Prevalence of *Adenovirus* and *Coxsackievirus B4* in Fresh Produce Plants from Egypt

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Abstract   Viruses have been increasingly recognized as important causes of foodborne disease. One category of implicated foods is those that are minimally processed, such as fresh produce. The initial attachment of enteric viruses to green vegetables is a critical step in the chain of contamination events. Therefore, the primary target of this research is study of the prevalence of enteric viruses (*Adenovirus* and *Coxsackievirus B4*) to draws attention to the threat of viruses as a risk to public health and accordingly, can provide insight into appropriate intervention methods that can be used to either prevent attachment or remove the attached viral pathogens. A total of 135 leafy green vegetables samples from three governorates (Giza, Kafr El-Sheikh, and Qalyubia) were collected, where 45 samples from each governorate. The vegetable items which chosen for the current study were lettuce, spinach, and green onions, where 15 samples from each item. From our findings, it was found that *Adenovirus* was present in some examined vegetables samples from the three locations but in different prevalence contamination percentages. Lettuce, spinach and green onion were contaminated by 13.3%, 20% and 13.3% for fields irrigated with water from Elmaryotia canal (Giza governorate), respectively. While, by 60%, 46.6% and 26.6% for fields irrigated with water from the Kitchener drain (Kafr El-Sheikh governorate), respectively. As well as, by 33.3%, 13.3% and 6.6% for El-Gabal El-Asfar farm (Qalyubia governorate), respectively. On the other hand, CoxB4 was not detected in any samples from the region of Elmaryotia canal. In contrast, the three vegetable items of the area of Kitchener drain were contaminated by 40%, 26.6%, and 30%, respectively. In the same trend samples of El-Gabal El-Asfar farm showed contamination percentages were 40%, 26.6%, and 20%, respectively. It is worth mentioning statistically found that, there is a significant difference between locations regarding *Adenovirus* and Cox B4 present on lettuce plant (p ≤ 0.05). While no statistically significant difference between locations regarding *Adenovirus* and CoxB4 present on spinach and green onion plants (p > 0.05). However, there was a highly statistically significant difference between locations regarding *Adenovirus* and CoxB4 at (p ≤ 0.001).

Keywords   *Adenovirus*, *Coxsackievirus B4*, Fresh produce, Elmaryotia canal, Kitchener drain, El-Gabal El-Asfar, Egypt

1. Introduction

Viruses are estimated to be the responsible for 59% of the total foodborne illnesses, 27% of the hospitalizations, and 12% of the deaths [1]. Enteric viruses in water and food are a significant cause of mortality in infants and young children in less developed countries [2]. *Human enteric virus transmission is known to occur through contaminated food or water (by the fecal-oral route), person to person, aerosols, and contact with contaminated surfaces. Human enteric*

viruses have a low infectious dose, and a few, as ten virus particles are capable of causing illness [3]. Foods that are associated with transmission include shellfish, fresh produce and ready-to-eat (RTE) foods that do not undergo further processing [4]. The main viruses associated with foodborne diseases are *Adenovirus*es and other enteroviruses including *Coxsackievirus B4* [5].

Human adenovirus (HAdV), belonging family *Adenoviridae*, and are icosahedral, non-enveloped viruses with a double-stranded DNA genome [6]. HAdV includes more than 70 HAdV types, classified into seven species (HAdV A–G) [7, 8]. *Human adenoviruses* can cause various diseases such as gastrointestinal tract (gastroenteritis), respiratory diseases (acute respiratory infection), eye (conjunctivitis), genitalia (urethritis), and central nervous system (meningoencephalitis) [9]. They have been detected in shellfish, wastewater, and surface waters in many

locations [10]. *Adenovirus*es can spread not only via droplet infection but also via the fecal-oral route. They cause 10% of gastroenteritis in children and are the second most common cause of hospitalization due to diarrhea of children [11].

The human enteroviruses are ubiquitous, enterically transmitted viruses that cause a broad spectrum of illnesses among infants and children [12]. Enteroviruses may persist on fresh fruit and vegetables for several days under conditions commonly used for storage in households. Therefore, there will be a risk of infection from consumption of those foods if they are contaminated with viruses. Though enteroviruses are mainly transmitted via the fecal-oral route, the spread of certain species via aerosol is also a cause for concern [13]. Viral particles are shed with feces and symptoms of the diseases caused by them are different from typical gastroenteritis. They enter the host with contaminated food and multiply in the digestive tract. Symptoms of infection are often slight, moderate. However, viruses may spread into other organs and cause diseases that are serious or even fatal such as aseptic meningitis, and occasionally paralysis [14]. Cases of infection giving evidence of association with eating soft fruit, green vegetables and other foods have been recognized [15, 16]. Among enteroviruses, the group B coxsackieviruses, particularly *Coxsackievirus B4* (CVB4) [17]. This virus was first isolated in 1948, in New York State during a paralytic poliomyelitis investigation in the town of Coxsackie [18]. CVB4 is a small, nonenveloped, positive-strand RNA enterovirus in the *Picornaviridae* family, which also includes poliovirus, echovirus, and coxsackievirus A and enterovirus [17]. These viruses cause a wide range of diseases includes gastroenteritis and many other severe illnesses. Therefore, the primary target of this research is drawing attention to the threat of viruses as a risk to public health when they are present in foods (fresh produce) that do not or minimal undergo further processing so, it is important as we seek to develop mitigation and intervention strategies for increment of the efficacy of food safety.

2. Materials and Methods

2.1. Samples Collection

A total of 135 fresh produce samples were collected from three governorates [45 samples from fields irrigated with water from Elmaryotia (Giza governorate), 45 samples from fields irrigated with water from Kitchener drain (Kafr El-Sheikh governorate), and 45 samples from El-Gabal El-Asfar farm (Qalyubia governorate)]. Three produce items (lettuce, spinach and green onions) were chosen for this study, 15 samples from each item. The samples of each produce were kept in plastic bag and stored at (-20°C) until use for examination and further studies.

2.2. Isolation of Virus

2.2.1. Pre-Analytical Sample Processing

Fresh plant tissues (2 kg) were sterilized by soaking in 70% ethanol for 5 min then socked in 2% sodium hypochlorite for 1 min, washed in sterilized distilled water for 2 min and dried. To investigate the presence of *Human adenovirus* (HAdV) and *Coxsackievirus B4* (CVB4) in the samples, fresh plant tissues of each sample was cut and mixed in sterile distilled water and macerated using an electric blender. The extraction was filtrated with two layers cheesecloth. The crude sap was clarified by filter paper (Whatman No 4) and stored at -20°C until used for concentration.

2.2.2. Concentration of Virus

Primary concentration: Viruses were eluted and concentrated from foods surface and crude sap samples via 150 ml of 10% (w/v) beef extract was added to 100 g raw fresh foods and to 100 ml crude sap samples, the mixtures were stirred for 30 min at room temperature and centrifuged at 12000 rpm for 15 min at room temperature [19]. The pellet was discarded, and the supernatant was then concentrated by organic flocculation method.

Secondary concentration: All samples were secondary re-concentrated using an organic flocculation method according to Katzenelson *et al.* [20], Anonymous [21] and Mäde *et al.* [22], then the elutes were acidified to pH 3.5 using HCl (5N), (Merck-Schuchardt) and centrifuged at 3000 rpm for 15 min. The supernatant was discarded, and the pellet was dissolved in 1ml of Na_2HPO_4 (El Nasr Pharmaceutical Chemical Co.). All samples were kept at -70°C for further analysis.

