

Original Research Article

## The effect of *Allium sativum* (Garlic) extract on infectious bronchitis virus in specific pathogen free embryonic egg

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**Abstract**

**Objective:** Garlic is a plant has been used as a flavor, and anti-microbial and anti-diarrheal agent. Infectious bronchitis virus (IBV) is a coronavirus. The available vaccines against IBV cannot cover new variants. This study evaluated the inhibitory effects of garlic extract on IBV.

**Materials and Methods:** The constituents of garlic extract were detected by gas chromatography. This study was done in four groups of embryonic SPF eggs; first group was used for virus titration; second group received the mixture of different virus titration and constant amount of garlic extract; third group received  $10^{-3}$  titration of virus and after 8 hr received garlic extract and the last group received different dilutions of garlic extract.

**Results:** Based on our results, in the second group, IBV vaccine strain (4/91) at all titration and M41 in  $10^{-2}$  and  $10^{-3}$  titration and in the third group both variants of virus the embryonic Index (EI) was significantly increased.

**Conclusion:** The garlic extract had inhibitory effects on IBV in the chickens embryo.

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**Introduction**

Avian infectious bronchitis virus (IBV) is the microorganism that causes a significant economic loss in the poultry industry worldwide. IBV is a positive-sense, single-stranded RNA virus that belongs to the coronaviruses. All viruses in the family of coronavirus replicate in the cytoplasm of infected cells (Maclachlan and Dubovi, 2010; Cavanagh, 2007). Virion of IBV has four kinds of

proteins in its structure including the spike (S), the membrane (M), the envelope (E) and the nucleocapsid (N). The major target of IBV is the respiratory system and can also affect the reproductive system and kidneys (Raj and Jones, 1997). There is no exact cure for infectious bronchitis (IB) due to IBV so, the vaccination is the best way to prevent. However, the variations of IBV that is made during replication may reduce the efficacy of some IBV vaccines

(Jackwood et al., 2007). Although the vaccination strategy has been adopted, medicinal plants that have antiviral effects can be regarded as an alternative to vaccination or at least can be added to the vaccination programs (de Wit and Cook, 2014). *Allium sativum*, garlic in English, belongs to the Alliaceae family (Delahaand Garagusi, 1985). It has different minerals such as Ca, Fe, K, Cu and Mg and also contains variety of vitamins (This plant is full of water-soluble organosulfur compounds like S-allyl cysteine (SAC), S-ethyl cysteine (SEC), and S-propyl cysteine (SPC) (Suleria et al., 2015).

Traditionally, garlic has been used for curing disease (Bayanet et al., 2014) and some of its medicinal effects like hypoglycemic (Johnson et al., 2006) anticancer (Dion and Milner, 1997) and antimicrobial activities (Rose et al., 2005) have been proven. Experimental trial of garlic gave it a place in veterinary medicine industry and it is used for health improvement and disease prevention in animal sciences. Due to industrialization, in the 21st century, a part of our diet is synthetic. Not only our daily food but also most of the medicines are synthetic. Although the most of the chemical medicines are effective, their wide side effects are known. Although it is thought that the medicinal plants will go aside in human life little by little, people gradually figure out the complications of using the chemical medicines and attentions come back to the medicinal plants (Bayan et al., 2014). For infectious bronchitis due to IBV, no certain treatment exists. Removal of cold stress, high-quality air and management factors can help to control this disease. Antibacterial agents reduce death due to airsacculitis (Maclachlan and Dubovi, 2010). Therefore, it is logical to use medicinal plants for treatment of the disease for which the only way to control is vaccination and the vaccination in some

region is not impressively effective. The aim of this study is evaluation of the effect of *Allium sativum* (garlic) extract on infectious bronchitis virus in SPF embryonic egg as a pilot study.

## Materials and Methods

### Garlic extract

To prepare garlic extract, outer layer of garlic was detached and the bare garlic was washed. Then, 80 g fresh garlic was added to 200 ml aquaporin and mixed completely. The mixture was centrifuged at 2000 rpm twice and the upper layer of liquid was separated. This extract was kept at room temperature. Finally, it was filtered through a 0.25  $\mu$ m filter and was stored in sterile falcon. The extract was examined by head-space GC-mass in the Reference Laboratory of Veterinary, University of Tehran, Tehran, Iran. In this study, 0.1 ml of extract was used for each egg (Wang et al., 2015).

