Zagazig J. Agric. Res., Vol. 34 No .(4) 2007 717-736

TESTING MUTAGENIC ACTIVITY OF MAIDENHAIR FERN PLANT (*Adiantum capillus- veneris L.*) EXTRACT USING SOME MICROBIAL SYSTEMS

Hassan, Amina A[1]. and Eman A. H. Khattab[2]

[1.] Genetics Dept., Fac. Agric., Zagazig Univ., Egypt

[2.] Virus and Phytoplasma Dept., Plant Pathol. Res. Inst. Agric. Res. Center, Giza, *Egypt.*

Accepted 23/5/2007

ABSTRACT: The mutagenic activity of *Adiantum capillus-veneris L.*, a medicinal herb that has been used as a medication treatment in many developing countries was assessed using some bacterial models (prophage induction and transduction).The results showed that the plant extract has no mutagenic activity in the range of used concentrations from 0.25 to 30%.The transduction assay introduced another proof that this plant has no mutagenic activity, whereas transduction frequency at high concentration (30%) was $2.03x10^{-6}$ while at zero concentration (control) was $6.3x10^{-6}$. This might due to the inability of plant extract to increase the number transducing particles responsible for transducing the marker gene. On the other hand, the antimicrobial properties were studied against bacteria, bacteriophage and plant viruses. The results showed that *A. capillus-veneris L.* extract had a slight toxicity towards tested *Pseudomonas aeruginosa* bacterial strains,but it had remarkable anti-bacteriophag property against phage Fll6, whereas pfu/ml reached $0.4x10^{11}$ at the highest concentration (30%)while it was $12.6x\ 10^{11}$ at zero concentration (control). The *A. capillus-veneris L.* extract was able to inhibit the infection of tomato mosaic virus (TMV) and cucumber mosaic virus (CMV) viruses which were isolated from natural infected tomato and squash plants respectively. Systemic and local infections were carried out pre- and post inoculation at three different time intervals (direct, after 24h. and after 48h.) at two different dilutions.

These results demonstrated that plant *A. capillus-veneris L.* extract was able to inhibite both TMV and CMV infection. The inhibitory effect was slight in case of post inoculation, while it was

more prononced in case of pre- inoculation. In general the extract of *A. capillus-veneris L.* had a high inhibitory effect against TMV and CMV by direct inoculation. The inhibitory effect in systemic infection (90%, 95%, for TMV and 80%, 85%, for CMV) was higher than in local infection (98.3%, 75% for TMV and 20%, 60% for CMV).

Key words: *Adiantum*, mutagen, transduction, bacteriophage, mosaic virus.

INTRODUCTION

Since the beginning of time, man was searching for a remedy to cure his illness. One of the best methods was herbs. He also used chemicals as alternative but lately figured out that it was not the ideal solution due to its unexpected side effects. As well as medicinal herbs had long thought to curative powers while present medical knowledge recognizes that some herbs have a healing properties. Some herbs were harmless while others were dangerous if consumed (Bresolin and Varagas, 1993; and Ferrdira and Varagas, 1999).

Medicinal plants are widely used to treat various diseases and are commonly used in popular medicine. The use of these plants by the general population is old and still a widespread practice. This makes study of their genotoxicity an essential target. More than 150.000 plant species have been studied and many of them contain therapeutic substances (Hoyes *et al.*, 1992). Many diseases have been treated using these plants such as diarrhea, hemorrhoid, digestive, diuretic, antiseptic, wound healer, antispasmodic, antiseptic, astringent, emollient... etc. The therapeutic substances of these plants can be extracted and used in preparation of drugs, or the plant itself can be used directly as a medication.Some plant extracts were used as inhibitors of plant viruses. Baranwal and Verma (1993) reported that out of 16 of plant extracts tested against infection of *Cyamopsis tetragonoloba* (guar) by *Sunn-hemp mosaic tobaccovirus,* 10 were 86- 100% inhibitors when applied for 24h, before challenge inculation. Non of the active extracts induced systemic resistance against virus.

Patel *et al.* (2000) tested leaf extracts of 29 plants for their effects on lesion development of

TMV on *Nicotiana glutinosa*. The results showed that leaf extracts of *Madhuca indica* inhibited TMV infection completely.

Leaf extracts of several plants were also potent inhibitors of TMV infection. Medicinal plants were able to synthesize toxic substances, which in nature was acted as a defense against infections, insects and herbivores and often affect the organisms that attack them. The medical uses of *A. capillus veneris L.* were variant, it used as a diuretic, and to treat urinary disorders, colds, rheumatism, heartburn, gallstones, alopecia and sour stomach, gravel and other impurities of the kidneys, dandruff, hair loss, menstrual difficulties, hydrophobia, catarrh, diabetes, boils, eczema and wounds. *A. capillus-veneris L.* was killed viruses, bacteria, and reduces phlegm, suppresses coughs, detoxifies, fights free radicals, support heart, clean blood, increases urination, lowers blood sugar and stimulates menstruation. Dried secretions could protect liver, reduce cholesterol, reduce blood pressure, stimulate, support gallbladder and heal wounds (Leslie, 2002).