2.3. *Adenovirus* Detection

2.3.1. DNA Extraction & PCR

DNA extraction was carried out using the phenol-chloroform-isoamyl alcohol method [23], centrifuged at 10,000xg for 5 min, and washed with ethanol 70%. The pellet was resuspended in 40 μl of TE low buffer (pH 8.0) and stored at -20°C before PCR analysis. To exclude any possibility of cross-contamination, all reagents were prepared in a laminar flow cabinet, and all extractions were made in duplicates and processed on different days. The oligonucleotide primers pairs were used according to Allard *et al.* [24] and Girones *et al.* [25], where hexAA1885[18858] 5′-GCCGCAGTGGTCTTACATGCACA TC-3′ (nucleotides 18858–18883, refers to the Ad2 hexon region), and hexAA1913[19181] 5′-CAGCACGCCGCGGA TGTCAAAGT-3′ (nucleotides 19158–19181, refers to the Ad2 hexon region) for conventional-PCR, and nehexAA1893[18961] 5′-GCCACCGAGACGTACTTCAGCC TG-3′ (nucleotides 18961–18937, refers to the Ad2 hexon region), and nehexAA1905[19108] 5′-TTGTACGAGTACGCG GTATCCTCGCGGTC-3′ (nucleotides 19079–19108, refers to the Ad2 hexon region) for nested-PCR. PCR amplification was performed according to Rigotto *et al.* [26] as follows: 5 μl of DNA and 85 pmoles of each primer (hexAA1885 and hexAA1913) were added to a reaction mix consisting of 20

mM Tris-HCl, pH 8.4, 50 mM KCl, 0.2 mM of dNTPs, 1.5 mM MgCl$_2$, and 5 U of *Taq* DNA polymerase. Mixtures were incubated for 1 min at 94℃, followed by 40 cycles of 1 min at 94℃, 1 min at 56℃ and 45s at 72℃, with a final incubation of 7 min at 72℃, and then, the reaction was held at 4℃. For nested-PCR, 1.0 ml of the first PCR product was added to a 50 ml reaction mix containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 0.2 mM of dNTPs, 3.0 mM MgCl$_2$, 85 pmol of each primer (nehexAA1893 and nehexAA1905), and 2.5 U of *Taq* DNA polymerase. Mixtures were incubated for 1 min at 94℃, followed by 25 cycles of 1 min at 94℃, 1 min at 57℃ and 45s at 72℃, with a final incubation of 5 min at 72℃, and then, the reaction was held at 4℃. PCR products were visualized on an ethidium bromide-stained 1.5% agarose gel [26].

2.4. *Coxsackievirus B4* Detection

2.4.1. RNA Extraction & RT-PCR

Viral RNA was extracted directly from concentrated extracts of vegetable samples using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. RT-PCR amplified the VP1-2A junction region of *Coxsackievirus B4* with primers that flanked the VP1-2A junction region [27]: CoxB4-F$_{3156}$ 5′-CGCATCTACTTCAAACCCAA-3′ (nucleotides 3156–3176, according to Jenkins *et al.* [28]), and CoxB4-R$_{3468}$ 5′-TTGTGCATTGGCATCTGGC-3′ (nucleotides 3450-3468, according to Jenkins *et al.* [28]). cDNA synthesis was performed with about 100 ng of RNA using 0.1 μM of the anti-sense CoxB4-R primer and the M-MLV RT enzyme (Promega, Cat #), according to the manufacturer's instructions. PCR amplification was performed according to Mulders *et al.* [27] in a final volume of 25 μl as follows: 2.5 μl of cDNA, 2.5 μl of 2.5 mM of dNTPs, 2.5 μl of 10X buffer, 2.5 μl of 25 mM MgCl$_2$, 1 μl of each forward and reverse primers at 10 μM, 0.2 μl *Taq* DNA polymerase, and water. Mixtures were incubated for 1 min at 94℃, followed by 35 cycles of 1 min at 94℃, 1 min at 42℃ and 2 min 30s at 72℃, with a final incubation of 10 min at 72℃, and then, the reaction was held at 4℃. PCR products were visualized on an ethidium bromide-stained 1.5% agarose gel.

2.5. Statistical Analyses

All statistical calculations were done using SPSS (statistical package for the social science version 25.00) statistical program at the 0.05 level of probability [29]. Qualitative data were done using Chi-square and Pearson correlation. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The *P-value* was considered non-significant (NS) at the level of >0.05, and significant at the level of <0.05.

3. Results

A total of 135 leafy green vegetable samples from three governorates (Giza, Kafr El-Sheikh, and Qalyubia) were collected to study the prevalence of enteric viruses (*Adenovirus* and *Coxsackievirus B4*) in Egypt. Since 45 samples from fields irrigated with water from Elmaryotia canal (Giza), 45 samples from fields irrigated with water from Kitchener drain (Kafr El-Sheikh), and 45 samples from El-Gabal El-Asfar farm (Qalyubia). The vegetable items which chosen from each studied region were lettuce, spinach, and green onions, where 15 samples from each item.

3.1. Prevalence of *Adenovirus*

The results showed that the prevalence contamination percentages of lettuce plants with *Adenovirus* were 13.3% (2/15), 60% (9/15) and 33.3% (5/15), respectively in the fields irrigated with water from Elmaryotia canal (Giza governorate), Kitchener drain (Kafr El-Sheikh governorate), and El-Gabal El-Asfar farm (Qalyubia governorate) (Table 1 & Fig. 1). On the other hand, the prevalence contamination of spinach plants was 20% (3/15), 46.6% (7/15) and 13.3% (2/15), respectively (Table 2 & Fig. 2). Whereas the prevalence contamination of green onion plants was 13.3% (2/15), 26.6% (4/15) and 6.6% (1/15), respectively (Table 3 & Fig. 3). Since the results revealed the statistically significant difference between locations regarding *Adenovirus* present on lettuce (p ≤ 0.05), spinach (p > 0.05), and green onion plants (p > 0.05). The highest prevalence of *Adenovirus* was found in Kafr El-Sheikh governorate by 44.4% (20/45), followed by 15.6% (7/45) in Giza governorate, and 13.3% (6/45) in Qalyubia governorate with high statistically significant difference between locations regarding *Adenovirus* (p ≤ 0.001) (Table 4 & Fig. 4). On the other hand, the results revealed no statistically significant difference between types of plant regarding irrigated with water from Elmaryotia canal (Giza governorate), Kitchener drain (Kafr El-Sheikh governorate), or El-Gabal El-Asfar farm (Qalyubia governorate) with *Adenovirus* (p > 0.05).

Table 1. Comparison between locations regarding *Adenovirus* present on lettuce plants

Adenovirus		Lettuce plants irrigated with water from			Chi-square	P-value
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	2	9	5	7.177	0.028 S
	%	13.3%	60.0%	33.3%		
-ve	N	13	6	10		
	%	86.7%	40.0%	66.7%		

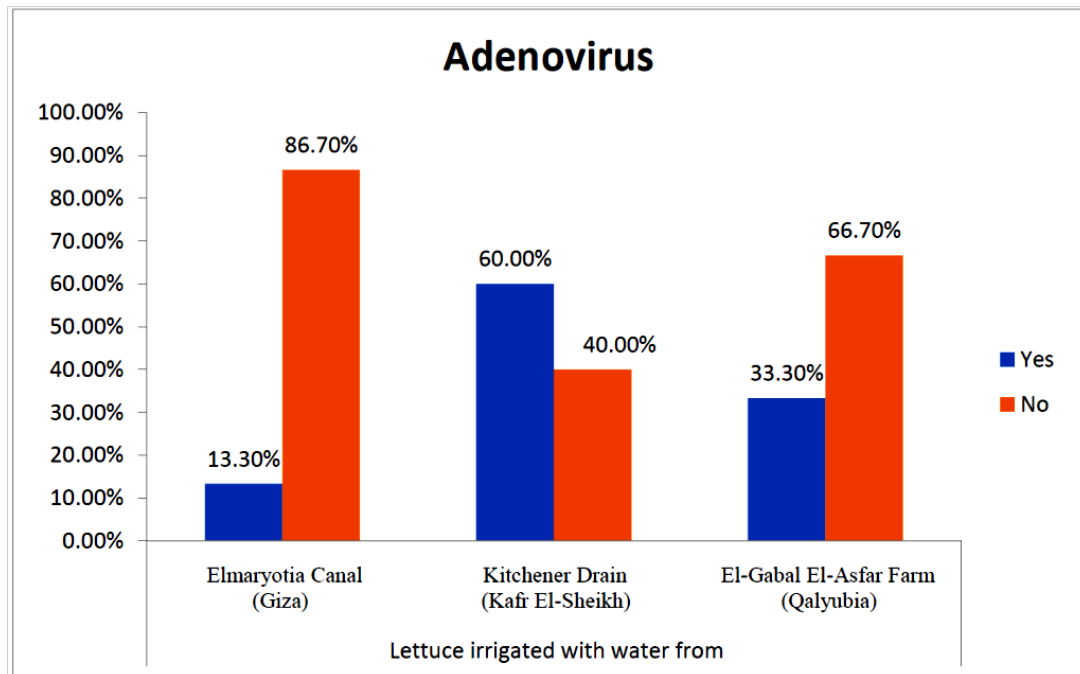S= significant at *P-value* ≤ 0.05

Figure (1).   Comparison between locations regarding *Adenovirus* present on lettuce plants