### Viruses

Two strains of IBV, 4/91 (Intervet) and M41 were separately targeted in this trial. Here, 1000 dose of each strain was used to prepare several dilutions.  $10^{-2}$  to  $10^6$  dilutions of each strain was prepared with PBS. In this study, 0.1 ml of each dilution was used for each egg.

### SPF eggs and Candling

Here, 9 day old SPF eggs were obtained from Razi Institute. All eggs were kept in incubator at 38°C for 24 hr for adaptation. Then, all eggs were checked through candling to check their viability. This study had two control groups. The first group consisted of 17 day old eggs and the second group consisted of 12 day old eggs. The study had 7 experimental groups. Each group that was inoculated with Intervet 4/91 and M41 contained 28 eggs. The group that was treated by the mixture of several dilutions of Intervet 4/91 ( $10^{-2}$  to  $10^{-6}$ ) and constant amount of garlic extract

contained 20 eggs and also the group was treated by the mixture of the several dilutions of M41 ( $10^{-2}$  to  $10^{-6}$ ) and constant amount of garlic extract contained 20 eggs. A group with 10 eggs was exposed to  $10^{-3}$  dilution of Intervet 4/91 and after 8 hr, was treated by constant amount of garlic extract and a group with 20 eggs was exposed to  $10^{-3}$  dilution of M41 and after 8 hr treated by constant amount of garlic extract. The last group contained 6 eggs that were treated only by garlic extract to evaluate its toxicity. All eggs in each group were observed under the candle twice a day and an egg with a dead embryo was transferred to the refrigerator. After 8 day candling, the evidence of embryo health such as existence, the number and the size of chorioalantoic membrane veins and the embryo movement were evaluated and compared with control group.

### **Embryo index**

At the end of the experiment, all eggs in each group (control and experimental) were weighed one by one. Then, the embryo of them were removed and washed. Embryos in each groups were weighed. The embryo index (EI) was calculated for all eggs using the following formula (Dhinakaret al., 2004)

$$EI = [\text{embryo weight (gr)} / \text{egg weight (gr)}] \times 10000$$

### **Statistical analysis**

To analyze the results of this study, SPSS (version 22.0), t-test in Microsoft Excel 2010 for Windows were utilized (Howitt and Cramer, 2014).

## **Results**

### **Candling**

No evidence of embryo death such as disappearance of chorioalantoic membrane veins and disappearance of the embryo

movement were seen during the 8 days of study.

### **Candling: Intervet4/91**

There was not no embryonic evidence but a decrease in growth rate at all dilutions of this strain was seen as compared to control group. This sign in  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilutions of virus was more marked.

### **Candling: M41**

Until the third day after the start of the study, there was no evidence of embryo death. At the end of the 3rd day of study, all embryos in  $10^{-2}$  and half of the embryos in  $10^{-3}$  dilution of virus died. At the end of the 4th day, the remaining embryos at  $10^{-3}$  dilution of virus died. Rest of embryos in other dilutions did not show any death evidence until the end of the study but the decrease in growth rate in  $10^{-4}$  dilution of virus as compared to control group was significant.

### **Candling: The mixture of the several dilutions of Intervet4/91 and constant garlic extract**

No embryo in none of dilution showed the death evidence and the growth rate was the same as control group during this study.

### **Candling: Mixture of the several dilutions of M41 and constant garlic extract**

In this group, until the 3rd day after injection, no embryo death was recorded. At the end of the 3rd day, one of the embryos in  $10^{-2}$  was died and at the end of the 4th day one embryo in  $10^{-2}$  and one embryo in  $10^{-3}$  dilution of virus were died. Finally, at the end of the 5th day after injection, the remaining embryos in  $10^{-2}$  were died. At other dilutions, until the end of the study, no embryo death was seen and the embryo growth rate in comparison with the control group did not show

## Effect of garlic extract on IBV

significant variation. It was observed that the delay of embryo death at  $10^{-2}$  and  $10^{-3}$  dilutions of the M41 was due to garlic extract.

