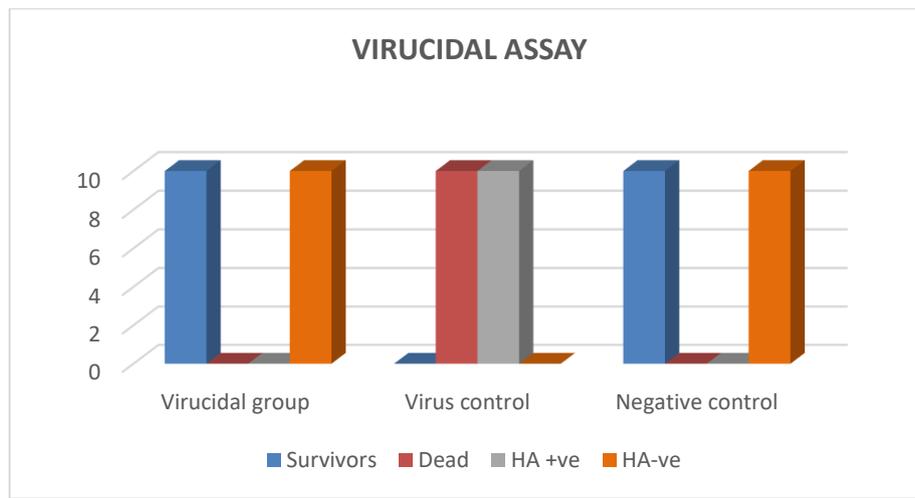
Depending on embryo survival rates throughout the 4 days of observation and results of rapid HA test. In comparison to virus control group, which was potent enough as it killed all the embryos within

48-72 hrs post inoculation with strong +ve HA activity, no embryo deaths were recorded from virucidal group and the negative control (uninoculated group) representing 100% embryo survival with –ve HA activity (**Table 2 & Fig. 3**).

**Table 2:** Results of virucidal assay in ECEs.

| Group                      | No of eggs | Mortality (4 days pi) |             |             |             | Mortality due to virus % | HA test   |           |
|----------------------------|------------|-----------------------|-------------|-------------|-------------|--------------------------|-----------|-----------|
|                            |            | 24h                   | 48h         | 72h         | 96h         |                          | +ve       | -ve       |
| <b>G1: Virucidal group</b> | <b>10</b>  | <b>0/10</b>           | <b>0/10</b> | <b>0/10</b> | <b>0/10</b> | <b>0 %</b>               | <b>0</b>  | <b>10</b> |
| <b>G2: Vc</b>              | <b>10</b>  | <b>0/10</b>           | <b>5/10</b> | <b>5/5</b>  | <b>0/0</b>  | <b>100 %</b>             | <b>10</b> | <b>0</b>  |
| <b>G3: Uc</b>              | <b>10</b>  | <b>0/10</b>           | <b>0/10</b> | <b>0/10</b> | <b>0/10</b> | <b>0 %</b>               | <b>0</b>  | <b>10</b> |

Key: Vc: virus control, Uc: uninoculated control, Pi: post inoculation

**Fig. 3:** Virucidal assay in ECEs.**Therapeutic assay:**

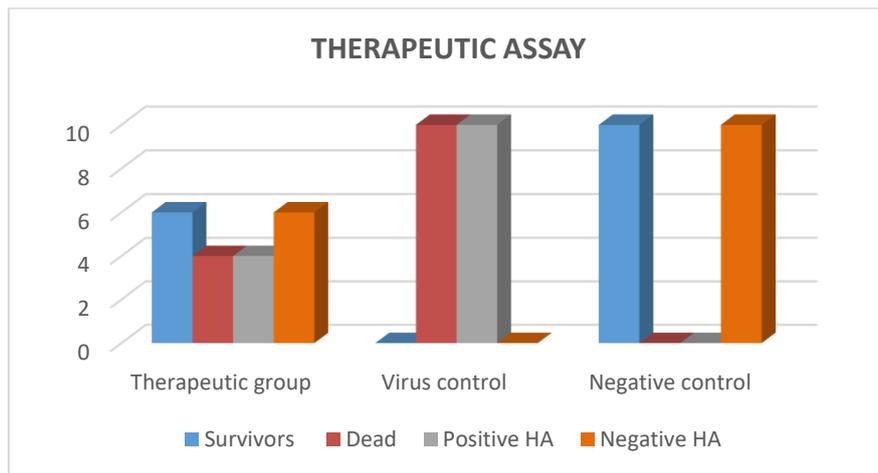
When tested Deca-Cel® oil as a therapeutic agent, it was found that 4 out of 10 ECEs died with +ve HA activity when infected with ND virus 2hrs before Deca-Cel® oil inoculation in therapeutic group resulting in 40% embryo mortality while the other 60%