Table 2.   Comparison between locations regarding *Adenovirus* present on spinach plants

Adenovirus		Spinach plants irrigated with water from			Chi-square	P-value
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	3	7	2	4.773	0.092 NS
	%	20.0%	46.7%	13.3%		
-ve	N	12	8	13		
	%	80.0%	53.3%	86.7%		

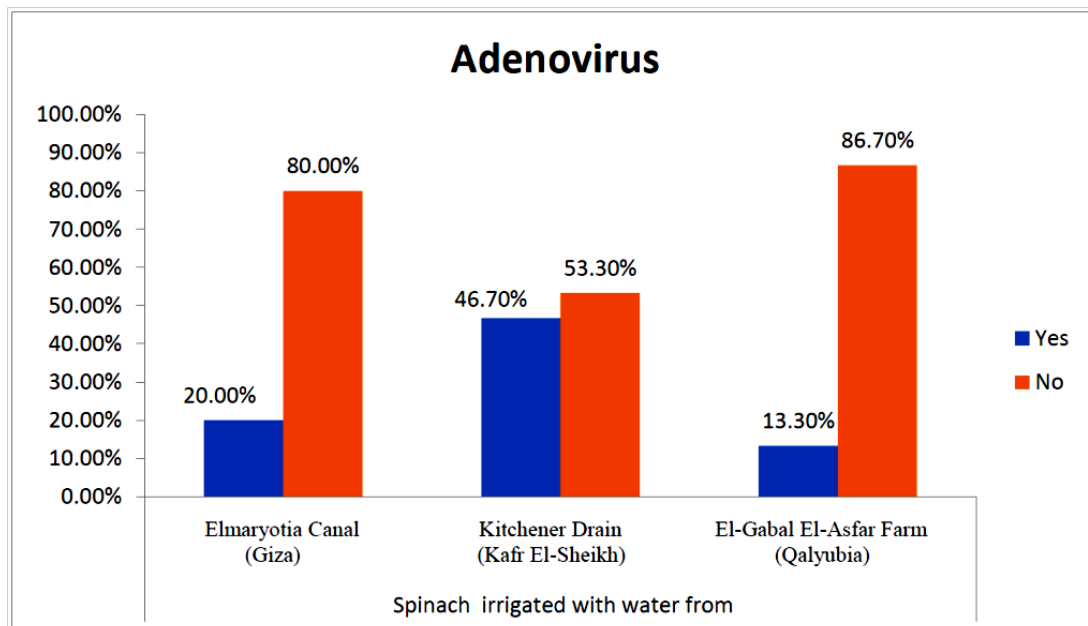NS= Non-significant at *P-value* > 0.05

Figure (2).   Comparison between locations regarding *Adenovirus* present on spinach plants

Table 3.   Comparison between locations regarding *Adenovirus* present on green onion plants

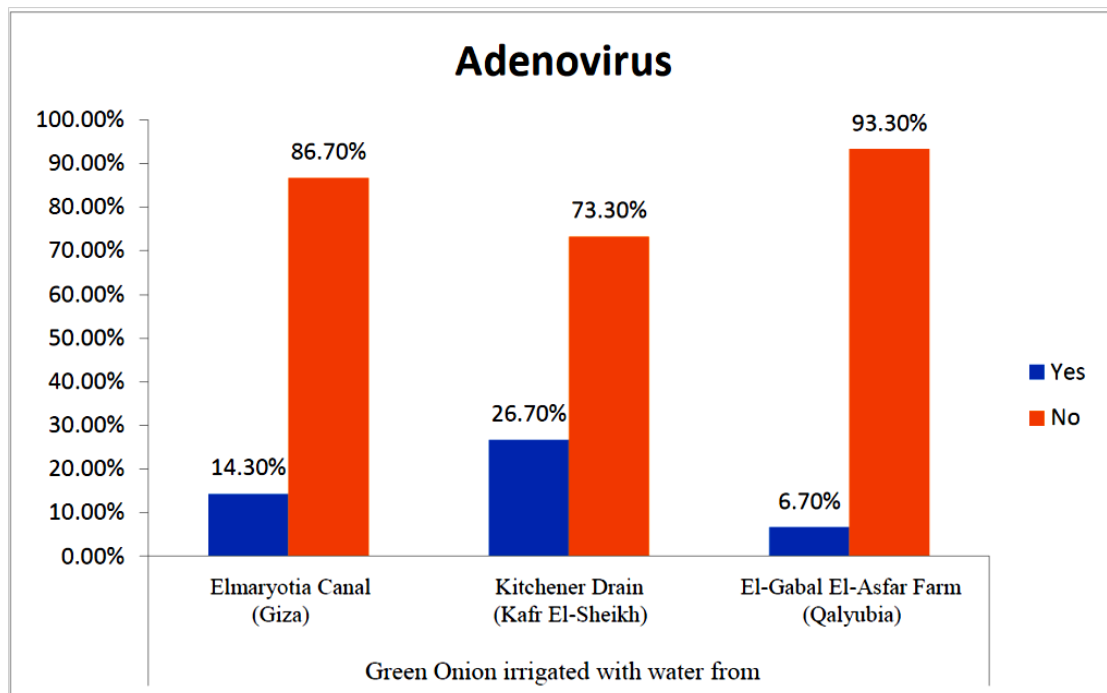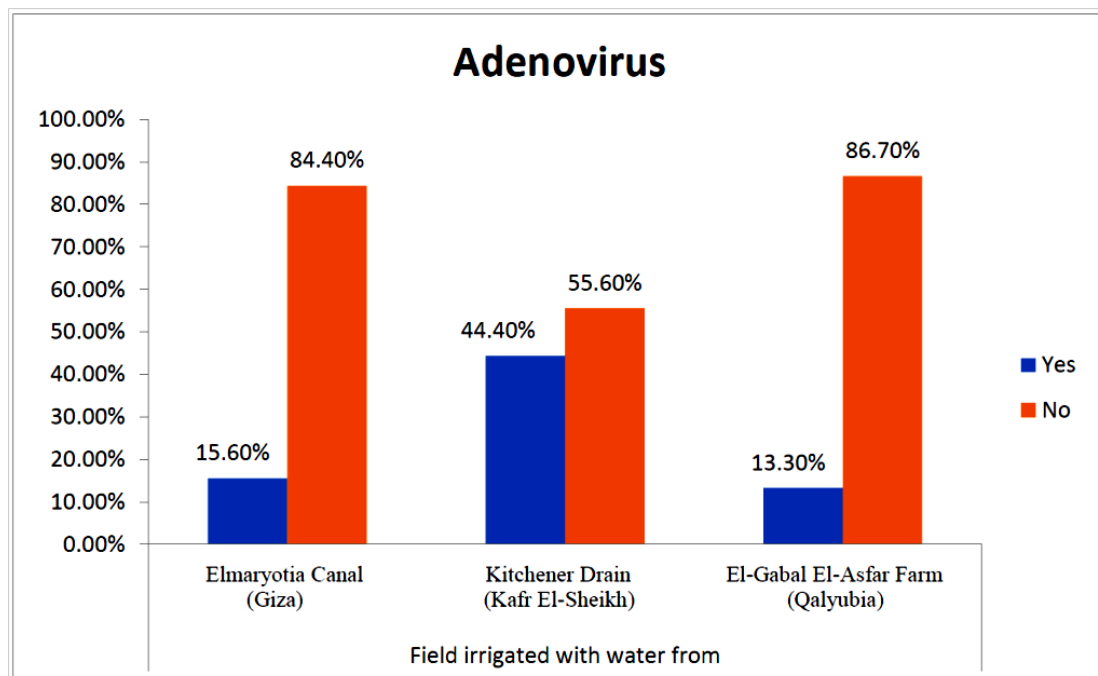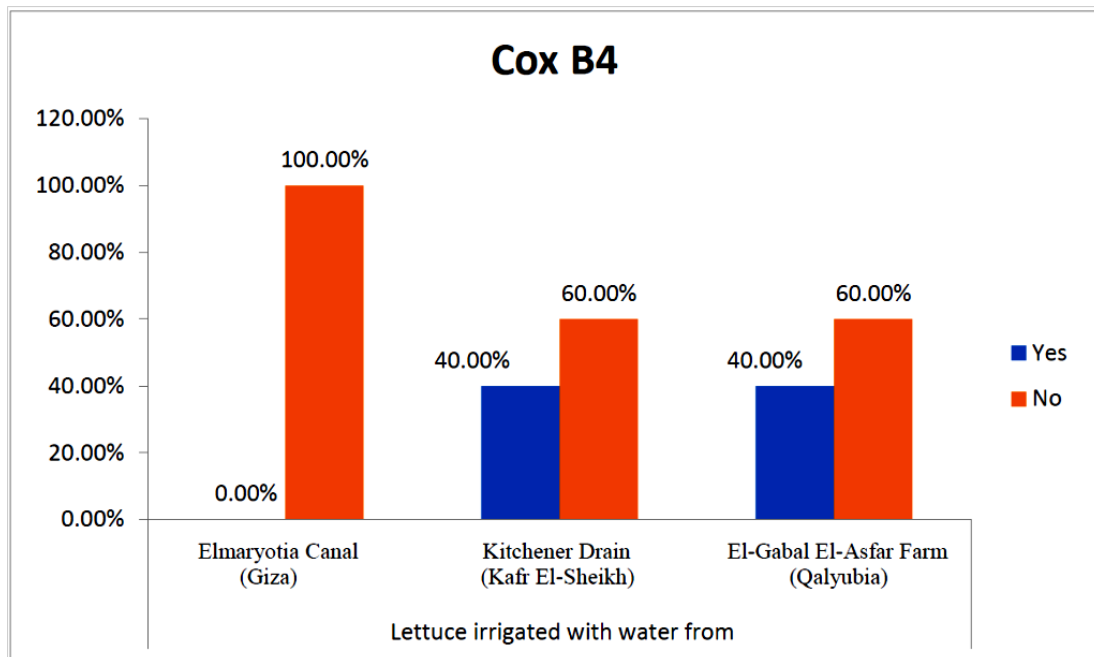| *Adenovirus*		Green onion plants irrigated with water from			Chi-square	P-value
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	2	4	1	2.268	0.306 NS
	%	14.3%	26.7%	6.7%		
-ve	N	13	11	14		
	%	86.7%	73.3%	93.3%		

