ISOLATION AND PARTIAL CHARACTERIZATION OF *SALMONELLA* PHAGE FROM MAROJARM PLANTS

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ABSTRACT

The marjoram plant is recorded as medicinal one, traditionally recommended as stimulant / tonic , and help to reduce the severity of asthma, indigestion, headache, rheumatism and toothache**.**

Results of this study showed high total count of contaminating bacteria in marjoram plants in non-packed samples collected from eight Egyptian governorates as compared with the pre-packed ones from two local companies. The presence of *Bacillus* sp. and *E. coli*, but not *Salmonella* sp. are note-worthy.

One phage successfully infected *Salmonella typhimurium* was isolated using plaque assay which produced circular turbid plaques .The electron micrograph of negatively stained preparation indicated that the phage particles have hexagonal head (~80 nm) with short tail (~20 nm), which is classified to family *Podoviridae*. The biological characterization of the phage isolate is found to be : inactivation point at 62°C for 10 min., longevity *in vitro* for 72 hrs, dilution end point of 10^{-3} and stability for 1.5 hrs at pH 7.2.

Keywords: Marjoram plants, Microbial contamination, *Salmonella* phages.

INTRODUCTION

Marjoram plants (*Marjorena hortensis* L.) and marjoram-related microbial populations with their corresponding phages should have received more research in Egypt.

In Egypt, the plant has a cultivated area of nearly 4000 feddan, mostly in the governorates of Giza, Fayum, and Menia **(Ministry of Agriculture, 2005)**. It is traditionally recommended as stimulant / tonic , and help to reduce the severity of asthma, indigestion, headache, rheumatism and toothache. **Purdue University (1997)** reported some antifungal and anticancer activities.

Sources of contamination of medicinal plants are multiple, among which are practices applied during harvesting, handling, drying, packing and storage. **Kneifel and Berger (1974)** emphasized the importance of drying and storage as potential sources of contamination. Specifically, contamination with *Salmonella* is mostly due to polluted surface / standing water **(Tindall *et al* ., 2005 and Ryan & Ray, 2004).**

Egyptian researches on isolation of phages from medicinal plants are non-existent. The present study aimed to fill that gap.

MATERIALS AND METHODS

1. Source of plant samples

From local shopes in eight governorates (five samples per each), non-packed marjoram plants were taken in consideration. Additionally, two local companies offered pre-packed samples (five per each) to be included in the study. All samples were maintained in plastic bags at 2-5°C until analyses.

2. Bacterial load

Steps for the total count of bacteria and yeast and the presence / absence of *Bacillus* sp., *E. coli* and *Salmonella* sp. were carried-out according to procedures detailed in the following references:

- Total count of:

Bacteria	**Kunene *et al* . (1999)**
Yeast	**Yamamato *et al.* (1999)**

- Presence / absence of

Bacillus sp.	**Power *et al*. (1975)**
E. coli	**Jones & Stevens (2002)**
Salmonella sp.	**Giannella (1996)**

3. Source of bacterial isolates

Ten isolates of the following bacteria from the Virology Lab of Department of Microbiology , Ain Shams University Faculty of Agriculture are provided:

Salmonella typhimurium	*Staphylococcus* sp.1
Salmonella sp.1	*Staphylococcus* sp.2
E. coli strain B	*Esercheii* sp.1
E. coli strain $H_1D_1B_1$	*Esercheii* sp.2
Bacillus subtilis	*Bacillus megatherium*

4. Sources of phages

The phage was isolated from the plant samples as recorded in number (1).

5. Detection and isolation of phages

A lytic phage was assayed qualitatively (spot test) and quantitatively (plaque assay) according to **Robert *et al.* (1970).** The single plaque isolation technique was applied to produce the pure lysate phage.

6. Determination of biological characters of the isolated phage

Tests / assays were carried-out to determine the biological characters of the isolated phage. The following references were used table (3):

Test / assay	Reference
- Thermal inactivation point	Robert *et al* . (1970)
- Dilution end point	Robert *et al* . (1970)
- Longevity *in vitro*	Stringer (1986)
- Stability at different pH levels	Stringer (1986)

The phage morphology was described using a transmission electron microscope (Type Jeol-Jem 1010, Regional Centre of Fungi, Al-Azhar University, Cairo , Egypt) used negative staining technique utilizing phosphotengestic acid (PTA) at PH 6.8.

RESULTS AND DISCUSSION

Data represented in table (1) show a source / microbe matrix for the highest total count (HTC) is presented as follows:

		HTC of	
		Yeast	Bacteria
Source	Shops	54×10^5	71×10^6
	Companies	52×10^4	77×10^4

It appears that the HTC was greater for shops than for companies , and for bacteria than for yeast. Results reported by **Frank (1989), Kunene *et al.* (1999)** and **Miwa *et al.* (1999)** showed that in marjoram the bacterial load is much higher than that of yeast.