### **Candling: Treatment with garlic extract 8 hours after the exposure to Intervet4/91**

In this group, although no significant evidence of embryo death was seen, the embryo size was increased compared to the group that was treated only with Intervet 4/91.

### **Candling: Treatment with garlic extract 8 hours after the exposure to M41**

In this group, among ten eggs that received only the M41, 3 days after the injection, 4 embryos were died and on the 4th day, 3 embryos were died. On the other hand, the eggs treated with garlic extract 8 hours after virus injection, until 3 days after the virus injection no embryo was died but on the 4th and 5th day, 2 and 4 embryo deaths were seen.

### **Embryo index:**

The average of embryo index for 17 day old group was 3804.6 and 3728 unit and this average for 12 day old group was 1047.4.

### **Embryo index: Treatment with several dilutions of Intervet4/91 and also treatment with the mixture of several dilutions of Intervet4/91 and constant amount of garlic extract**

The comparison of the results of these two groups shows that at all dilution of virus, EI is increasing due to garlic extract and at all dilutions, the differences between EI are statistically significant. Garlic extract at all virus dilutions can significantly reduce the effect of virus on embryos (Figure 1 and Table 1).

### **Embryo index: Treatment with several dilutions of M41 and also treatment with the mixture of the several dilutions**

### **of M41 and constant amount of garlic extract**

The comparison of the results of these two groups shows that in  $10^{-2}$  and  $10^{-3}$ , the increase in EI is statistically significant due garlic extract. At these dilutions, the garlic extract can inhibit the effects of virus on the embryos (Figure 2 and 3 and Table 2).

Table 1. The volatile components of aqueous extract of garlic obtained by head-space GC-MS.

NO	compound	Percentage	
1	Acetic acid	0.84	--
2	Allyl methyl sulfide	1.85	33.94
3	3,3, thiobis, 1, propan	3.16	30.53
4	Methyle 2, propenyle disulfide	8.94	--
5	Methyle- trans propenyl- disulfide	1.33	--
6	Diallyl disulfide	53.91	--
7	Disulfide, di-2-propenyl	12.76	--
8	Trisulfide, di-2-propenyl	1.26	--
9	Hexadecanoic acid	1.87	--

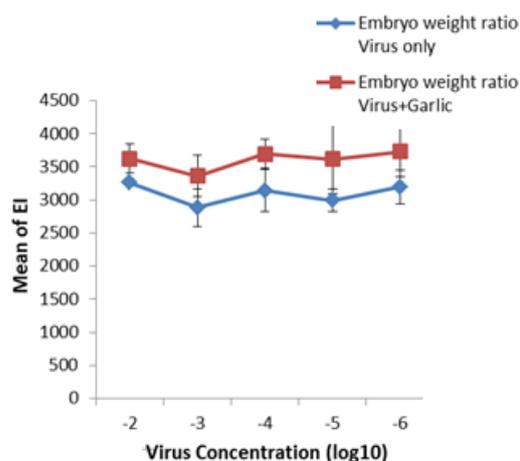


Figure 1. Effect of garlic extract on EI in SPF embryonic eggs. Eggs were treated with the mixture of Intervet4/91 at several dilutions and constant amount of garlic extract. Concentrations of -2 to -6 for Intervet4/91 are  $10^{-2}$  to  $10^{-6}$ . Bars show the standard deviation from three independent assays.

### **Embryo index: Treatment with garlic extract, 8 hours after exposure to Intervet4/91**

The results in this group showed that the EI in the group that was treated with the garlic extract is significantly increased compared to the group that was not treated.

Injection of Intervet 4/91 alone causes a reduction in EI as compared to the control group. The treatment with the garlic

extract, after 8 hr showed that this extract can inhibit the effects of Intervet 4/91 on embryos (Figure 4 and Table 3).