NS= Non-significant at *P-value* > 0.05

Figure (3).   Comparison between locations regarding *Adenovirus* present on green onion plants

Figure (4).   Comparison between locations regarding *Adenovirus*

Table 4.   Comparison between locations regarding *Adenovirus*

Adenovirus		Fields irrigated with water from			Chi-square	P-value
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	7	20	6	14.679	0.001 HS
	%	15.6%	44.4%	13.3%		
-ve	N	38	25	39		
	%	84.4%	55.6%	86.7%		

HS=Highly significant at *P-value* ≤ 0.001

Table 5.   Comparison between locations regarding *Coxsackievirus B4* present on lettuce plants

CoxB4		Lettuce plants irrigated with water from			Chi-square	*P-value*
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	0	6	6	8.182	0.017 S
	%	0.0%	40.0%	40.0%		
-ve	N	15	9	9		
	%	100.0%	60.0%	60.0%		

S= significant at *P-value* ≤ 0.05

Figure (5).   Comparison between locations regarding CoxB4 present on lettuce plants

Table 6.   Comparison between locations regarding *Coxsackievirus B4* present on spinach plants

CoxB4		Spinach plants irrigated with water from			Chi-square	*P-value*
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	0	4	4	4.865	0.088 NS
	%	0.0%	26.7%	26.7%		
-ve	N	15	11	11		
	%	100.0%	73.3%	73.3%		

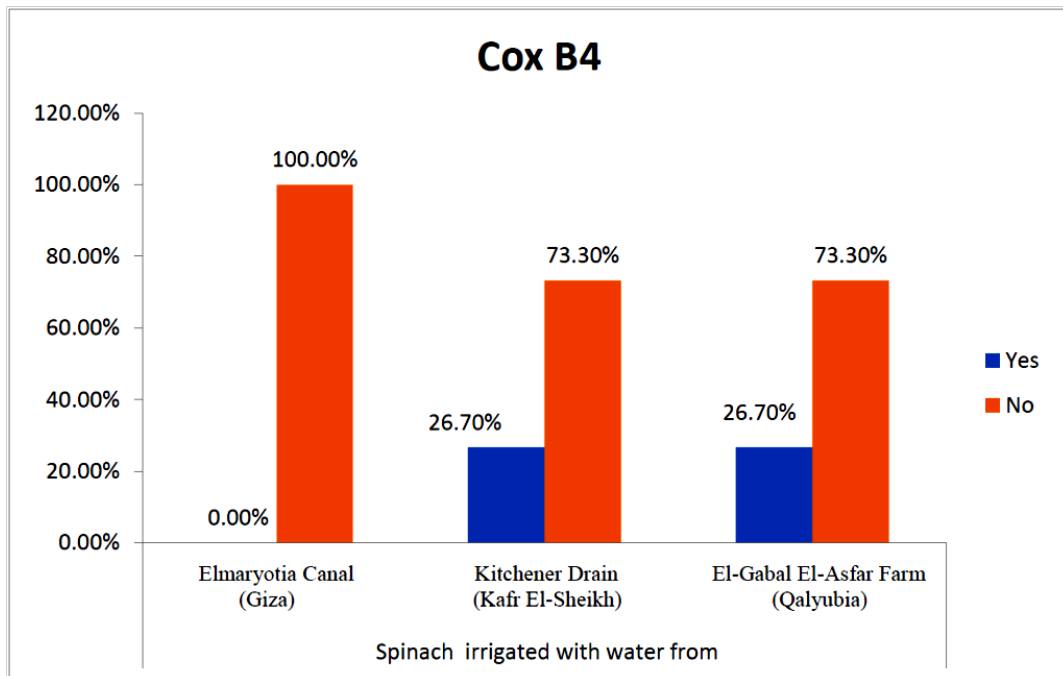NS= Non-significant at *P-value* > 0.05

Figure (6).   Comparison between locations regarding CoxB4 present on spinach plants

Table 7.   Comparison between locations regarding *Coxsackievirus B4* present on green onion plants

CoxB4		Green onion plants irrigated with water from			Chi-square	*P-value*
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	0	3	3	3.462	0.177 NS
	%	0.0%	20.0%	20.0%		
-ve	N	15	12	12		
	%	100.0%	80.0%	80.0%		