Antimicrobial and antifungal activities of *A. capillus-veneris L.* were reported.The plant was found to inhibite the growth of *Streptococcus pyogens, Bacillus subtilus, Corynebacterium ovis, Aspergillus flavus, Aspergillus niger, Trichophyton mentagrophyte, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa* (Mahmoud *et al.*, 1989, Mahran *et al.*, 1990 and Pradeep *et al.*, 2002 a and b).An ethanol extract of the rhizome of *A. capillus-veneris L. plant* evidenced antiviral properties *in vitro* against *Vesicular stomatitis* virus (Leslie, 2002).The phytochemical screening revealed the presence of volatile oil in the fronds of *A. capillus-veneris L.* growing in Egypt. The analysis showed 16 compounds of the volatile oil (Mahran *et al.*, 1990).

Therefore, this study was conducted to evaluate mutagenic activity of *A. capillus-veneris L.* plant extract using prophage induction and transduction assaies. No much studies were carried out to evaluate its mutagenic activity.

MATERIALS AND METHODS

Bacteriophage and Bacterial Strains

The generalized transducing F116 phage and the original

bacterial strains of *Pseudomonas aeruginosa* PAO1,PU21 and MAM2, were obtained from M. Day, University of Wales, Cardiff, UK.The strains that were used in this study listed in Table 1.

Table 1. Bactrial strains of *Pseudomonas aeruginosa*

Strains	Genotypes	References
MAM2A1	Lysogen of MAM_2 (F116), Str^r	This study
PU21A3	Non- lysogen, Str^s, Amp^r	This study

Str^s = Streptomycin sensitive.
Str^r = Streptomycin resistance.
Amp^r = Ampcillin resistance.

Plant Extract of Maiden- Hair Fern (*Adiantum capillus-veneris L.*)

The fresh plants of maidenhair fern were obtained from natural source. The whole plant dried at room temperature and then blended to give a homogenate material. Fourty gram of dried homogenate soaked in 300 ml of ethyle alcohol 60% for 2-3 days, filtered and soaked again in 100 ml of ethyle alcohol 60% for 2-3 days. Finally, the extract was filtered. A volume of plant extract was transferred into the rotary evaporator to get powder. The powder was dissolved using dimethylesulfoxid (DMS). This solution was sterilized by filtration through filter membrane (0.2 µm Whatman) and then a stock of plant extract was prepared. Several concentrations (0.25, 0.5, 1, 5, 10, 15, 20, 25, 30%) were used in this study .

Growth Media

The nutrient agar (NA) and nutrient broth (NB) media were used. Soft agar (0.8% w/v agar) was prepared in distilled water and kept at 45°C on waterbath. Phosphate buffer was prepared from 1/15M potassium phosphate (KH_2Po_4) and 1/15M disodium phosphate (Na_2Hpo_4. $2H_2O$). Streptomycin (12 mg/ml) and ampcillin (600 µg/ml) were added as sterilized solutions by filtration through 0.2 µm filter membrane to the media after autoclaving.

Treating Plant Extract of Bacterial Strains

The plant extract was added to NB medium and inoculated by individual MAM2A1and PU21A3 strains. After inoculation, the cultures were incubated at 30°C overnight. Serial dilutions were prepared and counts of donor and recipient were recorded on NA

medium. The survival of donor and recipient were calculated as colony forming units (cfu / ml).

Treating Plant Extract of Lysogenic Strain to Prophage Induction

The NB flasks with different concentrations of plant extract were prepared, inoculated by lysogenic strain MAM2A1 and incubated at 30°C for 24h. A few drops of chloroform were added and centrifugated at 5000 room for 30 min, filtred. The supernatant was assayed, the plaque forming units (pfu/ml) were calculated for each concentration.

Phage Titration

Serial hundred- fold dilutions of phage were prepared in phosphate buffer (PH 7.0). Phage titer was determined by mixing equal volumes (0.1 ml) of a phage dilution with host cells (growing overnight in NB at 30°C), adding soft agar, and pouring immediately onto an NA plate (Gulig *et al.*, 2002) . Plates were incubated at 30°C for 24h, and pfu/ ml was recorded.

Transducing Streptomycin Resistance gene

The induced phage particles from treated lysogenic strain were used to transduce streptomycin resistance gene. Recipient cells were grown in NB overnight. Viable count of the recipient strain was made. Equale volumes (0.5 ml) of phage lysate and recipient cell suspensions were mixed (Multiplicity of infection MOI = 1: 10). The mixture was kept for 15-30 min at room temperature, to allow phage adsorption. Serial dilutions were prepared and placed onto selective media. Number of colonies (transductants) were recorded and transduction frequencies were calculated.

