# EFFECT OF SOME ANTIVIRAL SUBSTANCES ON SOME FOOD-BORNE VIRUSES

By


## NAGLAA ABOZIED SEIF EL-ALFY

B.Sc. Agric. Sci, (Technology and Management of Agricultural Projects, Open Education Center), Fac. of Agric., Ain Shams Univ., 2009

M.Sc. Agric. Sci. (Agricultural Viruses), Ain Shams University, 2016


**A Thesis Submitted in Partial Fulfillment**
**Of**
**The Requirements for the Degree of**

## DOCTOR OF PHILOSOPHY
## in
## Agriculture Science
## (Agricultural Viruses)


**Department of Microbiology**
**Faculty of Agriculture**
**Ain Shams University**


**2019**

**Approval Sheet**

# EFFECT OF SOME ANTIVIRAL SUBSTANCES ON SOME FOOD-BORNE VIRUSES

By


## NAGLAA ABOZIED SEIF EL-ALFY

B.Sc. Agric. Sci, (Technology and Management of Agricultural Projects, Open Education Center), Fac. of Agric., Ain Shams Univ., 2009

M.Sc. Agric. Sci. (Agricultural Viruses), Ain Shams University, 2016


**This thesis for Ph.D. degree has been approved by:**

**Dr. Ahmed Anwar Mohamed Shahin ……**

Prof. of Microbiology, Fac. of medicine, Zagazig University

**Dr. Abdalla Mohamed Eid El Ahdal …….**

Prof. Emeritus of Agric. Virology, Fac. of Agric., Ain Shams University

**Dr. Khalid Abd El-Fattah El-Dougdoug …….**

Prof. Emeritus of Agric. Virology, Fac. of Agric., Ain Shams University

**Dr. Badawi Abd El-Salam Othman …….**

Prof. Emeritus of Agric. Virology, Fac. of Agric., Ain Shams University

**Date of examination:** 14 / 11 / 2019

# EFFECT OF SOME ANTIVIRAL SUBSTANCES ON SOME FOOD-BORNE VIRUSES

By

**NAGLAA ABOZIED SEIF EL-ALFY**

B.Sc. Agric. Sci, (Technology and Management of Agricultural Projects, Open Education Center) Ain Shams Univ., 2009

M.Sc. Agric. Sci. (Agricultural Viruses), Ain Shams University, 2016

**Under the supervision of:**

**Dr. Badawi Abd El-Salam Othman**

Prof. Emeritus of Agric.Virology, Agric. Microbiol. Dept., Fac. of Agriculture, Ain Shams University (Principal Supervisor).

**Dr. Khalid Abd El-Fattah El-Dougdoug**

Prof. Emeritus of Agric.Virology, Agric. Microbiol. Dept., Fac. of Agriculture, Ain Shams University.

**Dr. Ali Fahmy Mohamed**

Head Researches of Microbiology, Holding Company for Biological Product and Vaccines (VACSERA).

# ABSTRACT

**Naglaa Abozied Seif EL-Alfy: Effect of some Antiviral Substances on some Food-Borne Viruses. Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2019.**

The antiviral role of *Moringa* Oleifera leaves extracts ((chloroform (CL), Ethyl acetate (E.A), methanol 80% (M) and n.butanol (n.b)) to control Coxsackie B (COX-BV) and Hepatitis A (HAV) viral infections were monitored in vitro and in vivo compared with human interferon alpha (IFNă-2a) in order to evaluate the antiviral activity of Moringa leaves extracts. The total phenolic content of CL, E.A, M and n.b extracts of Moringa leaves extracts were estimated using spectrophotometry, it was found that, the E.A extract contained the highest ratio of phenol followed by n.b extract. In vitro, cytotoxicity was determined for *Moringa* leaves extracts on viability of HEP-2 and Vero cells using MTT assay. It was found that, The 97.7 µg/ml concentrations of four extracts were considerably non-toxic for HEP-2 and Vero cell lines culture. Antiviral activity of four *Moringa* leaves extracts compared with IFNă-2a to HEP-2 cell lines against COX-BV and Vero cell lines against HAV viral infection was determined by assessment of the COX-BV and HAV viruses infectivity titer declining rate and relative residual living cell count using End Point Assay, using three ways pre-treatment, co-treatment and post-treatment cell line was treated with *Moringa* extracts. It was found that, the extract E.A and n.b were better as antiviral activity of COX-BV in pre-treatment and HAV in co-treatment respectively compared with IFNă-2a. In vivo, toxicity of Moringa extracted by E.A and n.b were evaluated using male albino Wistar rats, the E.A and n.b safe concentration extracts 97.7 and 390 µg/ml toxic concentration were given oral gavage administered daily for 28 days. It was found that, the toxic concentrations of E.A and n.b Moringa extracts at 390 µg/ml were toxic on both the rats and the cell culture and the nontoxic concentration of E.A and n.b extracts