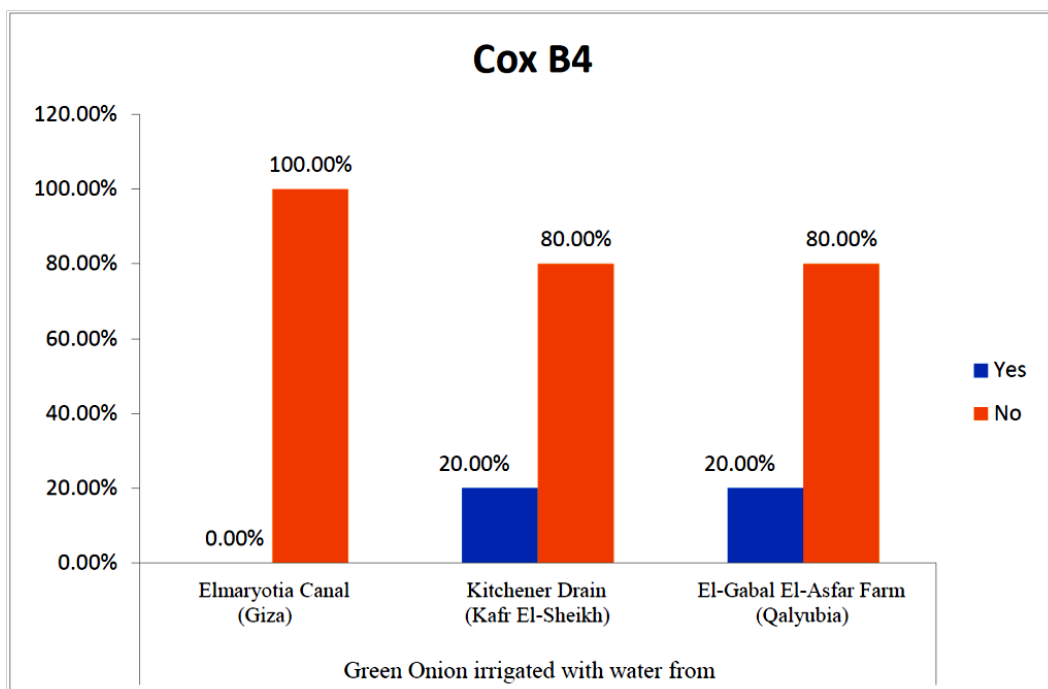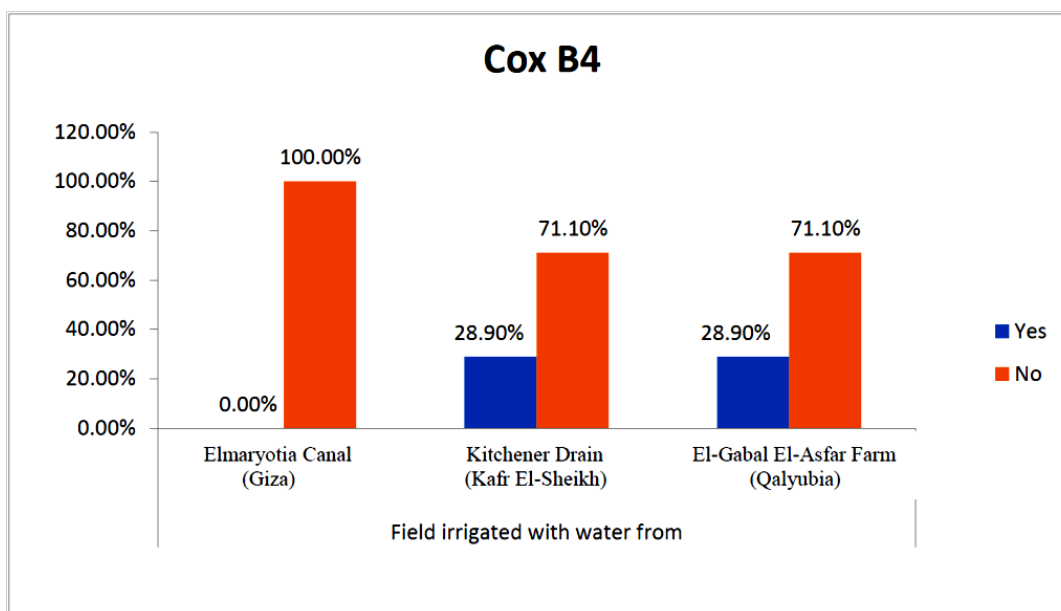NS= Non-significant at *P-value* > 0.05

Figure (7).   Comparison between locations regarding CoxB4 present on green onion plants

Table 8.   Comparison between locations regarding *Coxsackievirus B4*

CoxB4		Fields irrigated with water from			Chi-square	*P-value*
		Elmaryotia canal	Kitchener drain	El-Gabal El-Asfar farm		
+ve	N	0	13	13	16.101	0.000 HS
	%	0.0%	28.9%	28.9%		
-ve	N	45	32	32		
	%	100.0%	71.1%	71.1%		

HS=Highly significant at *P-value* ≤ 0.001

Figure (8).   Comparison between locations regarding CoxB4

3.2. Prevalence of *Coxsackievirus B4*

The results showed that the prevalence contamination percentages of lettuce plants with *Coxsackievirus B4* was 40.4% (6/15) in fields irrigated with water from both Kitchener drain (Kafr El-Sheikh governorate), and El-Gabal El-Asfar farm (Qalyubia governorate) (Table 5 & Fig. 5). Also, the prevalence contamination of spinach plants was 26.7% (4/15) in each region (Table 6 & Fig. 6). Whereas the prevalence of contamination of green onion plants was 20% (3/15) in each area (Table 7 & Fig. 7). The results revealed the statistically significant difference between locations regarding CoxB4 present on lettuce plants (p ≤ 0.05), while no statistically significant difference between locations regarding CoxB4 present on spinach and green onion plants. On the other hand, *Coxsackievirus B4* was not detected in any samples from the region of Elmaryotia canal (Giza governorate), where the prevalence of *Coxsackievirus B4* was found in Kafr El-Sheikh and Qalyubia governorates by 28.9% (13/45) for each governorate with highly statistically significant difference between locations regarding CoxB4 (p ≤ 0.001) (Table 8 & Fig. 8). On the other hand, the results revealed no statistically significant difference between types of plant regarding irrigated with water from Elmaryotia canal (Giza governorate), Kitchener drain (Kafr El-Sheikh governorate), or El-Gabal El-Asfar farm (Qalyubia

governorate) with *Coxsackievirus B4* (p > 0.05).

3.3. Correlation between *Adenovirus* and *Coxsackievirus B4*

As shown in tables (9, 10, 11, 12 & 13), there were statistically significant positive correlations as the following: First correlations were between *Adenovirus* contaminated lettuce plants with *Adenovirus* contaminated spinach plants (Fig. 9-A), with *Adenovirus* contaminated green onion plants (Fig. 9-B), with *Coxsackievirus B4* contaminated lettuce plants (Fig. 9-C), with *Coxsackievirus B4* contaminated spinach plants (Fig. 9-D), and with *Coxsackievirus B4* contaminated green onion plants (Fig. 9-E). Second correlations were between *Adenovirus* contaminated spinach plants with *Adenovirus* contaminated green onion plants (Fig. 10-A), with *Coxsackievirus B4* contaminated lettuce plants (Fig. 10-B), with *Coxsackievirus B4* contaminated spinach plants (Fig. 10-C), and with *Coxsackievirus B4* contaminated green onion plants (Fig. 10-D). Third correlations were between *Adenovirus* contaminated green onion plants with *Coxsackievirus B4* contaminated lettuce plants (Fig. 11-A), with *Coxsackievirus B4* contaminated spinach plants (Fig. 11-B), and with *Coxsackievirus B4* contaminated green onion plants (Fig. 11-C). Fourth correlations were between *Coxsackievirus B4* contaminated lettuce plants with

Coxsackievirus B4 contaminated spinach plants (Fig. 12-A), and with *Coxsackievirus B4* contaminated green onion plants (Fig. 12-B). Finally, the last correlation between *Coxsackievirus B4* contaminated spinach plants with *Coxsackievirus B4* contaminated green onion plants (Fig. 13).