of ECEs survived and did not show any HA activity. Also the virus control group showed the same result as that in virucidal assay as all ECE of this group died within 2-3 days pi with strong positive hemagglutination activity (**Table 3 & Fig. 4**).

**Table 3:** Results of therapeutic assay in ECEs.

| Group                                   | No of eggs | Mortality (4 days pi) |             |             |             | Mortality due to virus % | HA test   |           |
|---|------------|-----------------------|-------------|-------------|-------------|--------------------------|-----------|-----------|
|   |            | 24 h                  | 48 h        | 72 h        | 96 h        |                          | +ve       | -ve       |
| <b>G1:(Therapeutic treatment group)</b> | <b>10</b>  | <b>0/10</b>           | <b>0/10</b> | <b>0/10</b> | <b>4/10</b> | <b>40 %</b>              | <b>4</b>  | <b>6</b>  |
| <b>G2: Vc</b>                           | <b>10</b>  | <b>0/10</b>           | <b>5/10</b> | <b>5/5</b>  | <b>0/0</b>  | <b>100 %</b>             | <b>10</b> | <b>0</b>  |
| <b>G3: Uc</b>                           | <b>10</b>  | <b>0/10</b>           | <b>0/10</b> | <b>0/10</b> | <b>0/10</b> | <b>0 %</b>               | <b>0</b>  | <b>10</b> |

Key: Vc: virus control, Uc: uninoculated control, Pi: post inoculation



**Fig. 4:** Therapeutic assay in ECEs.

**Prophylactic assay:**

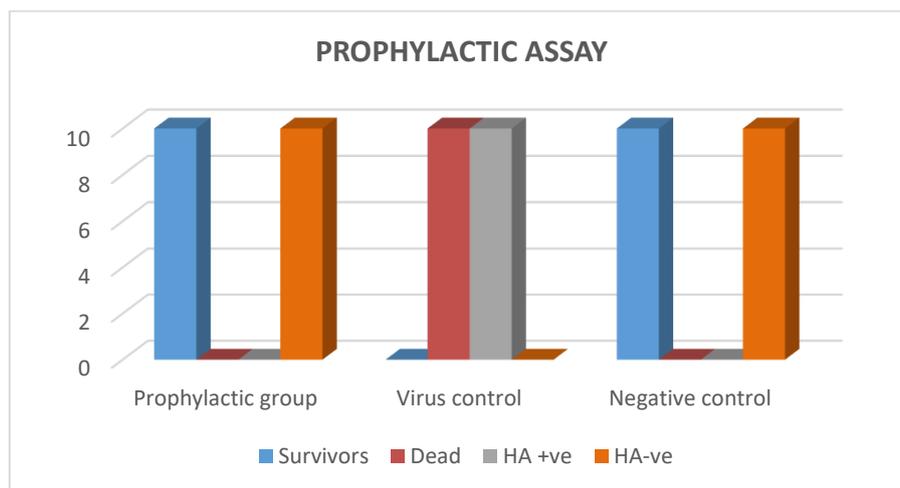
According to our findings, Deca-Cel® oil can be used successfully as a prophylactic agent as all ECEs of prophylactic group survived along the whole experimental period with 100% embryo survival rate and

negative HA results which was the same as that in negative control group (uninoculated group), furthermore virus control group also showed the same results as that previously recorded in the virucidal and therapeutic assays (**Table 4 & Fig. 5**).

