BIO-CONTROL OF SOME PATHOGENS INFECTING DATE PALM USING VIRUSES

By

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B.Sc. Agric. Sc. (Microbiology), Ain Shams University,2008.

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ABSTRACT

Loay Lewaa El-Hamd Abd-allah: Bio-control of some pathogens infecting date palm using viruses. **Unpublished Ph. D. Thesis, Department of Microbiology, Faculty of Agriculture, Ain Shams University, 2018.**

Pink rot inflorescence disease in date palm (*Phoenix dactylifera* L.) is considered important disease in date palm. The causal agent was isolated from trunk using specific media. The causal agent was identified as *Serratia marcescen*s using 16s rRNA gene sequencing and was recorded in GenBank. *B.subtilis* bacterium is considered pathogenic bacteria in date palm in tissue culture stage that obtained from ARC.

Three different specific lytic phages of *S. marcescens* were isolated from soil and trunk of the diseased date palm tree using single plaque isolation technique and named SP1, SP2 and SS1 and 3 phages for *B.subtilis* were isolated from soil and named BS1, BS2 and BS3.

The morphological characters revealed that the isolated bacteriophages for *S. marcescens* and *B.subtilis* are belonging to *Myoviridae* family and *Siphoviridae* family. Thermal inactivation points (TIP) of phages were 54±1, 78±1, 52±1, 58±1, 46±1 and 54±1°C and pH range were 7-9, 6-9, 5-9, 6-8, 6-9 and 5-8 for phages SP1, SP2, SS1, BS1, BS2 and BS3 respectively. Freezing and thawing of phages showed that phages still active for 3, 3, 2, 4, 2 freezing and thawing times and UV irradiation for 25, 20, 25, 20, 15 and 20 min for SP1, SP2, SS1, BS1, BS2 and BS3 respectively. Longevity *In Vitro* (LIV) of all phages appeared active more than 90 days. Host range for serratia phages infect *S. marcescens* A, B and C with SP1 and SP2 while SP1 and SS1 infected *S. marcescens* D. All *B.subtilis* phages couldn’t infect any identified

bacteria. Bradford showed the total protein of phages that 0.29, 0.35, 0.28, 0.23, 0.14 and 0.28 mg/ml for SP1, SP2, SS1, BS1, BS2 and BS3 respectively. SDS-PAGE showed molecular weight of protein of SP1 was ~66, 35 and 29 KDa. SP2 was~ 38, 35, 30 and 29 KDa. SS1 was ~97, 38, 35, 30 and 29 KDa. BS1 was ~97, 70, 37 and 35KDa. BS2 was ~120, 97, 70, 35 and 25 KDa. BS3 was ~97, 70, 35 and 27 KDa. DNA concentration was was 10.13, 9.76, 11.48, 12.14, 10.75 and 7.26 µg/ml. RAPD-PCR showed SP1 amplified to 4 fragments with 2000, 1500, 1000 and 600 bp, phage SP2 amplified to 4 fragments with 1500, 1000, 700 and 600 bp, SS1 phage amplified to 5 fragments with 1500, 1000, 800, 700 and 600 bp. BS1 amplified to 3 fragments with 1000, 600 and 500 bp, phage BS2 amplified to 2 fragments with 1000 and 600 bp, BS3 phage amplified to 2 fragments with 1000 and 500 bp

Five formulas were used to protect phages from UV irradiation the best used formulas were Beet-root juice and Carrot juice for all *S.marcescens* phages and Beet-root juice, Carrot juice and Casein formulas for all *B.subtilis* phages.

As phage therapy, Phages were used for preservation and prevention *In vivo* and *In vitro* after 0, 30 and 60min. Results showed that using of phages for prevention better than the preservation.

Total phenolic compounds and indols decreased in preservation comparing with control while the level of activity of PPO enzyme increased in preservation comparing with control.

Key words: date palm, *Phoenix dactylifera*, Bacteriophage, Phage, *Serratia marcescens*, *Bacillus subtilis*, pink rot inflorescence, tissue culture formula, phage therapy, phenols.

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