Negative Control Test

The ascorbic acid (Vitamin C) was used as a negative control with prophage induction from lysogenic strain and transduction assay. One gram of ascorbic acid was dissolved in 100 ml distilled water as a stock.

Positive Control Test

The ethyl methane sulfonate (EMS) was used as a positive control as the same procedure described in the negative control test.

Anti-Viral Activity

Treating plant extract of bacteriophage F116

The phage lysate was treated with the same previous concentrations of plant *Adiantum*

capillus-veneris L. extract for 24h. The plaques were counted and the pfu/ ml was calculated. The treated phage lysate was used in transduction assay as previously mentioned.

Treating plant extract of TMV and CMV viruses

Two virus isolates, tomato mosaic virus (TMV) and cucumber mosaic virus (CMV) were used in this study. These viruses were isolated from naturally infected tomato plants *(Lycopersicon esculentum L.)* and squash plants *(Cucurbita pepo L.)* respectively. *A. capillus-veneris L.* extract was applied pre- and post- inoculation by different dilutions (1: 1 and 1: 2 v/v) at different intervals (immediately, after 24h. and after 48h.) on either TMV or CMV infection on systemic host *(Lycopersicon esculentum L.)* and local host *(Chenopodium amaranticolor).* Local lesions produced on the leaves were counted three to five days after inculation.Inoculated plants were examined for symptoms development. Plants used as controls were inoculated with distilled water. The inhibition percentage of virus infectivity was caculated according to Baranwal and Verma, (1992).

Percent inhibition= (C- T/ C) x 100

Where:

C= average number of lesion on control leaves.

T= average number of lesion on treated leaves .

This investigation was carried out at the Microbial -Genetics Lab.,Genetics Dept., Fac., Agric., Zagazig Univ. and at Virus and Phytoplasma Dept.,Plant Pathology Res. Inst., Agric. Res.Center, Giza.

RESULTS AND DISCUSSION

Effect of *Adiantum caplillus-veneris L.* Extract on Survival Percentages of *Pseudomonas aeruginosa* Strains

The effect of *A. capillus-veneris L.* extract on survival percentage of two different strains of *P. aeruginosa* was investigated Table 2. The survival percentage (S%) ranged from 64.4 up to 93.3 % in strain MAM2A1 upon exposure to different concentrations of the tested plant. The survival percentages of the other strain was nearly the same. The killing effect of 30% of the plant extract reached 35.6 and 37.9% in strains MAM2A1 and PU21A3 respectively. These

results showed that plant *A. capillus-veneris L.* had a slight toxicity to the bacteria at used concentrations. Previous studies demonstrated that the *A. capillus-veneris L.* extract showed high activity against *Bacillus subtilus, Staphylococcus aureus, E. coil* and *Pseudomonas aeruginosa* (Mahmoud *et al.*, 1989). Moreover, Mahran *et al.* (1990) found that *A. capillus-veneris L.* inhibited the growth of *Streptococccus pyogenes, Bacillus subtilus, Corynebacterium ovis, Aspergillus flavus, Aspergillus niger* and *Trichophyton mentagrophyte.*The *A. capillus-veneris L.* extract had antimicrobial action against *Bacillus, E. coli, Staphylococcus, Proteus, Pseudomonas* and *Candida* (Leslie, 2002).

Table 2. Survival percentage (S%) of *Pseudomanas aeruginosa bacterial* strains upon exposure to plant *Adiantum capillus-veneris L.* extract.

Conc. %	MAM2A1		PU21A3	
	Cfu/ml 10^9	S%	Cfu/ml 10^9	S%
0.0	4.5±0.7	100	2.9±0.1	100
0.25	4.2±0.3	93.3	2.7±0.2	93.1
0.50	4.1±0.4	91.1	2.6±0.3	89.7
1.0	3.9±0.5	86.7	2.5±0.08	86.2
5.0	3.8±0.3	84.4	2.3±0.07	79.3
10.0	3.7±0.1	82.2	2.2±0.06	75.9
15.0	3.2±0.2	71.1	2.1±0.1	72.4
20.0	3.1±0.3	68.9	2.0±0.04	68.9
25.0	3.0±0.06	66.7	1.9±0.03	65.6
30.0	2.9±0.04	64.4	1.8±0.02	62.1

Effect of *Adiantum capillus-veneris L.* Extract on Prophage F116 Induction

Data in Table 3 show the mutagenic activity of *A. capillus-veneris L.* plant extract using prophage induction assay. The plant extract in concentrations ranged from 0.25 up to 30% did not increase the induction of prophage F116 from the lysogenic strain MAM2A1. However, the pfu/ml was increased from 15.5 to 175.7 x 10^9 in the range of 0.25 up to 15.0% of plant extract. So, this

plant extract showed no mutagenic activity in the range of concentrations used in this study. According to Houk and DeMarini (1988) and DeMarini *et al.* (1990), the positive response of any compound is recommended to be corresponding to a three- fold increase in pfu/ ml over the spontaneous background release of the lysogenic strain. Accumulating evidences indicated that prophage induction is a broader genetic endpoint than any other bacterial assay(Varagas *et al.*, 2001 and Cizmas *et al.*, 2003). Since the prophage induction assay is a sensitive test among the other bacterial assay, presence or absence any substances to activate the test will not change the results .