Table2. The inhibitory effect off garlic extract on IBV (M41) in ovo as a primary mixture of different dilutions of virus and constant amount of garlic extract

Parameter	Dilution of virus	Number of egg	Candling evidences Number of death/ reduction of growth in comparison to control group (17 days old)							Mean of EI	p Value
			1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day	7 <sup>th</sup> day		
i	10 <sup>-2</sup>	4	0/++	0/++	4/++	-	-	-	-	804.1±122	<0.05
	10 <sup>-3</sup>	4	0/++	0/++	2/++	2/++	-	-	820.8±98	<0.05	
	10 <sup>-4</sup>	4	0/++	0/++	0/++	0/++	0/++	0/++	4043.6±227		
	10 <sup>-5</sup>	4	0/+	0/+	0/-	0/-	0/-	0/-	3644.2±476		
	10 <sup>-6</sup>	4	0/+	0/+	0/-	0/-	0/-	0/-	3691.4±347		
ii	10 <sup>-2</sup>	4	0/++	0/++	0/++	2/++	2/++	-	-	1296.4±269	<0.05
	10 <sup>-3</sup>	4	0/+	0/+	0/+	1/+	1/+	0/-	0/-	2604±1251	<0.05
	10 <sup>-4</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	4047.1±301	
	10 <sup>-5</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3864.1±321	
	10 <sup>-6</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3789±300	
iii	0	8	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3728	

All data expressed as the mean±standard deviation of triplicate samples. Mean values were compared among different groups by t-test analysis using EXCEL 2010 for Windows. The embryo egg groups were: IBV-infected control that was exposed to several dilutions of M41 (group i); Exposure to several dilutions of M41 and constant amount of garlic extract (group ii); Non treatment control (group iii). High grade reduction of growth in comparison to group iii (++); low grade reduction of growth in comparison to group iii (+); equal growth in comparison to group iii (-).

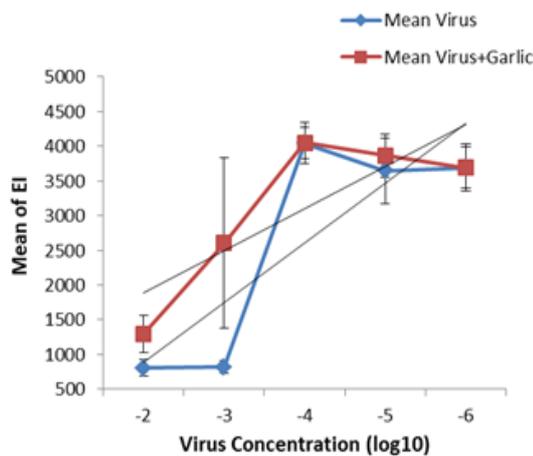


Figure 2. Effect of garlic extract on EI in SPF embryonic eggs. Eggs were treated with the mixture of M41 at several dilutions and constant amount of garlic extract. Concentrations of -2 to -6 for Intervet4/91 are 10<sup>-2</sup> to 10<sup>-6</sup>. Bars show the standard deviation from three independent assays

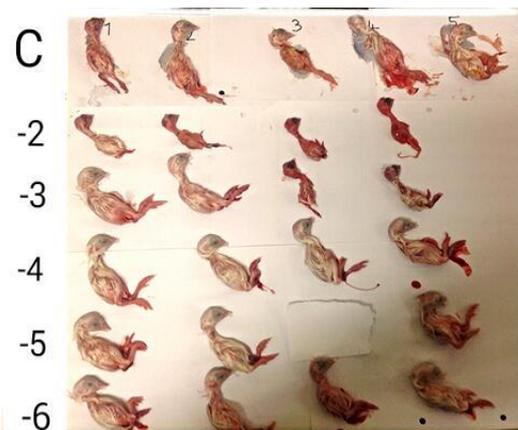


Figure 3. Effect of garlic extract on EI in the mixture of M41 at several dilutions and constant amount of garlic extract. SPF embryos were used for evaluation of the effect of garlic extract on IBV infection in ovo. Each row represents a concentration of virus. The first row represents a control group and the second to sixth rows for 10<sup>-2</sup> to 10<sup>-6</sup> concentrations of virus plus 0.1ml garlic extract for each egg.