**Table 4:** Results of prophylactic assay in ECEs.

| Group                                    | No of eggs | Mortality (4 days pi) |             |             |             | Mortality due to virus % | HA test   |           |
|--|------------|-----------------------|-------------|-------------|-------------|--------------------------|-----------|-----------|
|  |            | 24 h                  | 48 h        | 72 h        | 96 h        |                          | +ve       | -ve       |
| <b>G1:(Prophylactic treatment group)</b> | <b>10</b>  | <b>0/10</b>           | <b>0/10</b> | <b>0/10</b> | <b>0/10</b> | <b>0 %</b>               | <b>0</b>  | <b>10</b> |
| <b>G2: Vc</b>                            | <b>10</b>  | <b>0/10</b>           | <b>5/10</b> | <b>5/5</b>  | <b>0/0</b>  | <b>100 %</b>             | <b>10</b> | <b>0</b>  |
| <b>G3: Uc</b>                            | <b>10</b>  | <b>0/10</b>           | <b>0/10</b> | <b>0/10</b> | <b>0/10</b> | <b>0 %</b>               | <b>0</b>  | <b>10</b> |

Key: Vc: virus control, Uc: uninoculated control, Pi: post inoculation



**Fig. 5:** Prophylactic assay in ECEs.

**Results of antibacterial assay:**

The results of MIC of Deca-Cel® and selected antibiotics against *E. coli* and *Salmonella Typhimurium* isolates were

illustrated in (**Tables 5 & 6**), regarding *E.coli*, our findings showed that it was resistant to cefradine, and clindamycin with MIC >128 µg/mL and to erythromycin with

MIC 128 µg/mL while *Salmonella Typhimurium* was found to be resistant to erythromycin and clindamycin with MIC 32 µg/mL. On the other hand, Deca-Cel® oil showed good MICs value which was 0.2 and 0.4 µL/ml for *E. coli* and *Salmonella*

*Typhimurium* respectively, which means that these EOs can be used successfully as a good alternative to many antibiotics that showed resistance due to its wide use in poultry farms.

**Table 5:** MIC of Deca-Cel® oil and selected antibiotics against *Salmonella Typhimurium*.

| Antimicrobial agent | MIC       | Results   |
|---------------------|-----------|-----------|
| Deca-Cel®           | 0.4 µL/ml | sensitive |
| Erythromycin        | 32 µg/ml  | resistant |
| Clindamycin         | 32 µg/ml  | resistant |
| Tilmicosin          | 2 µg/ml   | sensitive |
| Spectinomycin       | 2 µg/ml   | sensitive |
| Apramycin           | <1 µg/ml  | sensitive |
| Neomycin            | <1 µg/ml  | sensitive |

**Table 6:** MIC of Deca-Cel® oil and selected antibiotics against *E. coli*.

| Antimicrobial agent | MIC        | Results   |
|---------------------|------------|-----------|
| Deca-Cel®           | 0.2 µL/ml  | sensitive |
| Cefradine           | >128 µg/ml | resistant |
| Clindamycin         | >128 µg/ml | resistant |
| Erythromycin        | 128 µg/ml  | resistant |
| Spectinomycin       | 64 µg/ml   | sensitive |
| Tylosin             | 16 µg/ml   | sensitive |
| Streptomycin        | 8 µg/ml    | sensitive |

## DISCUSSION

Veterinary reviews are limited in this area of study. Because of this, it is difficult to compare the findings obtained by various studies because of the variations in the composition of the same EOs and the techniques used to assess the agents' sensitivity. Also in our study we selected Deca-Cel® oil for studying its antiviral and antibacterial efficacy against NDV and some enterobacteriaceae because it contains large number of EOs that are widely distributed, easily available and also have strong record in the literature for their antiviral and antibacterial activity and as far as we know, this is the first study conducted on Deca-Cel® oil. Nonetheless, despite these variations, it is possible to infer the suitability of some compounds as promising

candidates for treating viral and bacterial affections.

Using an in ovo model, three distinct approaches—virucidal, therapeutic, and prophylactic—were employed in this work to systematically investigate Deca-Cel® oil's potential against NDV.

The 0% embryo survival rate with strong positive HA activity recorded in virus control group (Untreated/Challenged) either on virucidal or prophylactic and therapeutic assays indicated the velogenicity of the used ND virus titer as it was potent enough that killed all the embryos within 48-72 hrs post inoculation with severely hemorrhagic embryos exhibited clear pathological lesions that did not appear in the other groups. These findings corroborated those reported

by (Andleeb, Ashraf *et al.*, 2020), who found that all chicken embryos died 48 hours after being injected with NDV.

On the other hand, 100 % embryo survival rate with –ve HA activity which recorded on negative control (uninoculated group) indicated the high safety and hygienic conditions of incubation throughout the whole duration of the experiment.