Table 3. Effect of *Adiantum capillus-veneris L.* extract on prophage F116 induction from lysogenic strain

Conc.%	Pfu/ml 10^9	Fold increase	Mutagenic activity
0	208.8±0.9	-	-
0.25	15.5±0.9	-	-
0.50	26.1±0.8	-	-
1.0	52.8±1.3	-	-
5.0	126.3±1.7	-	-
10.0	128.4±2.1	-	-
15.0	175.7±2.3	-	-
20.0	52.8±1.9	-	-
25.0	8.5±0.7	-	-
30.0	7.1±0.8	-	-

- = no fold increase or no mutagenic activity.

Transduction Assay

The phage induced upon exposure to different concentrations of plant extract was not able to enhance the transduction frequency of streptomycin resistance gene Table 4. No fold increased than those observed in control was recorded. Transduction frequency per recipient at concentration 30% was $2.03x10^{-6}$ whereas at zero concentration was $6.3x10^{-6}$. So plant extract was not able to increase the transducing particles responsible for transducing the marker gene.Transduction frequency might depend on the percent of transducing phage particles that might generate among the whole progeny phage

particles produced from the lysogenic strain (Miller, 2001, Lang and Beatty, 2001 and Kang *et al.*, 2002). Since the number of transductants was lower than control, it seemed that *A. capillus-veneris* did not increase the rate of host DNA fragmentation, introducing another proof that this plant had no mutagenic activity in concentrations ranged from 0.25 up to 30%.

Table 4. Phage induction by plant *Adiantum capillus-veneris L.* extract and tranducing streptomycin resistance gene

Plant extrat concentrations %	No. transductants 10^3	Trensduction frequency	Fold increase
0	18.3±0.8	6.3×10^{-6}	-
0.25	16.8±0.9	5.8×10^{-6}	-
0.50	16.3±0.7	5.6×10^{-6}	-
1.0	16.0± 0.5	5.5×10^{-6}	-
5.0	14.8±0.4	5.1×10^{-6}	-
10.0	10.8±0.3	3.7×10^{-6}	-
15.0	10.3±0.9	3.6×10^{-6}	-
20.0	7.4±0.8	2.6×10^{-6}	-
25.0	6.2±0.9	2.1×10^{-6}	-
30.0	5.9±0.9	2.03×10^{-6}	-

The recipient strain PU21A3 was 2.9×10^9 cfu/ml.

Effect of Ascorbic Acid (Negative Control) and Ethyl Methan Sulfonate (Positive Control) on Prophage Induction and Transduction

In order to evaluate the mutagenic activity of plant *A. capillus-veneris L.* extract, two different experiments were carried out as negative control Table 5 using ascorbic acid and the other, as positive control using the mutagenic agent EMS Table 6. Upon using ascorbic acid no enhancement in either plaque forming or transduction frequencies were detected. All values were below the level of control. These results confirmed obtained data presented in Tables 3

and 4. Data in Table 7 clearly showed that EMS agent had a powerful effect as a mutagenic agent in the induction of the prophage (up to 6-fold increase) and so, transducing streptomycin resistance gene.

Table 5. Effect of ascorbic acid on prophage induction and transduction mechanisms (negative control)

Conc. %	Pfu/ml 10^9	No transductants 10^4	Transduction frequency
0	21.6	4.6	6.3×10^{-6}
0.25	19.5	0.97	1.3×10^{-6}
0.50	5.5	0.89	1.2×10^{-6}
1.0	3.4	0.71	9.7×10^{-7}
5.0	1.6	0.65	8.9×10^{-7}
10.0	1.2	0.51	6.9×10^{-7}
15.0	0.79	0.52	7.1×10^{-7}
20.0	0.57	0.62	8.5×10^{-7}
25.0	0.07	0.71	9.7×10^{-7}
30.0	0.05	0.65	8.9×10^{-7}

Recipient cell counts = 7.3×10^9 Cfu /ml.

Table 6. Effect of EMS on prophage induction and transduction mechanism (positive control)

Conc.%	Pfu/ml10^9	Mutagenic index	Mutagenic response	No. transductants 10^4	Transduction frequency
0.0	21.4	1.0	-	4.6	6.3×10^{-6}
0.25	25.3	1.18	-	5.7	7.8×10^{-6}
0.50	30.1	1.4	-	7.9	10.8×10^{-6}
1.0	84.8	3.96	+	16.9	23.1×10^{-6}
5.0	91.6	4.28	+	19.8	27.1×10^{-6}
10.0	113.6	5.3	+	19.9	27.2×10^{-6}
15.0	129.5	6.05	+	21.3	29.2×10^{-6}
20.0	129.9	6.07	+	21.7	29.7×10^{-6}
25.0	122.1	5.7	+	23.4	32.05×10^{-6}
30.0	130.4	6.09	+	26.6	36.4×10^{-6}

Recipient cell counts = 7.3×10^9 cfu/ ml.