According to Table (2), samples from the companies did not exhibit any sign of *Salmonella* presence. However, they showed mild presence of *Bacillus sp.* and *E. coli.* Shops, on the other hand, gave six out of 8 samples free from *Salmonella,* together with mild presence of *Bacillus* sp. and extensive presence of *E. coli*.

From one sample (taken from BeniSuef governorate), a lytic phage was isolated and characterized as specific for *Salmonella typhimurium* (Fig. 1). After incubation, the isolate produced turbid and circular single plaques (Fig. 2). The isolated of *Salmonella* phage shows signs of remarkable stability in terms of thermal inactivation point of 62°C/10 min., longevity *in vitro* for 72 hrs., dilution end point of 10^{-3} , stability for 1.5 hrs at pH 7.2. These results are in general agreement with those reported previously **by Robert *et al.* 1970, and Stringer, 1980.**

According to transmission electron micrographs (Fig. 3), the isolated *Salmonella* phage appeared to be of hexagonal head (~80nm) and short tail (~20 nm), indicating that it belongs to family *Podoviridae* as pointed out by **Robert *et al* .(1970), Ackerman & DuBow (1987) and Carey-Smith *et al* . (2006).**

In light of the present results it is safe to use packed marjoram plant due to the absence of *salmonella* sp. In case of using cold non-packed of marjoram plant, it is

suggested to use *salmonella* phage as biological control after sequencing the genomes of lytic bacteriophages e.g: *Salmonella* phage vil for safety prior to food applications **(Anany *et al*. 2011).**

Table (1): Load of yeast and bacteria in samples of marjoram plants, non-packed (from local shops) and pre- packed (from local companies)

	Microbial total count (mean ± SD)*	
Source of samples	Yeast	Bacteria
Non-packed from local shops in: Cairo governorate	($31 \times 10^4 \pm 24.23$)	($41 \times 10^5 \pm 1.89$)
Kalyoubia governorate	($80 \times 10^4 \pm 40.00$)	($69 \times 10^6 \pm 56.94$)
Giza governorate	($56 \times 10^4 \pm 39.23$)	($42 \times 10^4 \pm 42.42$)
Alexandria governorate	($38 \times 10^4 \pm 32.81$)	($71 \times 10^6 \pm 50.59$)
Ismailia governorate	($54 \times 10^5 \pm 30.33$)	($53 \times 10^4 \pm 29.87$)
Fayum governorate	($52 \times 10^3 \pm 20.21$)	($52 \times 10^4 \pm 20.23$)
BeniSuef governorate	($57 \times 10^3 \pm 20.68$)	($49 \times 10^4 \pm 42.49$)
menia governorate	($34 \times 10^3 \pm 31.44$)	($219 \times 10^2 \pm 16.48$)
Pre-packed from local companies:		
Company I	($32 \times 10^4 \pm 27.27$)	($61 \times 10^4 \pm 36.77$)
Company II	($52 \times 10^4 \pm 28.17$)	($77 \times 10^4 \pm 20.47$)

* found in 10 g of dry leaf plant sample diluted in 90 ml sterile saline solution.

Table (2): Presence / absence of *Bacillus sp., E. coli* and *Salmonella sp*. in samples of marjoram plants, non-packed (from local shops) and pre-packed (from local companies).

Source of samples (5 per source)			
	Bacillus sp.	*E. coli*	*Salmonella sp.*
Non-packed from local shops in:			
Cairo governorate	+	+	-
Kalyoubia governorate	+	++	+
Giza governorate	+	++	-
Alexandria governorate	+	++	-
Ismailia governorate	+	++	-
Fayum governorate	+	++	-
BeniSuef governorate	+	++	-
Menia governorate	+	++	+
Pre-packed from local companies:			
Company I	+	+	-
Company II	+	+	-

Extensive (++) / Mild (+) / Absence (-).