Table 9. Correlation between *Adenovirus* contaminated lettuce plants with *Adenovirus* contaminated green onion plants, with CoxB4 contaminated lettuce plants, with CoxB4 contaminated spinach plants, and with CoxB4 contaminated green onion plants

Adenovirus contaminated lettuce plants (Lettuce + Adenovirus)			
Item	**Pearson correlation coefficient**	**P. value**	***Significance**
Spinach + Adenovirus	0.707	0.000	Significant
Green onion + Adenovirus	0.450	0.002	Significant
Lettuce + CoxB4	0.707	0.000	Significant
Spinach + CoxB4	0.626	0.000	Significant
Green onion + Cox B4	0.528	0.000	Significant

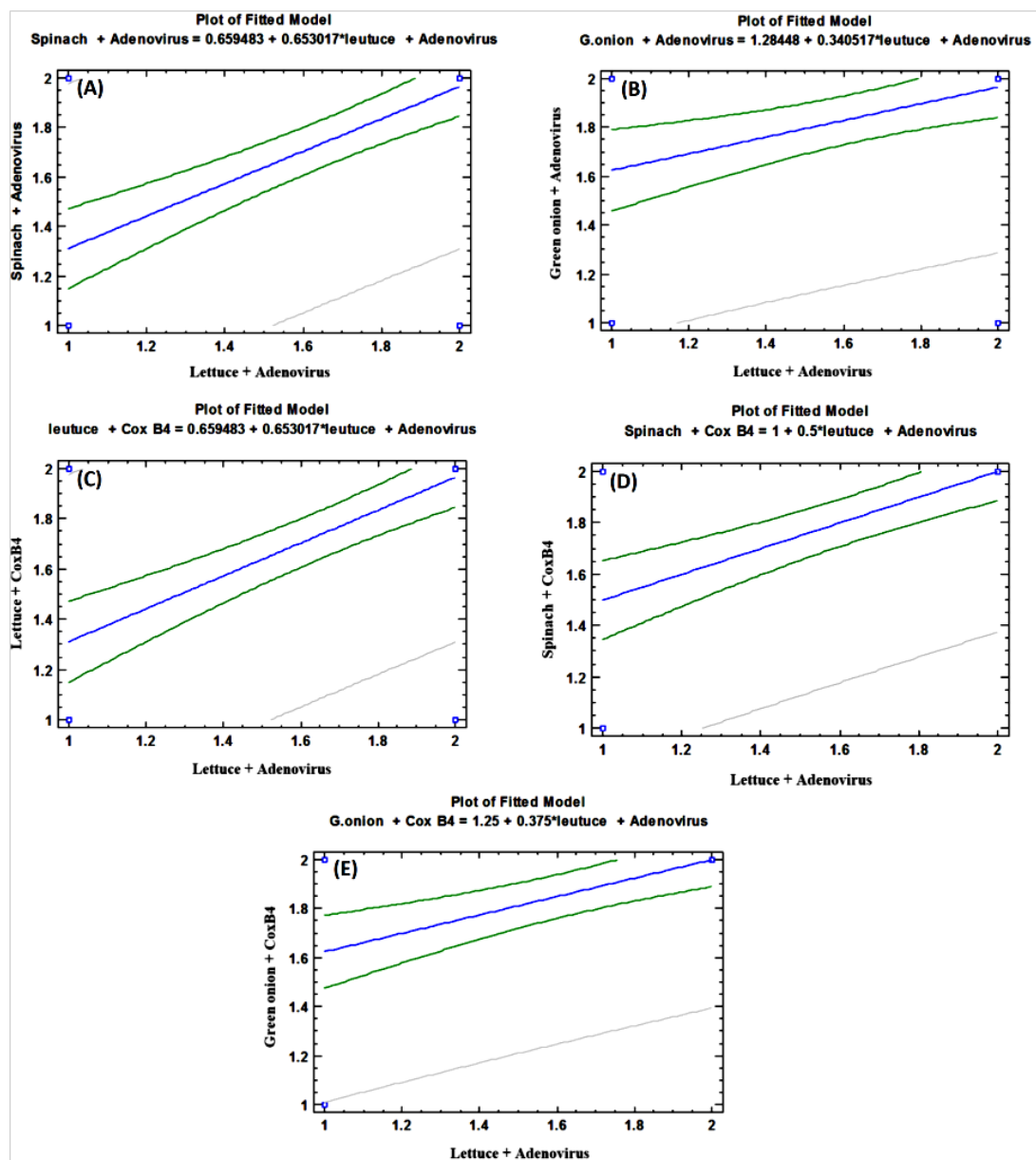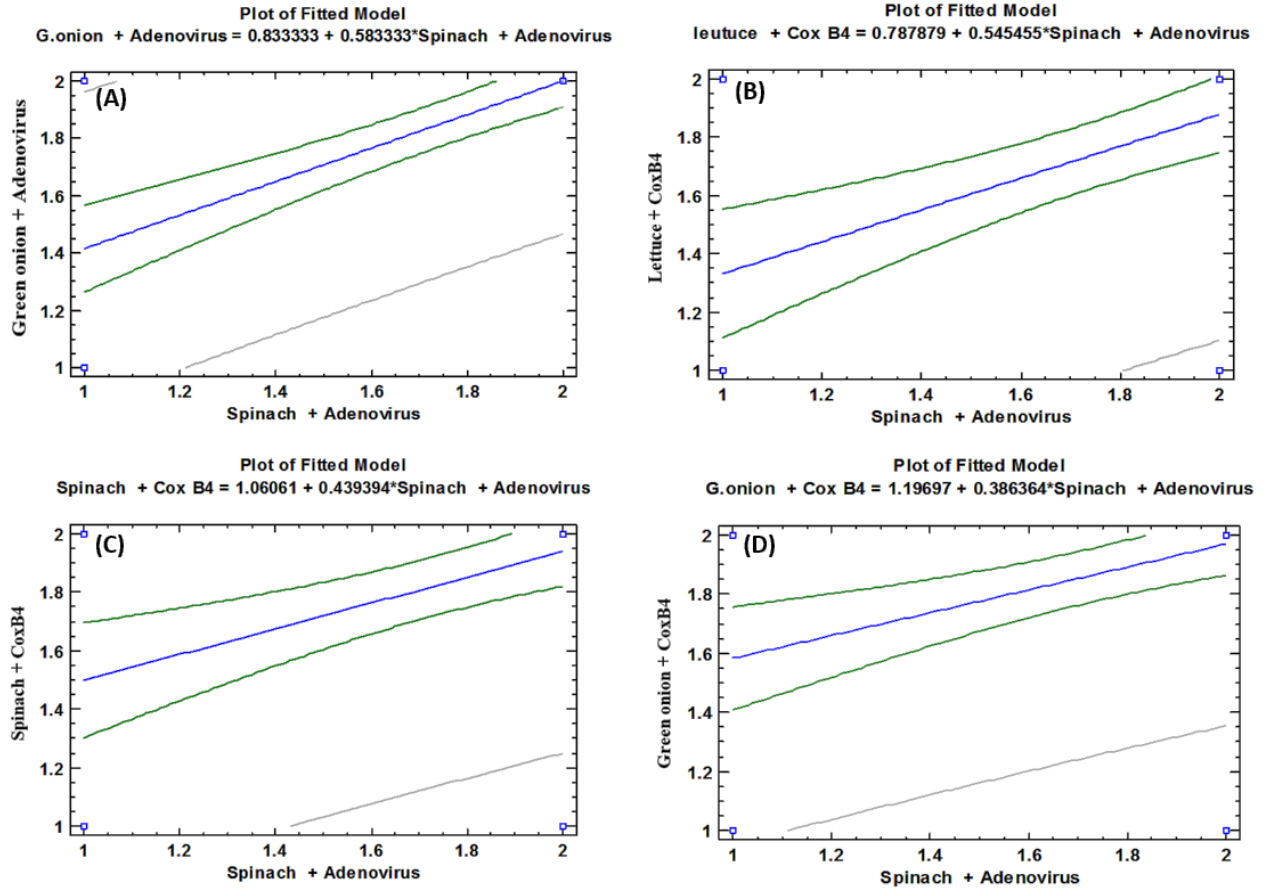*Correlation is significant at the 0.05, and 0.001 level

