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# Antiphytoviral Activity of some Natural Substances on Tobacco Mosaic Virus (Menoufia Strain) Infecting Tomato in Egypt and the Physical Properties of the Viral Inhibitors

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## ABSTRACT

Aqueous extracts of four plants were tested *in vitro* and *in vivo* for their antiviral properties against heat resistant strain (HRS) of tobacco mosaic virus (TMV). The effect of different dilutions of these extracts as well as the physical properties of the extract inhibitors was also studied. Obtained results showed that all the tested extracts had inhibitory effect on the virus, but in varying degrees. The inhibitory effect of the extracts increased with the increment of the storage period from 15 to 50 to 90 minutes both *in vitro* and *in vivo*. Using the *in vitro* technique showed higher inhibitory effect than that induced by *in vivo*. Pre-inoculation rubbing also showed more inhibition to virus infection than post inoculation rubbing. The most effective extract was that of *Crocus sativus*. The least effective one was that of *Ocimum basilicum*. Complete virus inhibition (100%) was obtained using the extract from *C. sativus* and *Avena sativa*. The antiviral activity of the used extracts was found to be highly affected by dilutions. The inhibitors were completely inactivated when kept at 40-85 $^{0}$C for 10 minutes, survived for more than 85 days at room temperature and active at pH 2-11. The inhibitors in the extracts of *C. sativus* and *A. sativa* were not precipitated by a saturated solution of ammonium sulfate, while that of *Tussilago farfara* and *O. basilicum* were precipitated.

**Key words:** Antiphytoviral activity, natural substances, Tobacco Mosaic Virus (Menoufia strain), tomato, physical properties, viral inhibitors, Egypt.

## INTRODUCTION

Viral diseases are considered the main factors affecting tomato plants causing a great loss in yield and thus effective methods for control are required. Many studies on the inhibition of viruses using water plant extracts have been attempted by several workers (Bhamuprakash *et al.*, 2008; Adman and Rubase, 2009 and Yan *et al.*, 2010).

Most of the virus inhibitors were extracted from leaves, few were extracted from roots and very few from bark, latex, seeds, flowers and fruits of higher plants (Schuster *et al.*, 1995; El-Shamy and Shaaban, 1999; Tarus *et al.*, 2006 and Chen *et al.*, 2009).

Tomato plants *Lycopersicon esculentum* cv. Money Maker have been found to be naturally infected with a severe virus disease in Menoufia, Egypt. The virus induced mosaic mottling, blistering and severe abnormalities on leaves of infected plants. The virus was sap transmissible. Physical properties, host range, electron microscopy and serological studies identified the virus as a heat resistant strain of TMV and called the Menoufia strain (El-Shamy, 1987).

In the present study, preliminary experiments both *in vitro* and *in vivo* (pre-inoculation and post-inoculation) were carried out to study the effect of four plant extracts on the infectivity of heat resistant strain of tobacco mosaic virus (TMV) infecting tomato plants. Also, physical properties of the potent inhibitors were included in this study.

## MATERIALS AND METHODS

Antiviral effect of aqueous extracts from four different plants belong to four different families was studied. These plants were: *Crocus sativus* L (Iridaceae), *Tussilago farfara* L (Compositae), *Avena sativa* L (Graminae) and *Ocimum basilicum* (Labiatae). Effect of different dilutions of these extracts on the infectivity of the virus strain was also studied.

### Preparation of partially purified inoculums

Virus inoculum was prepared by grinding 5 gm of frozen diseased tomato leaves in a sterile mortar with about 50 ml distilled water. The pulp was squeezed through muslin cloth and the filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant was partially purified by further centrifugation and then diluted suitably with distilled water (1/100) to obtain 100-150 local lesions per leaf (using *Datura metel* as a test plant).

### Preparation of inhibitors

Crude water extract was prepared by grinding the dried plant shoots in distilled water (1: 3 w/v). They were kept over night at room temperature (25-30 $^{0}$C). The pulp was squeezed through cheesecloth and centrifuged at 2000 rpm for 15 minutes. The undiluted supernatant extract and the extracts serially diluted with distilled water in ten-fold dilutions starting from $10^{-1}$ to $10^{-4}$ were prepared. The antiviral effect of the crude extract of the dried shoots of the above mentioned four plant species were studied. Effect of different dilutions of these extracts and the physical properties of the inhibitors

(thermal inactivation point "heat sensitivity", pH, longevity *in vitro* and precipitation by ammonium sulfate) were also studied for their antiviral activity. Antiviral effect of the mentioned plant extracts was studied *in vitro* and *in vivo* (pre-inoculation and post-inoculation). Increasing storage period of the virus with the plant extract from 15 to 50 to 90 minutes was also studied. The antiviral effect of different dilutions of these extracts *in vitro* at 15 minutes was also studied. Infectivity of the virus was examined by local lesion assay on detached leaves of 50-60 day old *Datura metel* plants using the Latin Square Design. The total number of lesions for each treatment as well as the mean number of lesions per leaf was calculated. Mean percentage of inhibition = C-T/C ×100, where (C) is the mean number of lesions of control and (T) is the mean number of lesions of treated inoculum.