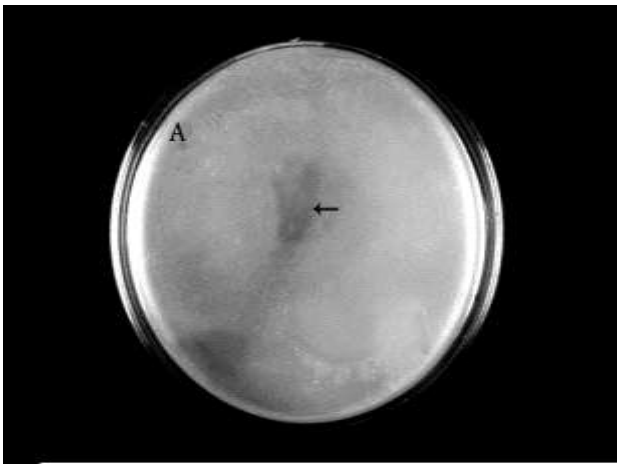

Fig . (1): A spot test showing the lysis of *Salmonella typhimurium.*

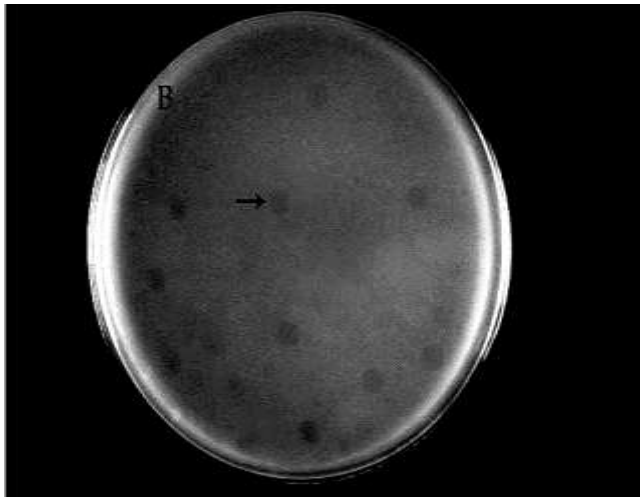

Fig . (2): plaque assay technique show plaques resulting from the of the lytic *Salmonella* phage.

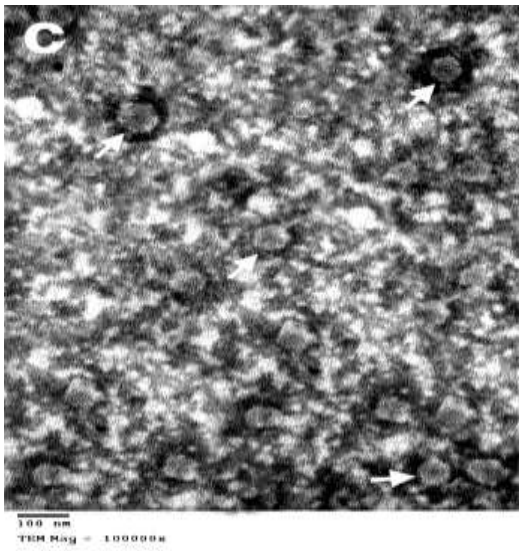

Fig. (3): Electron micrograph of lytic *Salmonella* phage (arrows) stained with 2% phosphotengestic acid (PTA) at pH 6.8.

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عزل ودراسة بعض الخصائص فاج السالمونيلا المعزول من نباتات البردقوش

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معمل الفيرولوجي ، قسم الميكروبيولوجيا الزراعية ، كلية الزراعة ، جامعة عين شمس ، شبرا الخيمة ، القاهرة ، مصر.

يعتبر نبات البردقوش من النباتات الطبية الهامة حيث انه يلعب دورا هاما فى الحد من أزمات الربو التى تصيب الجهاز التنفسى ويعالج كثير من الأمراض مثل عسر الهضم والروماتيزم والصداع وكذلك الم الاسنان.

وقد أظهرت نتائج هذه الدراسة ارتفاع العدد الكلي للبكتريا الملوثة لعينات نباتات البردقوش الغير معبأة والمجمعة من ثماني محافظات مصرية بالمقارنة بالعينات البردقوش المعبأة والمجمعة من شركتين محليتين. حيث تواجد كل من ميكروبات *Bacillus sp.* الباسيلس و*E.coli* الإيكولاي بنسبة عالية في حين تواجدت السالمونيلا بنسبة قليلة في بعض العينات الغير معبأة.

وقد تم عزل فاج واحد قادر على إصابة ميكروب السالمونيلا تيفاي من عينة من عينات نباتات البردقوش الغير معبأة وكانت عزله الفاج تحدث بلاكات دائريه الشكل – معكرة.

وعند التعرف على الشكل المورفولجي لهذا الفاج بإستخدام تكنيك الصبغ السالب والفحص بالميكروسكوب الإليكتروني وجد أن هذا الفاج له رأس سداسيه الشكل ذات أبعاد ٨٠ نانومتر وذات ذيل قصير ذات أبعاد ٢٠ نانومتر وبهذه الخصائص يكون هذا الفاج يتبع عائلة Podoviridae.

وعند الكشف عن ثبات هذا الفاج وجد أنه يفقد قدرته على إحداث العدوى عند تعرضه لحرارة ٦٢°م لمدة ١٠ق ، وعندما يخفف إلى $١٠^{-٣}$ ، وبعد ٧٢ ساعة عندما يحفظ على درجة حرارة المعمل عند PH ٧,٢ لمدة ساعة ونصف من التحضين.

الكلمات الدالة :

نباتات البردقوش – التلوث الميكروبي – فاجات السالمونيلا.