Figure (9). Linear Pearson Correlation between *Adenovirus* contaminated lettuce plants with *Adenovirus* contaminated spinach plants (**A**), with *Adenovirus* contaminated green onion plants (**B**), with CoxB4 contaminated lettuce plants (**C**), with CoxB4 contaminated spinach plants (**D**), and with CoxB4 contaminated green onion plants (**E**)

Table 10.   Correlation between *Adenovirus* contaminated spinach plants with *Adenovirus* contaminated green onion plants, with CoxB4 contaminated lettuce plants, with CoxB4 contaminated spinach plants, and with CoxB4 contaminated green onion plants

Adenovirus contaminated spinach plants (Spinach + Adenovirus)			
Item	**Pearson correlation coefficient**	**P. value**	***Significance**
Green onion + Adenovirus	0.712	0.000	Significant
Lettuce + CoxB4	0.545	0.000	Significant
Spinach + CoxB4	0.508	0.000	Significant
Green onion + CoxB4	0.503	0.000	Significant

*Correlation is significant at the 0.05, and 0.001 level

Figure (10).   Linear Pearson Correlation between *Adenovirus* contaminated spinach plants with *Adenovirus* contaminated green onion plants (**A**), with CoxB4 contaminated lettuce plants (**B**), with CoxB4 contaminated spinach plants (**C**), and with CoxB4 contaminated green onion plants (**D**)

Table 11.   Correlation between *Adenovirus* contaminated green onion plants with CoxB4 contaminated lettuce plants, with CoxB4 contaminated spinach plants, and with CoxB4 contaminated green onion plants

Adenovirus contaminated green onion plants (Green onion + Adenovirus)			
Item	**Pearson correlation coefficient**	**P. value**	***Significance**
Lettuce + CoxB4	0.434	0.003	Significant
Spinach + CoxB4	0.602	0.000	Significant
Green onion + CoxB4	0.553	0.000	Significant

*Correlation is significant at the 0.05, and 0.001 level

Plot of Fitted Model
leutuce + Cox B4 = 0.755639 + 0.530075*G.onion + Adenovirus

Plot of Fitted Model
Spinach + Cox B4 = 0.650376 + 0.635338*G.onion + Adenovirus

Plot of Fitted Model
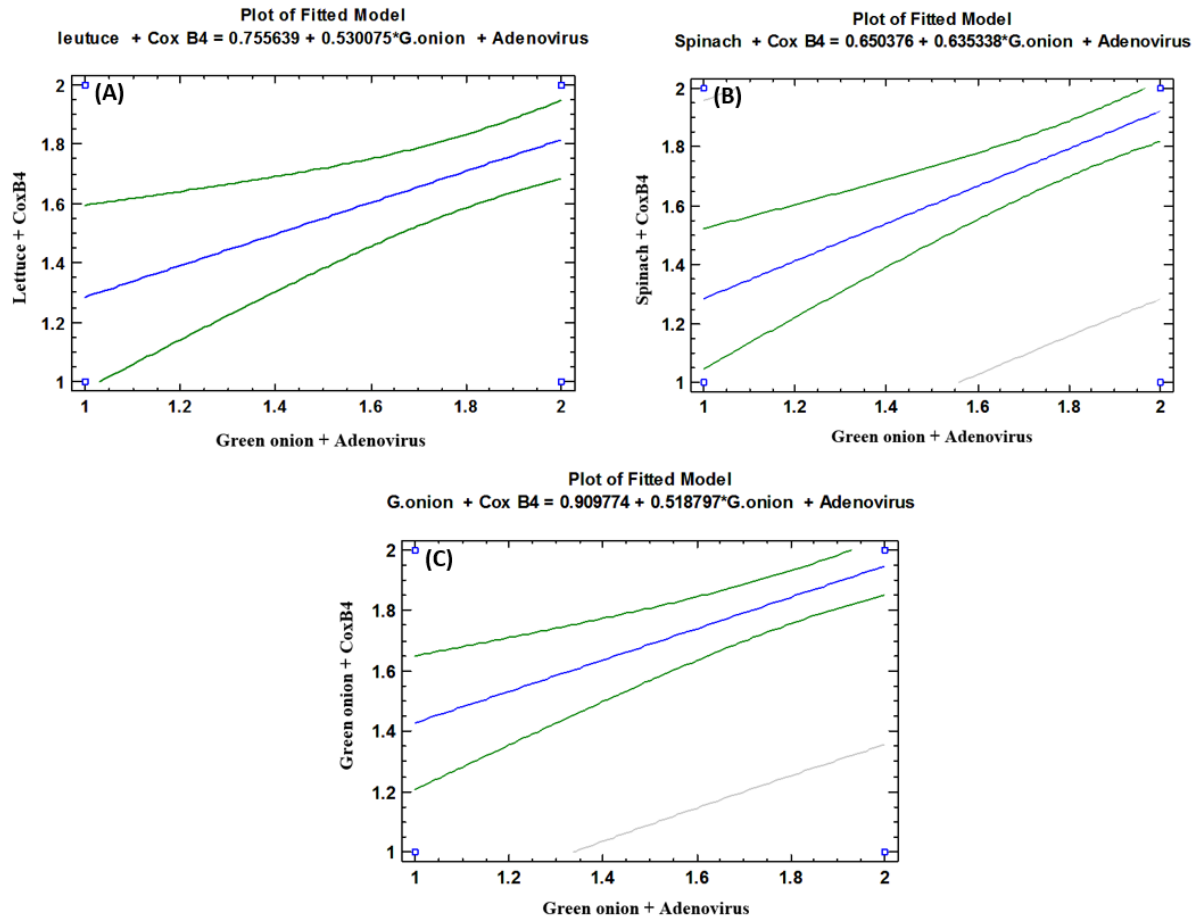G.onion + Cox B4 = 0.909774 + 0.518797*G.onion + Adenovirus

Figure (11). Linear Pearson Correlation between *Adenovirus* contaminated green onion plants with CoxB4 contaminated lettuce plants **(A)**, with CoxB4 contaminated spinach plants **(B)**, and with CoxB4 contaminated green onion plants **(C)**

Table 12. Correlation between *Coxsackievirus B4* contaminated lettuce plants with CoxB4 contaminated spinach plants, and with CoxB4 contaminated green onion plants

Coxsackievirus B4 contaminated lettuce plants (Lettuce + CoxB4)			
Item	Pearson correlation coefficient	P. value	*Significance
Spinach + CoxB4	0.771	0.000	Significant
Green onion + CoxB4	0.650	0.000	Significant