+ = mutagenic response.

- = no mutagenic response.

Anti-Viral Activity

Effect of *A. capillus-veneris L.* extract on bacteriophage F116 activity

In order to assay the anti- viral activity of plant *A. capillus-veneris L.* extract, phage F116 was treated with different concentrations of plant extract. The ability of treated phage to form plaques and to transduce was tested (Table 7) . High concentrations of plant extract (25 to 30%) decreased the number of formed plaques wherease pfu/ml reached 0.9 to 0.4×10^{11}. These results showed that the plant extract had a remarkable anti-viral activity. However, number of transductants and subsequently transduction frequency did not affect by treating the phage. So, the plant extract did not increase the number of transducing particles that formed among the progeny particles. Previous studies demonstrated that an ethanol extract of the rhizome *Adiantum capilus-veneris L.* plant evidenced antiviral properties *in vitro* against *Vesicular stomatitis* virus (Leslie, 2002).

Table 7. Anti-bacteriophage activity of plant *Adiantum capilus-veneris* extract

Conc.%	Pfu/ml 10^{11}	No. transductants 10^4	Transduction frequency
0.0	12.6	7.1	2.6×10^{-6}
0.25	9.7	6.9	2.5×10^{-6}
0.50	9.4	6.8	2.5×10^{-6}
1.0	8.3	6.6	2.4×10^{-6}
5.0	5.12	6.6	2.4×10^{-6}
10.0	4.9	6.5	2.3×10^{-6}
15.0	4.2	6.5	2.3×10^{-6}
20.0	4.1	6.4	2.3×10^{-6}
25.0	0.9	6.4	2.3×10^{-6}
30.0	0.4	6.2	2.3×10^{-6}

Recipient cell counts was 2.7×10^{10} cfu/ ml.

The plant extract treated phage particles were allowed to transduce streptomycin resistance gene to treated recipient cells Table 8. Number of transductants were also decreased when the recipient strain treated with different concentrations of plant extract.

Table 8. Effect of plant extract treated phage and recipient on transduction frequency

Conc.%	No. recipient 10^{10}	No. transductants 10^{4}	Transduction frequency
0.0	2.7	7.1	2.6×10^{-6}
0.25	2.6	5.8	2.23×10^{-6}
0.50	2.5	4.8	1.92×10^{-6}
1.0	2.4	3.5	1.45×10^{-6}
5.0	2.3	3.4	1.5×10^{-6}
10.0	2.2	3.3	1.5×10^{-6}
15.0	2.1	3.2	1.5×10^{-6}
20.0	1.9	3.0	1.57×10^{-6}
25.0	1.8	2.9	1.6×10^{-6}
30.0	1.7	2.8	1.6×10^{-6}

Effect of *A. capillus-veneris L.* extract on plant viruses (TMV and CMV) activity

Data in Tables 9 and 10 indicated that plant *A. capillus-veneris L.* extract inhibited both TMV and CMV viruses infection with different degrees. The inhibitory effect on each of the two viruses depended on the host tested, concentration of inhibitor and the time of application. In general, the inhibitory effect of plant extract was not obvious in case of post inoculation while it was more announced in case of pre-inoculation. In case of TMV systemic infection at three different intervals (direct, after 24 hours and after 48 hours) by two different dilutions (1: 1 and 1: 2) the results were (90% and 95%), (75% and 85%) and (50% and 45%) respectively. In case of CMV systemic infection the results were (80% and 85%), (70% and 75%) and (40 % and 50%) respectively. The above results were in case of pre-inoculation. But in case of post-inoculation the results were as follows: in case of TMV systemic infection at the same intervals by same dilution, the results were (45% and 45%), (35% and 20%) and (20% and 10%), respectively. In case of CMV systemic infection the results were (50% and 30%), (15% and 15%) and (10% and 18%) respectively Table 9.In case of TMV local infection at the same intervals by same dilutions the results were (98.3 and 75%), (81% and 58.3%) and (41.4% and 40%), respectively Figure 1. In case of CMV local infection, the results were (76.9% and 60%), (30.8% and 50%) and (15.4% and 15%), respectively Figure 2.

Table 9. The inhibitory effect of *Adiantum capillus-veneris L.* extract on TMV and CMV infection using systemic host plant (*Lycopersicon esculentum* L.)