## Effect of garlic extract on IBV

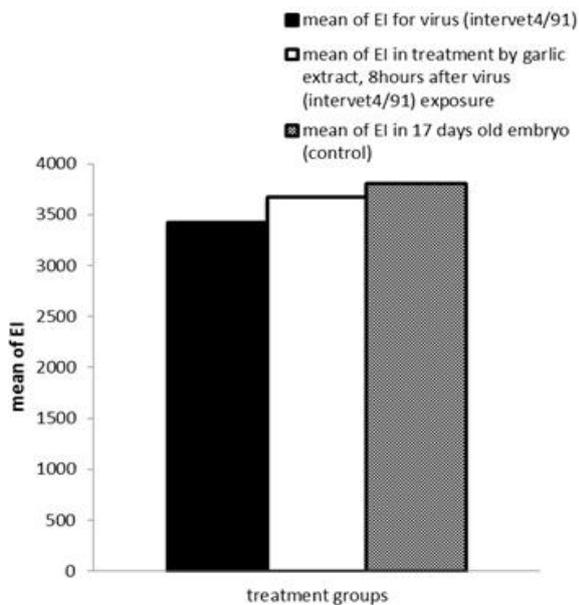


Figure 4. Effect of garlic extract, 8 hr after the exposure to Intervet 4/91. The mean of EI for control group that received only PBS is shown as a grey column (3804.6), the mean of EI for the group that received  $10^{-3}$  concentration of Intervet 4/91 is shown as black column (3419.6) and the mean of EI for embryos that were infected by  $10^{-3}$  concentration of Intervet 4/91 and were treated with garlic extract after 8 hr is shown as a white column (3674.6).

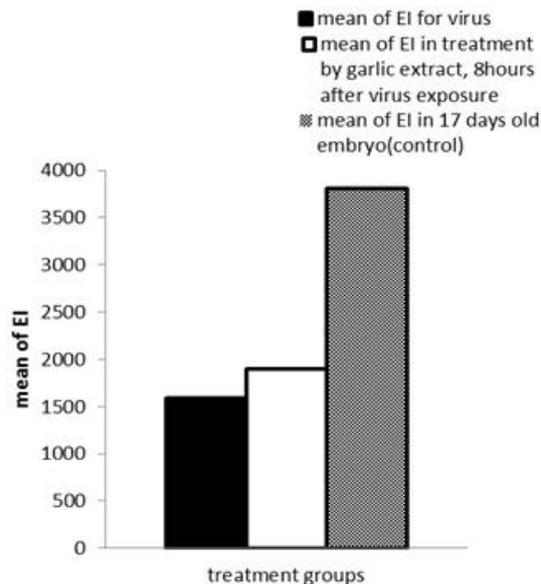


Figure 5. Effect of garlic extract, 8 hr after exposure to M41. The mean EI for control group that received only PBS is shown as a grey column (3804.6), the mean EI for the group that received  $10^{-3}$  concentration of M41 is shown as black column (1587.6) and the mean EI for embryos that were infected by  $10^{-3}$  concentration of M41 and were treated with garlic extract after 8 hr is shown as a white column (1903.2).

### Embryo index: Treatment with garlic, 8 hours after exposure to M41

M41 by itself can cause a significant decrease in EI at high concentration of virus. Treatment with garlic extract, 8 hr after exposure to M41 can increase the EI as compared to the control group. Garlic extract treatment can inhibit the effect of virus on the embryo (Figures 5 and 6 and Table 3).

### Head-space GC-mass

The result of headspace GC-mass shows that some components are more abundant than the others and resulted in higher peaks. By using the data bank analyzing, the components that were structurally so close to the components of garlic extract were recommended (Figure 7 and Table 4).