*Correlation is significant at the 0.05, and 0.001 level

Plot of Fitted Model
Spinach + Cox B4 = 0.666667 + 0.666667*leutuce + Cox B4

Plot of Fitted Model
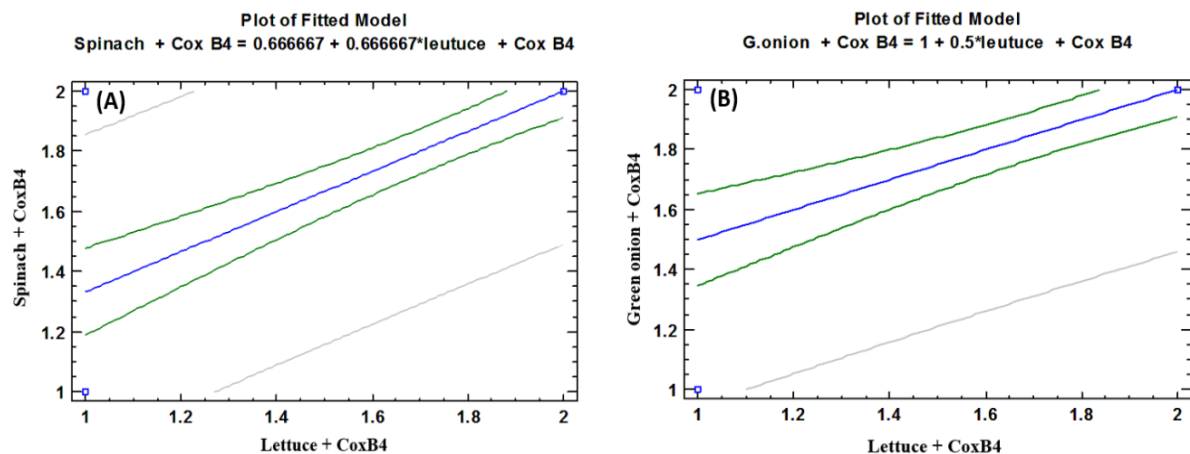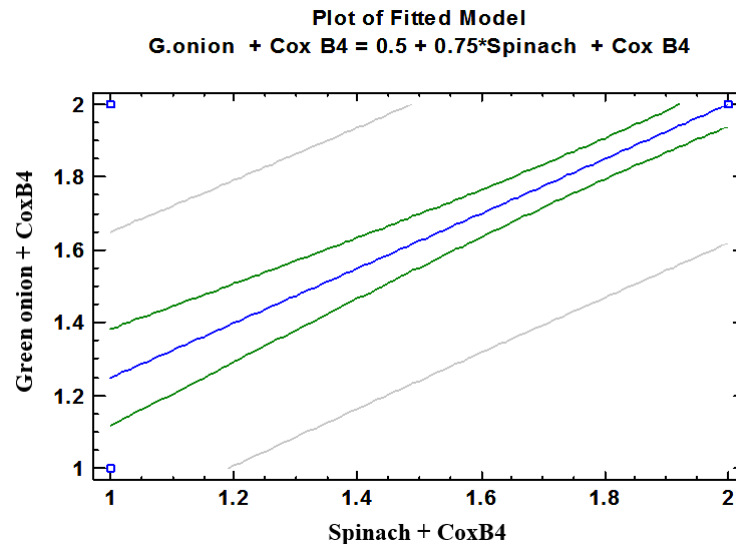G.onion + Cox B4 = 1 + 0.5*leutuce + Cox B4

Figure (12). Linear Pearson Correlation between *Coxsackievirus B4* contaminated lettuce plants with CoxB4 contaminated spinach plants **(A)**, and with CoxB4 contaminated green onion plants **(B)**

Table 13.   Correlation between *Coxsackievirus B4* contaminated spinach plants with CoxB4 green onion plants

Coxsackievirus B4 contaminated spinach plants (Spinach + CoxB4)			
Item	Pearson correlation coefficient	P. value	Significance
Green onion + CoxB4	0.844	0.000	Significant

*Correlation is significant at the 0.05, and 0.001 level

Plot of Fitted Model
G.onion + Cox B4 = 0.5 + 0.75*Spinach + Cox B4

Figure (13).   Linear Pearson Correlation between *Coxsackievirus B4* contaminated spinach plants with CoxB4 contaminated green onion plants

4. Discussion

Foodborne viruses are transmitted via contaminated food, as well as in combination with person-to-person contact or via contamination of the environment, e.g., water [15]. The contamination of fresh produce with enteric viruses which not subjected to further treatments to be taken must draw attention to the threat of these viruses as a risk to public health and provide insight into appropriate intervention methods that can be used to prevent such contamination with viral pathogens. The obtained results found that *Adenovirus* was present in some examined vegetables samples from the three locations but in different prevalence contamination percentages. Lettuce, spinach and green onion were contaminated by 13.3%, 20%, and 13.3%, respectively, for fields irrigated with water from Elmaryotia canal (Giza governorate). While by 60%, 46.6% and 26.6%, respectively, for fields irrigated with water from Kitchener drain (Kafr El-Sheikh governorate). As well as, by 33.3%, 13.3% and 6.6%, respectively for El-Gabal El-Asfar farm (Qalyubia governorate). On the other hand, CoxB4 was not detected in any samples from the region of Elmaryotia canal. In contrast, the three vegetable items of the area of Kitchener drain were contaminated by 40%, 26.6%, and 30%, respectively. In the same trend samples of El-Gabal El-Asfar farm showed contamination percentages were 40%, 26.6%, and 20%, respectively. Many foods have been implicated as vehicles in the transmission of enteric viruses. Among the more common ones are shellfish, fruits, vegetables, salads, sandwiches, and bakery items, among others. Indeed, any food that has been handled manually and is not further heated before consumption has the potential for contamination [30].

Food contaminated by fecal sources, such as fruits, and vegetables irrigated with water containing sewage or picked by infected workers, and ready-to-eat foods prepared by infected handlers are means of infection with enteric viruses. Enteric viruses cannot multiply in food or water but are generally very environmentally stable outside the host and are acid-resistant such as *Human adenovirus* and other enteroviruses [15]. Several studies reported that HAdVs are third only to rotaviruses and noroviruses as causative agents of acute gastroenteritis in infants and young children [31]. HAdVs are more frequently associated with acute gastroenteritis, more subtypes explicitly 40 and 41 [32]. *Adenovirus* transmission occurs by the fecal-oral route, and by ingestion of contaminated food and water [33]. It is known that HAdVs show high stability in the environment [34, 35] and food matrices [34]. As well as, many previous studies have also demonstrated that enteric HAdVs are high disseminated in the aquatic environment and may contaminating vegetables herbs and fruits [36-38]. In addition, a high occurrence of EVs and AdVs was detected in a variety of water environments, including groundwater [39, 40], indicating that raw vegetables can be potentially contaminated with these viruses through irrigation water. Therefore, many studies suggested that, the source of contamination was the discharge of sewage into river water used for irrigating crops. Moreover, Insufficient treatment of sewage can lead to an increase in the numbers of viruses being discharged. Many places worldwide have very limited or no treatment facilities for sewage and consequently semi-treated or raw sewage are pumped straight into the

aquatic environment. The role water plays in the epidemiology of HAdV, as well as the potential health risks constituted by these viruses in water environments, is widely recognized [41]. The presence of enteric viruses in sewage and hence in environmental surface waters reflects the infectious status of the population [42] and constitutes a public health risk [43]. In most instances, contamination of fruits and vegetables with enteric viruses is believed to occur before the product reaches food service [44]. Sources of such contamination include the use of contaminated soil, irrigation or washing water, or infected food handlers who harvest the product [45]. Treatment of sewage sludge by drying, pasteurization, anaerobic digestion and composting can reduce but not eliminate viruses, especially the more thermo-resistant ones [46]. Therefore, using recycled sewage effluent and sludge to irrigate or fertilize crops intended for human consumption carries with it the danger of virus contamination [47]. Likewise, soils can also become contaminated by land disposal of sewage sludge and through the use of fecally-impacted irrigation water. Viruses can survive in contaminated soil for long periods of time, the degree to which depends upon factors such as growing season, soil composition, temperature, rainfall, resident microflora, and virus type [48]. El-Senousy *et al.* [49] reported that *Adenovirus* may be a suitable candidate viral indicator of human viral contamination in Egyptian water and sewage samples. They recorded the prevalence percentage of *Adenovirus*es in raw sewage samples collected from Zenin wastewater treatment plant, and Nile water samples collected from El-Giza water treatment plant from July 2009 to June 2011, where was 91.7% (22/24), and 66.7% (16/24), respectively [49].

5. Conclusions and Recommendations

In conclusion, the obtained results demonstrated that a large number of samples from the three studied areas showed positive results for *Human adenovirus* and *Coxsackievirus B4*. Samples from the area of Kitchener drain (Kafr El-Sheikh governorate) showed the highest number of contaminated samples. While, the samples examined from the area of Elmaryotia canal (Giza governorate) showed the lowest number of contaminations, with no detection of CoxB4. So, we can say that vegetables may contain the risk of infection with enteric viruses that may cause serious danger to the public health of humans. Therefore, we recommended that control measures should be targeted at prevention of contamination (e.g., preventive measures at source, sewage treatment, improved food handling). Prevention of environmental sewage and fecal contamination in irrigation of vegetables and crops areas should be a particular focus for risk management activities. Researchers and risk managers need to be on alert to consider the likelihood of foodborne transmission of newly emerging viruses. Studies of the prevalence and levels of virus contamination of foods commonly implicated in outbreaks need to be completed and are essential to enable Quantitative Microbiological Risk Assessment (QMRA) to be conducted.

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