Virus	Time of Application	Pre-inoculation						Post-inoculation					
		Dilution 1:1			Dilution 1:2			Dilution 1:1			Dilution 1:2		
		AV. Untreated	AV. Treated	% of inhibition	AV. Untreated	AV. Treated	% of inhibition	AV. Untreated	AV. Treated	% of inhibition	AV. Untreated	AV. Treated	% of inhibition
TMV	Direct	2	18	90%	1	19	95%	9	11	45%	8	12	45%
	24 hours	5	15	75%	3	17	85%	7	13	35%	4	16	20%
	48 hours	10	10	50%	8	12	45%	4	16	20%	2	18	10%
CMV	Direct	4	16	80%	3	17	85%	10	10	50%	6	14	30%
	24 hours	6	14	70%	5	15	75%	3	17	15%	3	17	15%
	48 hours	12	8	40%	10	10	50%	2	18	10%	2	18	10%

20 Plants were used in each trial

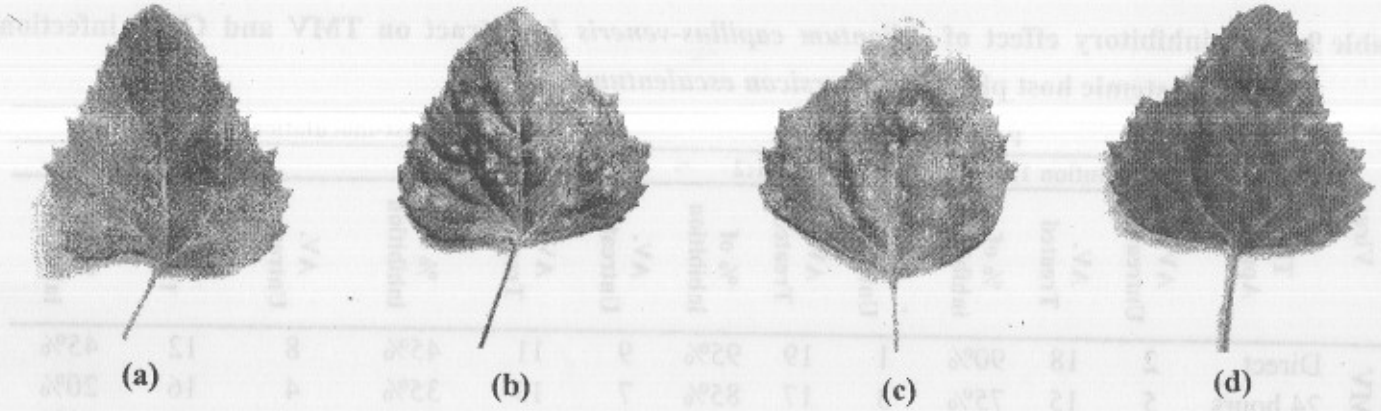

Fig 1. The inhibitory effect of TMV infection using local lesion host plant (*Ch. amaranticolor)* in case of pre inoculation by (1: 1 dil.) with the plant extract.

a) Control plant. b) Direct inoculation.

c) After 24 hours inoculation. d) After 48 hours inoculation.

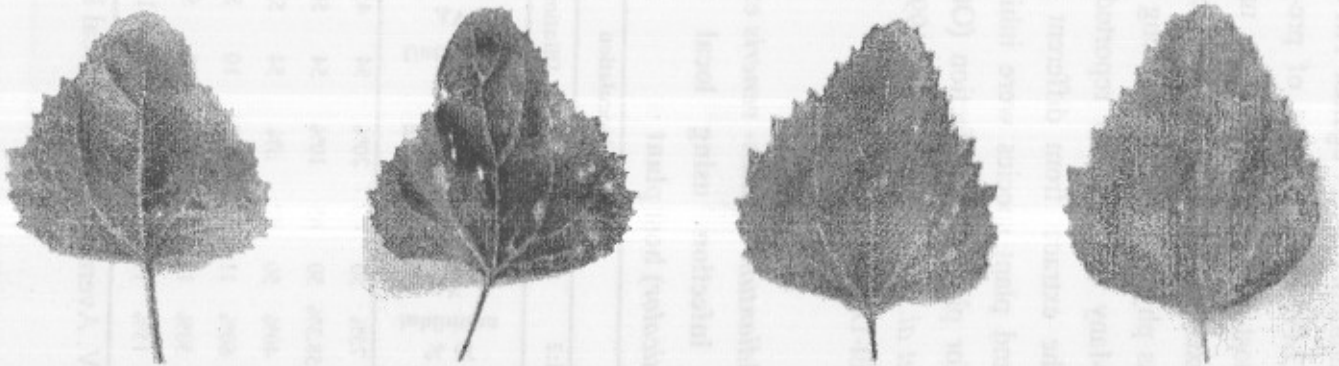

Fig 2. Inhibitory effect of CMV infection using local lesion host plant *(Ch. amaranticolor)* in case of pre inoculation by (1: 1 dil.) with the plant extract.

a) Control plant. b) Direct inoculation.

c) After 24 hours inoculation. d) After 48 hours inoculation.