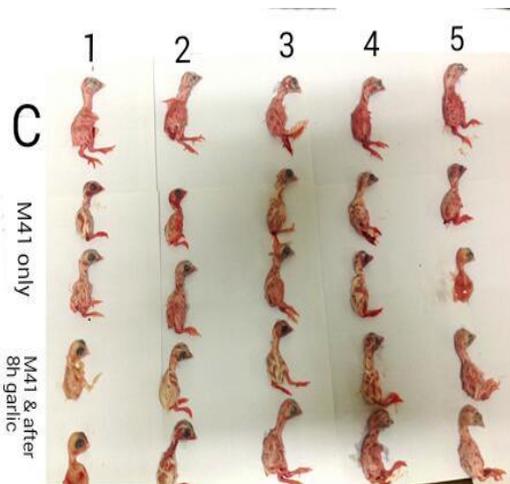


Figure 6. Effect of garlic extract, 8 hr after exposure to M41. The first row represents the control group that only received PBS. The second and third rows are the groups they were injected with  $10^{-3}$  concentration of M41 and the evidence of dwarfism is clear in them. The fourth and fifth rows were the groups treated with 0.1 ml garlic extract, 8 hr after they were exposed to  $10^{-3}$  concentration of M41 and the evidence of dwarfism is seen less commonly.

Table 3. The inhibitory effect off garlic extract on IBV (Intervet 4/91 and M41) in ovo by using the garlic extract as a treatment 8 hr after the exposure to the IBV

Parameter	Candling evidences										Mean of EI	p Value
	Dilution of virus	Number of eggs	Number of death/ reduction of growth as compared to control group(17days old)									
			1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day	7 <sup>th</sup> day			
Trial groups												
i	10 <sup>-3</sup>	5	0/++	0/++	0/++	0/++	0/++	0/++	0/++	0/++	3419.6	<0.05
ii	10 <sup>-3</sup>	5	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3674.6	<0.05
iii	10 <sup>-3</sup>	10	0/++	0/++	4/++	3/++	0/++	0/++	0/++	0/++	1587.6	<0.05
iv	10 <sup>-3</sup>	10	0/+	0/+	0/+	2/+	4/+	0/+	0/+	0/+	1903.2	<0.05
v	0	5	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3804	

Mean values were compared among different groups by t-test analysis using EXCEL 2010 for windows. The embryo egg groups were: IBV infected control that expose to 10<sup>-3</sup> dilution of Intervet 4/91 (group i); Treatment with garlic extract 8 hr after exposure to the 10<sup>-3</sup> dilution of Intervet4/91 (group ii); IBV-infected control that was exposed to 10<sup>-3</sup> dilution of M41 (iii); Treatment with garlic extract 8 hr after exposure to the 10<sup>-3</sup> dilution of M41 (group iv); Non-treatment control (group v). High grade reduction of growth in comparison to group iii (++); low grade reduction of growth in comparison to group iii (+); equal growth in comparison to group iii (-).

Table 4. The inhibitory effect of garlic extract on IBV (Intervet 4/91) in ovo as a primary mixture of different dilutions of virus and constant amount of garlic extract

Parameter	Candling evidences										Mean EI	p Value
	Dilution of virus	Number of eggs	Number of death/ reduction of growth in comparison to control group(17days old)									
			1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day	7 <sup>th</sup> day			
Trial groups												
i	10 <sup>-2</sup>	4	0/++	0/++	0/++	0/++	0/++	0/++	0/++	0/++	3194±222	<0.05
	10 <sup>-3</sup>	4	0/++	0/++	0/++	0/++	0/++	0/++	0/++	0/++	2992.2±314	<0.05
	10 <sup>-4</sup>	4	0/++	0/++	0/++	0/++	0/++	0/++	0/++	0/++	3143.7±220	<0.05
	10 <sup>-5</sup>	4	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+	2883.7±518	<0.05
	10 <sup>-6</sup>	4	0/+	0/+	0/+	0/+	0/+	0/+	0/+	0/+	3264±379	<0.05
ii	10 <sup>-2</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3625.5±57	<0.05
	10 <sup>-3</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3361±281	<0.05
	10 <sup>-4</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3694.5±313	<0.05
	10 <sup>-5</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3619.2±171	<0.05
	10 <sup>-6</sup>	4	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3730.5±256	<0.05
iii	0	8	0/-	0/-	0/-	0/-	0/-	0/-	0/-	0/-	3728	

All data are expressed as the mean±standard deviation of triplicate samples. Mean values were compared among different groups by t-test analysis using EXCEL 2010 for Windows. The embryo egg groups were: IBV-infected control that was exposed to several dilutions of Intervet 4/91 (group i); Exposure to several dilutions of Intervet 4/91 and constant amount of garlic extract (group ii); Non-treatment control (group iii). High grade reduction of growth in comparison to group iii (++); low grade degree reduction of growth in comparison to group iii (+); equal growth in comparison to group iii (-).