Depending on results of virucidal and prophylactic assays, it was found that Deca-Cel® oil completely inhibited virus growth in embryonated chicken eggs as revealed by the survival rate of embryos of the inoculated eggs. All ECEs received virucidal and prophylactic treatments showed 100% embryo survival rate and did not show any agglutination activity on rapid HA test with normal features of embryos on post mortem examination indicating that Deca-Cel® oil has direct antiviral activity against ND virus either as virucidal or prophylactic agent.

While in case of therapeutic assay, 40% embryo mortality were recorded while the other 60 % of ECEs survived and did not show any HA activity.

This survival rate recorded with Deca-Cel® as a therapeutic agent may be owing to that inoculation of the virus 2 hrs before the oil gave a time for the virus to propagate in ECE, while high prophylactic and virucidal activity might be due to that activity of different compounds in plants worked their best when added to the cells just around or before the time of virus adsorption.

General speaking, these potent antiviral properties of Deca-Cel® could be as a result of the potent antiviral activity of oregano oil, one of Deca-Cel®'s primary ingredients (Gilling, Kitajima *et al.*, 2014). Besides the powerful antiviral effect of galectin, as reported by (Yang, Lin *et al.*, 2021). Additionally, eucalyptus essential oil, which has been shown by (Sabo and Knezevic 2019) to possess significant antimicrobial

activities against different bacteria, fungi, and viruses.

Also, Thymus vulgaris which is a bioactive ingredient of Eucalyptus was found previously to be effective against NDV. As essential oils of this plant were able to disrupt viral envelopes and prevented attachment of the virion to the host cell (Rezatofghi, Seydabadi *et al.*, 2014)

Our findings supported the results obtained by (Al-Hadid 2016), as they stated that The fruit extract of Eucalyptus camaldulensis showed a potent inhibitory effect against NDV, as Eucalyptus camaldulensis fruits showed complete NDV inhibition at low and medium plant extract concentrations (250, 50 µg mL<sup>-1</sup>) with 0 % embryo mortality. However, a high concentration of Eucalyptus camaldulensis fruits extract (500 µg mL<sup>-1</sup>) resulted in 20% embryo mortality; this might be due to that the chicken embryo reached a certain degree of toxicity.

Mentofin® (a combination of eucalyptus oil and peppermint) in another study has been reported to have complete virucidal activity against ND virus in the presence of organic matter (skimmed milk) (Barbour, Yaghi *et al.*, 2010).

Also our results for Deca-Cel® as a prophylactic agent corroborated those of (Elaissi, Rouis *et al.*, 2012), who found that compared to cell pretreatment, virus pretreatment with Eucalyptus bicostata essential oil revealed superior antiviral activity. The antiviral effect of Eucalyptus astringens essential oil was detected just when the virus was cultured before cell invasion.

Regarding the antibacterial activity, the minimum effective concentrations of Deca-Cel® that we measured were 0.4 µL/ml for *Salmonella Typhimurium* and 0.2 µL/ml for *E. coli*. These results are consistent with a 2007 study by (Busatta, Mossi *et al.*, 2007) that determined the minimum inhibitory

concentration (MIC) of oregano essential oil against *Salmonella* isolate to be 0.460 mg/mL.

With MIC values ranging from 0.23 to 0.69 mg/mL, a different study by (Lu, Dai *et al.*, 2018) also showed that oregano oil had strong antibacterial activity against multidrug-resistant *Salmonella* isolates. Concerning *E. coli* (De Medeiros Barbosa, Da Costa Medeiros *et al.*, 2016) showed that oregano essential oil exhibited antibacterial activity against *Escherichia coli* with the minimum inhibition concentration of 0.6 µL/ml.

It was demonstrated that the strain of *Salmonella Typhimurium* was susceptible to tilmicosin, spectinomycin, apramycin, and neomycin but resistant to both erythromycin and clindamycin. This finding was consistent with those of (Jassim, Obead *et al.*, 2022) and (Adzitey 2018). Additionally, it was demonstrated that a certain strain of *E. coli* was resistant to cefradine, clindamycin, and erythromycin, but susceptible to spectinomycin, tylosin and streptomycin. According to (Kibret and Abera 2011), *E. coli* had high rates of resistance to erythromycin, also (Rubab and Oh 2020) found that *E. coli* had resistance to both cefradine and erythromycin and (Sohail, Khurshid *et al.*, 2015) found that *E. coli* had high levels of resistance to cephalixin (95%), cephradine (95%), and amikacin (91%). Finally, we can say that nowadays a large number of antibiotics showed resistance and Searching for alternatives from natural sources has become indispensable, and according to our findings, Deca-Cel® oil can be used as a good alternative to antimicrobial agents with good MIC values.