However in case of post inoculation, the results were as follows: in case of TMV local infection, the results were (20% and 16%), (10% and 7.4%) and (6% and 3.7%), respectively and in case of CMV local infection the results were (18.2% and 20%), (9.1% and 10%) and (0% and 0%), respectively Table 10. In general the extract of *A. capillus-veneris* by direct inoculation showed high inhibitory effect against TMV and CMV in both cases of pre- and post-inoculation.The natural extracts of higher plants were used as phyto- antivirals for long time. Many investigators reported that the extracts from different seeds and plant species were inhibitors for plant virus infection (Othman *et al.*, 1991, Meyer *et al* 1995 and El-Dougdoug, 1997).

Table 10. The inhibitory effect of *Adiantum capillus- veneris* extract on TMV and CMV infection using local lesion on(*Chenopodium amaranticolor*) host plant

Virus	Time of Application	Pre- inoculation						Post-inoculation					
		Dilution 1:1			Dilution 1:2			Dilution 1:1			Dilution 1:2		
		AV. Untreated	AV. Treated	% of inhibition	AV. Untreated	AV. Treated	% of inhibition	AV. Untreated	AV. Treated	% of inhibition	AV. Untreated	AV. Treated	% of inhibition
TMV	Direct	58	1	98.3%	60	15	75%	50	40	20%	54	45	16%
	24 hours	58	11	81%	60	25	58.3%	50	45	10%	54	50	7.4%
	48 hours	58	34	60%	60	36	40%	50	47	6%	54	52	3.7%
CMV	Direct	13	3	20%	20	8	60%	11	9	18.2%	10	8	20%
	24 hours	13	9	20%	20	10	50%	11	10	9.1%	10	9	10%
	48 hours	13	11	20%	20	17	15%	11	11	0%	10	10	0%

5 leaves were used in each trial. AV. Average number of local lesions.

REFERENCES

Baranwal, V. K. and H. N. Verma. 1992 . Localized resistance against virus infection by leaf extract of *Celosia cristata*. Plant Pathology, 41: 633- 638

Baranwal, V. K. and H. N. Verma 1993. Virus inhibitor activity of leaf extracts from different taxonomic groups of higher plants. Indian Phytopathology, 46: 402- 403.

Bresolin, S. and V. Varagas. 1993. Mutagenic potencies of medicinal plants screened in the Ames test. Phytother. Res., 7: 260- 262.

Cizmas, I., R. Barhiumi, R. Burgardt and K. Donnelly. 2003. A comparison of two methods for fractionating complex mixtures in preparation for toxicity analysis. J. Toxicol. Environ. Health, 66: 1351- 1359.

De Marini, D., H. Brooks and D. Parkes. 1990. Induction of prophage Lambda by chlorophenols. Environ. Mol. Mutagen, 15: 1-9.

El- Dougdoug, K. A. 1997. Antiphyto- viral activity of khella ad black common on infectivity and chemical structure of tomato mosaic virus (TMV). Proc. 9th Con. of Microbiol., Cairo, Egypt. March, 25- 27, PP: 203- 221.

Ferrdira, I.C.F. and V.M.F. Varagas . 1999. Mutagenicity of medicinal plant extract in *Salmonella* Microsome assay. Phytotheraby. Research, 13: 397- 400.

Gulig, P.A ., K. E. Cerveny, A. DePaola, and D. H. Duckworth. 2002 .Phage therapy of local and systemic disease caused by *Vibrio vulnificus* in iron-dextran-treated mice.Infection and Immunity, 70 : 6251-6262.

Houk, V. and D. Demarini .1988. Use of microscreen phage induction assay to assess the genotoxicity of 14 hazardous industrial wastes. Environ. Mol. Mutagen., 11: 13- 29.

Hoyes, L., W. Au, M. Heo and M. Legator . 1992. Evaluation of the genotoxic effects of folk medicine, *Petiveria Alliacea.* Mut. Res., 280: 29- 34.

Kang, H.Y., C.M. Dozois, S.A. Tinge, T.H. Lee and R. Curtiss .2002. Transduction mediated transfer of unmarked detection and point mutations through use of counter selectable suicide vectors. J. Bacteriol., 184 (1): 307- 312.

Lang, A.S. and J.T. Beatty . 2001. The gene transfer agent of *Rhodobacter capsulatus* and constitutive transduction in prokaryotes. Canada. Arch. Microbiol., 175 (4): 241- 249.

Leslie, T. 2002. Herbal Secrets of the Rainforest. Sage press, Inc.

Mahmoud, M., A. L. M. Jawad, A.M. Hussain , M. Al-Omari and A. Al-Naib . 1989. In vitro antimicrobial activity of *Salsola rosmarinus and Adiantum capillus- veneris.* Int. J. of Crude Drug Res., 27: 1, 14- 16.

Mahran, G.H., T.M. El-Alfy, K.F. Taha and M. El-Tantawy. 1990. Chemical composition and antimicrobial activity of the volatile oil and extracts of fronds of *Adiantum capillus-veneris*. Bull. Fac. Agric., Cairo Univ., 41: 3, 555- 572.