## Effect of garlic extract on IBV

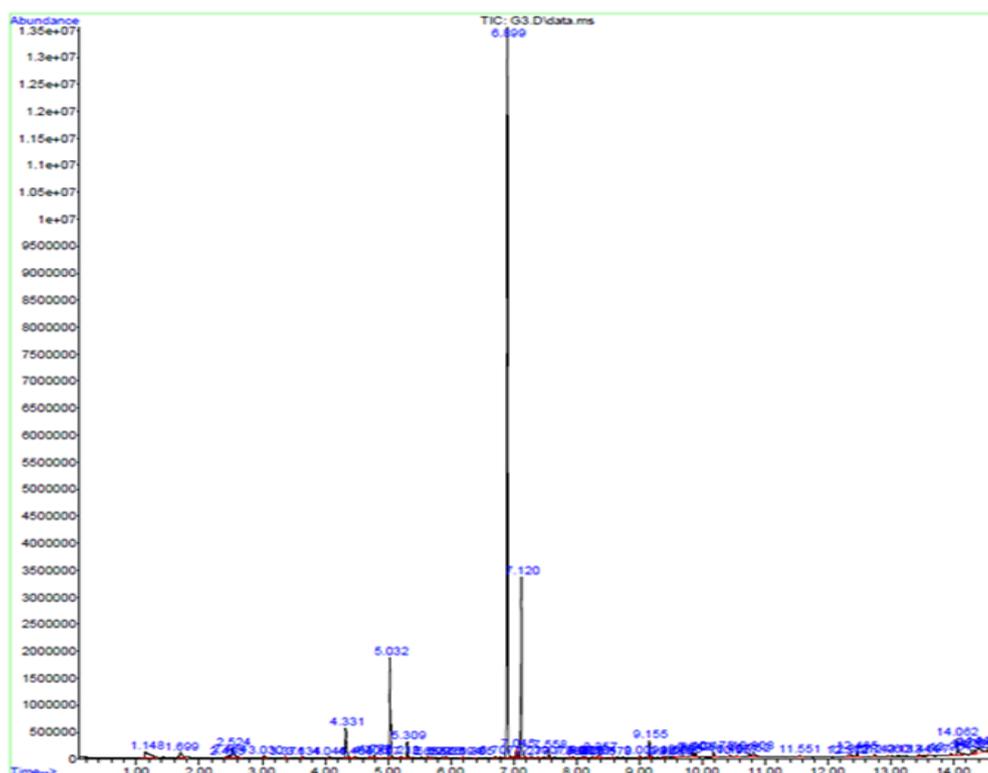


Figure 7. Head space GC-mass analyzing , Garlic extract

### Discussion

Infectious bronchitis (IB) due to IBV is a disease caused by coronavirus. Since it was appeared in the U.S. (1931) it has been one of the poultry industry problems around the world and also in Iran and has caused millions of deaths annually. However, only a few studies were done in the field of the effects of antiviral medicines on IBV. Since IBV is a coronavirus, it can be an appropriate model for other coronaviruses diseases like SARS and MERS and also because of using accessible, inexpensive and usable compounds in poultry industry that can be utilized in farms, garlic as an antiviral agent was chosen in this study. For the first time, this study evaluated the effects of garlic extract on infectious bronchitis virus (IBV). If the garlic extract has effect on pathogenesis process of the virus or not, it is important that complementary assays like negative staining and electronic microscope could also be done. The groups that were treated with garlic extract, 8 hr after virus exposure, were

evaluated to reveal that in which phase of virus infection, the garlic extract has an effect. The result showed that garlic extract could have an effect on the virus in replication phase. By comparing the results of two strain of IBV, it can be concluded that garlic extract has a significant effect on Intervet 4/91 as a sub-acute strain both in the mixture with several dilutions of virus and as a treatment, 8 hr after exposure to the virus. The EI in all experimental groups of Intervet 4/91 increased. Garlic extract has a significant effect on M41 as an acute strain in the mixture with only  $10^{-2}$  and  $10^{-3}$  dilution of virus and the increase in EI was observed only at these dilutions. As a treatment, 8 hr after exposure to the virus, garlic extract had a strong effect and significantly increased the EI . Based on this study, it can be said that garlic extract as a primary mixture with different strains of IBV has variable effects and its effects on sub-acute strains are more profound but as a treatment after virus exposure, it has a great effect on both strains (sub-acute and