## CONCLUSION

Based on the results gained in this study, it would be suggested that Deca-Cel® oil is a good potential antiviral agent against Newcastle disease virus and a good

alternative to antibiotics. However, further work is required to study the effect of these EOs in more depth such as determination of their effect on cell culture and employing other molecular and serological techniques for virus detection, and also to study their effect against another bacterial agents and hence warrants further studies.

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### تقييم التأثير المضاد للفيروسات والبكتيريا لبعض الزيوت النباتية ضد فيروس مرض النيوكاسل والسالمونيلا تيفيموريوم والإي كولاي

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المقدمة: نظرًا لقدرة النباتات الطبية على علاج مجموعة متنوعة من الأمراض الشائعة، فقد جذبت مؤخرًا الكثير من الاهتمام، كما يتم دعم تأثيراتها الطبية الآن من خلال وفرة من الأدلة العلمية، لذلك في الوقت الحاضر المنتجات الطبيعية مثل الزيوت العطرية والمستخلصات الخام توفر فرصًا لا حدود لها لتخليق الأدوية الجديدة. الهدف من الدراسة: أجريت هذه الدراسة لبحث النشاط المضاد للفيروسات والبكتيريا لمنتج الزيوت العطرية ديكاسيل ضد فيروس مرض النيوكاسل، سلالة لاسوتا، كنموذج فيروسي والإشريشية القولونية المقاومة للأدوية المتعددة والسالمونيلا تيفيموريوم كنماذج بكتيرية. المواد والطرق المستخدمة: تمت دراسة النشاط المضاد للفيروسات لهذه الزيوت النباتية بشكل منهجي في ثلاثة بروتوكولات تجريبية. المقاييس القاتلة والعلاجية والوقائية المستخدمة في اجنة البيض. فالبدائية، تم تقدير دراسة السمية لفحص التركيز الأمثل غير السام للنيوكاسيل في بيض الدجاج المخصب ومن ثم تم تحديد فعاليتها المضادة للفيروسات. تمت ملاحظة بقاء الأجنة عن طريق الفحص يوميًا وتم تسجيل معدلات بقاء الأجنة علي قيد الحياة في اليوم الرابع بعد الحقن. بعد انتهاء التجارب، تم قتل الناجين عن طريق تبريد البيض في الثلجة لمزيد من الفحص وتم جمع السائل الجنيني من البيض المحقون لاختبار التراص الدموي السريع (HA) للكشف عن فيروس نيوكاسل. يتم استخدام قيم التركيز المثبط الأدنى (MIC) لتحديد مدى حساسية بعض البكتيريا تجاه هذه العناصر لتقييم نشاطها المضاد للبكتيريا. النتائج: لدراسة نشاطها المضاد للفيروسات، وجد أن زيت الديكاسيل يمكن أن يثبط نمو فيروس مرض النيوكاسل تمامًا مع معدل بقاء أجنة مرتفع يصل إلى 100% مع نشاط سلبي HA كعامل مضاد للفيروسات ووقائي، في حين أنه تم تسجيل 60% معدل بقاء الأجنة ونشاط ترصص دموي سلبي كعامل علاجي، مقارنة بمجموعة اجنة البيض المحقونة بالفيروس فقط التي أظهرت معدل وفيات الأجنة بنسبة 100% خلال 48:72 ساعة مع نشاط HA إيجابي قوي. فيما يتعلق بالنشاط المضاد للبكتيريا، فقد وجد أن قيم MIC لهذه الزيوت كانت 0.4 ميكرو لتر / مل و 0.2 ميكرو لتر / مل ضد السالمونيلا تيفيموريوم والإشريشية القولونية على التوالي، مما يعني أنه يمكن استخدام هذه الزيوت النباتية كبديل جيد للمضادات الحيوية التي أظهرت مؤخرًا مقاومة. الاستنتاج: أظهرت النتائج الحالية أن هذه الزيوت لها خصائص واعدة مضادة للفيروسات ومضادة للبكتيريا ضد العديد من مسببات أمراض الطيور.