at 97.7 µg/ml were safe on the rats as well as the cell culture (HEP-2 and Vero cells). Antiviral experiment was performed using nontoxic conc. of E.A and n.b extracts comparing with the interferon on rats infected with COX-BV or HAV. The COX-BV and HAV titers in the infected intestinal of rats and treated rat with E.A, n.b and IFN -α2a at 5 weeks were determined by infecting a particular HEP-2 and Vero cell line respectively and determined the highest dilution producing cytopathic effect in 50% of the inoculated cells. It was found that, The COX-BV and HAV infectivity titer $10^3$ TCID50 as in post treatment rat with E.A, n.b and IFN -α2a showed antiviral activity in addition both antiviral showed an undetected virus infectivity titre on the 3rd week post infection. Ab level in blood collected from treated rat with E.A, n.b and IFN-α2a and infected with COX-BV virus and HAV was measured at the end of each week for 5 weeks using ELISA technique. The immunity against COX-BV and HAV it was noticed that, antiviral E.A and n.b extracts showed decreases level of ab at $4^{th}$ and $3^{rd}$ week respectively, compared with rats infected with COX-BV and HAV only as positive control, no treated rats as negative control and rats treated with IFN-α2a. Cytokines (Interlukin-6 (IL-6) and Interferon gamma (IFN-γ)) levels were measured in the infected treated rats with E.A and n.b extracts, infected treated rats with IFN-α2a and rat infected with COX-BV or HAV virus only on the 3rd, 7th and 15th days post viral infection using DAS ELISA technique. Regarding IL-6, data showed that the highest level of IL-6 was reported in infected rats with virus only on the 3rd day. While infected treated rats with E.A and n.b extracts and IFN-α2a showed a decreased IL-6 level at the same time. Regarding IFN-γ, data showed that, the highest level of IFN-γ was reported in infected treated rats with E.A and n.b extracts on the 3rd day post treatment then infected treated rats with FN-α2a then infected rats with virus only. MX gene expression values were estimated in HEP-2 and Vero cells (in vitro) and intestinal tissue of rats (in vivo) treated with non-toxic conc. E.A, n.b extracts and IFN-α2a that stimulate the induction of MX-mRNA as a marker of antiviral activity using specific primers by rt-

RT-PCR. It was found that, in vitro, the highest value for MX gene expression was in HEP-2 and Vero cells treated with IFN-α2a then HEP-2 and Vero cells treated with E.A and n.b extracts compared to control cells. In vivo, the highest value for MX gene expression was in intestinal tissue of rats treated with E.A extract, then intestinal tissue of rats treated with IFN-α2a, then intestinal tissue of rats treated with n.b extract compared to control intestinal tissues. Total Phenolic conc. (TPC) in rats serum were determined for serum of rats treated with E.A and n.b extracts and non-treated rats (control) using spectrophotometer. The result showed that, the TPC in serum of rat treated with E.A extract was higher that its conc.in serum of rat treated with n.b extract compared to control. The components present in the E.A and n.b extracts of Moringa leaf were identified by GC-MS analysis. It was found that, active compounds mutual between E.A and n.b leaves extracts of Moringa were: Phytol; (-)-Loliolide; 1 ,2-Benzenedicarboxylic Acid,3-Nitro; Methyl 15-Acetylhydroxypalmitate; Z,Z-8,10-Hexadecadien-1-ol; 2,6,10-Dodecatrien-1-ol,3,7,11-Trimethyl. Antiviral activity in Moringa extracts may be attributed to phenolic compounds and / or as a result of stimulation of COX-BV-sensitive HEP-2 cells and HAV-sensitive Vero cells or intestinal tissue of rats treated with E.A and n.b extracts to express MX gene protein. It was concluded that *Moringa* leaves extracts have antiviral activity against COX-BV and HAV.

**Key Words.**

*Moringa Oleifera* leaves extracts, phenol compound, IC50, Cytotoxicity, Antiviral activity, COX-BV and HAV Ab, IL-6, IFN-γ, MX gene, GC-MS.

# ACKNOWLEDGMENT

My sincerest gratitude is due to Allah, who provided me with the blessings and health to finish this thesis. He encompassed me with His mercy and guidance in every step on the way.

I would like to express my dearest gratefulness to my supervisor Prof. Dr. Badawi A. Othman, Professor Emeritus of Virology, Dept. of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, for his supervision, continuous guidance, and kindness and also for his faith in my research and his confidence in me as a person. His truly scientist nature was a continuous source of inspiration for me during the work and provided me with all the encouragement and support I needed throughout my thesis.

My deepest gratitude and sincere thanks to Prof. Dr. Khalid Abd El-Fattah El-Dougdoug, Professor Emeritus of Virology, Dept. of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, for his efforts and valuable assistance to follow up the progress of this work with his continuous guidance during his supervision.

I'm greatly indebted to express my special thanks and great appreciation to Prof. Dr. Ali Fahmy Mohamed, Professor in General Director of Applied Research of the Holding Company for Biological Product and Vaccines (VACSERA), for his efforts and valuable assistance to follow up the progress of this work with his continuous guidance during his supervision.

I'm greatly indebted to express my special thanks and great appreciation to Dr. Samar Ahmed El-Masry, lecture of Virology, Dept. of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, for continuous support and unlimited helping.

I would like to express my great appreciation to all staff members of the Dept. of Agric. Microbiol., Fac. of Agric., Ain Shams Univ. for their help and cooperation during the investigation.

At last, but never the least, a very heartfelt gratitude goes to my Family and my colleagues for their cooperation and kind encouragement during my study.

## CONTENTS

## LIST OF TABLES