acute). Raisi *et al.* (2011) examined the amount of garlic powder and period of use and evaluated the effects of garlic powder on the growth and the level of antibody against Newcastle and Gambro vaccines. They found that using one percent of garlic powder in last period of growing plan has significant effects on evaluated factors (unpublished data). Chen *et al.* (2014) evaluated the inhibitory effect of *Sambucus nigra* extract on IBV at an early point during replication. It was shown that virus titers in the treatment groups were significantly decreased as compared to control group (Chen *et al.*, 2014). Huawei *et al.* (2010) figured out that forsythoside A can inhibit IBV in cell culture. The data indicated that forsythoside A had had inhibitory effect on infected cells (Li *et al.*, 2011). Yin *et al.* (2011) studied on the effect of *Houttuynia cordata* on IBV-infected cells by using the plaque assay and PCR. This study showed that *H. cordata* resulted in more than 90% inhibition of the virus in Vero cells and chicken embryo kidney cells and it decreased the apoptotic rate by more than 90%. *H. cordata* can totally protect the SPF chicken embryos. Jackwood *et al.* (2010) showed that a mixture of oleoresins and essential oils (QR448) had antiviral effect on IBV *in vitro* and *in vivo*. It was found that this mixture can make a reduction in virus titre in Vero E6 and embryonating eggs. A 1:20 dilution of the mixture had the most marked effect on chickens. This treatment decreased the amount of RNA in trachea. Anti-viral activity of the mixture had the virucidal effect because it's activity greater to virus attachment and its entry (Jackwood *et al.*, 2010). Niu *et al.* (2008) indicated that Antheraea Pernyi nuclear polyhedrosis virus (ApNPV), as a member of the baculovirus family can offer protection against IBV in neonatal chickens. It showed that this virus can increase the inflammatory cytokine mRNA expression in chicken and also it was identified that budded virus can cause antiviral effects in

HD11 cells and the result showed that BV of this virus can enhance the immune activity and increase the protection against IBV in neonatal chickens (Niu *et al.*, 2008). Meer *et al.* (2007) showed that carbohydrate-binding agents have antiviral activity against Nidovirales in cell culture. The antiviral effects of *Hippeastrum hybrid agglutinin* (HHA), *Galanthus nivalis agglutinin* (GNA), *Cymbidium sp. Agglutinin* (CA) and *Urtica dioica agglutinin* (UDA) were evaluated. Cell viability assessed by MTT assay, the number of infected cell assessed by immunoperoxidase assay and the amount of viral protein expression assessed by luciferase-based assay were used to identify the antiviral effects and the results showed that these agents have antiviral activity against most of the members of Nidovirales (van der Meer *et al.*, 2007). Harrison *et al.* (2007) figured out that lithium chloride inhibits IBV cell culture. Several concentrations of LiCl and some types of cell line were used in this study (Vero cell, African Green monkey kidney-derived epithelial cell, and immortalized chicken embryo fibroblast cell lines). The result showed that the RNA of IBV, its protein, and viral progeny production were reduced in a dose-dependent manner. These effects were more close to cellular inhibition than virucidal effect and the protein synthesis in treatment groups was the same as control group so it was concluded that LiCl directly targeted IBV (Harrison *et al.*, 2007). The results showed that as a primary mixture of different strains of IBV and garlic extract had greater inhibitory effects on non-acute strain than acute one and using garlic extract as a treatment 8 hr after exposure to different strains of IBV had a significant inhibitory effect which was similar on both field and vaccine strain. Foregoing studies evaluated the antiviral effects of different agents such as chemical agents, natural agents and herbal extracts on the IBV *in vivo*, *in vitro* and different kinds of cells in cell culture.

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### Conflict of interest

No conflict of Interest.

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