Meyer, C., De. Dan and Z. Allam. 1995. Antiviral Protein in Higher Plant, Library of Congress. Cataloging in Publication Date Boca Raton Ann. Arab. London, Tokyo.

Miller, R.V. 2001. Environmental Bacteriophage- host Interactions: Factors contributed to natural transduction. Antonie Van Leeuu Wenhoew., 141- 147.

Othman, B.A., K. El-Dougdoug and M. Abo El-Nasr. 1991. Effect of garlic bubbilies extraction on *Tomato mosaic* virus. Annals Agric. Sci. Ain Shams Univ., Cairo, 36 (2): 423- 430.

Patel, K. D., P. M. Patel and B. N. Patel. 2000. Inhibition of *Tobacco mosiac* virus infection by some plant extracts. J. Myco. and Pl. Path., 30: 2, 229- 230.

Pradeep, P., A. Bohra and P. Parihar. 2002 a. Effect of *Adiantum* Against the toxigenic fungi, *Asperigillus flavus.* Current Agriculture, 26: 1-2, 77- 79.

Pradeep, P., A Bohra and P. Parihar. 2002 b. Screening of some ferns for their antimicrobial activity against *Salmonella typhi.* Advances in Plant. Sciences, 15: 2, 365- 367.

Varagas, V., S. Migliavacca, R. Horn and M. Pestana. 2001. Genotoxicity assessment in aquatic encvironments under the influence of heavy metals and organic contaminants. Mutat. Res., 490: 141- 158.

اختبار النشاط المطفر لمستخلص نبات كزبرة البئر باستخدام بعض النظم الميكروبية

أمينة أحمد حسن[١]- إيمان أحمد حسن خطاب[٢]

[١] قسم الوراثة- كلية الزراعة- جامعة الزقازيق.

[٢] قسم أمراض الفيروس والفيتوبلازما- معهد أمراض النبات- مركز البحوث الزراعية-الجيزة-القاهرة.

تم تقييم النشاط المطفرلنبات كزبرة البئر ذلك العشب الطبي الذي يستخدم في كثير من البلدان،و ذلك من خلال بعض النماذج البكتيرية (إستحثاث الفاج، النقل الجيني عن طريق البكتريوفاج)، أوضحت نتائج هذا الإختبار أن مستخلص النبات ليس له نشاط مطفر عند التركيزات التي إستخدمت في هذه الدراسة والتي تتراوح بين ٠,٢٥- ٣٠,٠%.

كما قدم النقل الجيني عن طريق البكتريوفاج دليل آخر علي أن هذا النبات ليس له نشاط مطفر حيث أن معدل النقل عند أعلي تركيز (٣٠%) كان ٢,٠٣× ١٠$^{-٦}$ بينما كان ٦,٣× ١٠$^{-٦}$ في الكنترول، وهذا يعزي إلي أن مستخلص النبات ليس له القدرة علي زيادة جزيئات الفاج المسئولة عن نقل الجين. بالإضافة إلى ذلك تم دراسة قدرة مستخلص نبات كزبرة البئر على مقاومة البكتريا والبكتريوفاج والفيروسات النباتية، وقد أوضحت النتائج أن مستخلص نبات كزبرة البئر له تأثير سمي طفيف علي سلالتين من بكتريا*Pseudomonas aeruginosa*، ولكن كان له تأثير أكبر سمية ضد بكتريوفاج F116، حيث وصلت Pfu/ ml إلي ٠,٤× ١٠^{١١} عند أعلي تركيز (٣٠%) بينما كانت ١٢,٦× ١٠ ^{١١} في الكنترول.

تم اختبار مستخلص نبات كزبرة البئر كمثبط لفيروس موزيك الطماطم وفيروس موزيك الخيار اللذان عزلا من نباتات الطماطم والكوسة المصابة طبيعياً علي التوالي، حيث تم استخدام تركيزين مختلفين (١:١، ١: ٢)، كمادة مثبطة لكلا الفيروسين قبل وبعد العدوى علي نباتات تظهر إصابة جهازيه وأخري تظهر إصابة موضعية علي ثلاث فترات هي: الحقن مباشرة، بعد ٢٤ ساعة وبعد ٤٨ ساعة من العدوى.

أوضحت النتائج أن مستخلص نبات كزبرة البئر أحدث تثبيط للإصابة بكلا الفيروسين، وكان التأثير التثبيطي طفيف في حالة الحقن بعد العدوي، ولكن هذا التأثير التثبيطي كان أكثر وضوحاً في حالة الحقن قبل العدوي، وكان هذا في كلا من الإصابة الجهازية والإصابة الموضعية، وأن هذا التأثير التثبيطي كان أعلي في حالة الإصابة الجهازية (٩٠%، ٩٥% لفيروس موزيك الطماطم، ٨٠%، ٨٥% لفيروس موزيك الخيار) عنها في حالة الإصابة الموضعية (٩٨,٣%، ٧٥% لفيروس موزيك الطماطم، ٢٠%، ٦٠% لفيروس موزيك الخيار).