**Inoculation procedure**

Inoculation was made by rubbing the detached leaves of the tested plants (*Datura metel)* with the forefinger. Leaves were dusted with 400-mesh carborandum prior to inoculation. The inoculated leaves were kept under observation in the greenhouse.

**Effect of natural substances extracts on virus infectivity**

**1- *in vitro***

For testing the effect of undiluted tested natural substances after 15, 50 and 90 minutes, as storage period, on virus infectivity *in vitro*, 0.1 ml of expressed sap containing virus was added to 0.9 ml of the undiluted substances, mixed well and allowed to stand for 15, 50 and 90 minutes. A corresponding control sample was also prepared by mixing crude sap of the virus (0.1 ml) with 0.9 ml distilled water and store for 15, 50 and 90 minutes. Virus-substance mixture and control were then inoculated into detached *D. metel* leaves. Results were tabulated after five days from inoculation; the mean number of lesions of treated leaves and the mean percentage of inhibition was calculated.

**2- *in vivo***

This trial was designed to show the effect of different extracts on virus infectivity *in vivo* in pre- and post-inoculation rubbing at 15, 50 and 90 minutes before and after inoculation with the virus.

**a- Pre-inoculation rubbing**

Detached *D. metel* leaves were rubbed by 3-5 ml of undiluted plant shoot extract. After 15, 50 and 90 minutes, they were inoculated by the sap containing virus. A corresponding control test was carried out by rubbing detached *D. metel* leaves with distilled water and after 15, 50 and 90 minutes, they were inoculated with the virus.

**b- Post-inoculation rubbing**

Detached *D. metel* leaves were inoculated by the virus 15, 50 and 90 minutes before they were rubbed by undiluted plant shoot extract. A corresponding control test was carried out by rubbing detached *D. metel* leaves by the virus 15, 50 and 90 minutes before they were rubbed by distilled water.

**Effect of different dilutions of natural substances extracts on their antiviral activity *in vitro***

Effect of different dilutions on the inhibitory activity of plant shoot extracts against virus infectivity *in vitro* was studied. Crude shoot extract of each tested plant was serially diluted by distilled water in ten-fold dilution starting from $10^{-1}$ to $10^{-4}$. 1.0 ml of virus sap was added to 9.0 ml of each dilution of $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ of the extract. The tubes were kept at room temperature for 15 minutes. A control tube contained 9.0 ml of undiluted extract mixed with 1.0 ml of virus sap. A comparative test for the detection of changing in the antiviral activity of the diluted extracts was made by adding 1.0 ml of untreated TMV inoculum to 9.0 ml of distilled water. The three treatments were inoculated into detached *D. metel* leaves. The mean number of lesions of each treatment, the mean number of lesions of control and the mean number of virus and distilled water were calculated and tabulated.

**Physical properties of the virus inhibitors**

**Effect of temperature**

Two ml of each separate undiluted extracts were placed in separate four tubes. The tubes were kept in an electric water bath adjusted at 40, 50, 60, 70, 80, 90 and 100°C for 10 minutes, cooled and then tested for its antiviral activity.

**Effect of pH**

The pH of the plant shoot extract was adjusted to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 by adding 0.2N HCl or 0.2N NaOH and allowed to stand for one hour at each pH. The extracts were then adjusted to pH7 by HCl or NaOH and tested for their antiviral activity.

**Longevity *in vitro***

About 5ml of each extract was stored in sterilized test tubes at room temperature (25-30 °C) for 3, 5, 10, 15, 27, 35, 45, 70 and 85 days and then tested for their antiviral activity.

**Precipitation by ammonium sulfate**

The plant extract was mixed with an equal volume of saturated ammonium sulfate. The mixture was left overnight, and then centrifuged at 3000 rpm for 15 minutes. The precipitate and the supernatant were each tested for their antiviral activity.

**Statistical analysis**

The data were statistically analyzed using t-test and ANOVA analysis. The tests were applied using SPSS software.

## RESULTS AND DISCUSSION

Obtained results showed that the tested extracts were more effective in reducing the virus infectivity at 15, 50 and 90 minutes both *in vitro* and *in vivo* and at 15 minutes *in vitro* for different dilutions of the extracts. Therefore, the study concentrated on the effect of these extracts on the virus infectivity at only 15, 50 and 90 minutes both *in vitro* and *in vivo* and at only 15 minutes *in vitro* for different dilutions in details to get more accurate results which might help to control the virus which causes a great loss of yield of many economic plants.

***In vitro* and *in vivo* effect of natural substances extracts on the infectivity of heat resistant strain (HRS) of TMV**

The results obtained and represented in tables 1, 2 and 3 showed that:

- All the tested extracts had inhibitory effect on heat resistant strain of TMV, but in varying degrees depending on the incubation period with the virus *in vitro* and the period at which the extract was rubbed pre-inoculation or post-inoculation with the virus.
- Inhibitory effect of the extracts increased with the increment of the storage period with the virus from 15 to 50 to 90 minutes both *in vitro* and *in vivo.*
- Using of the *in vitro* technique showed higher inhibitory effect than that induced by *in vivo.* This may be due to the fact that the used extract affects the virus directly *in vitro* (Tables 2 and 3).
- Pre-inoculation rubbing also showed more inhibition to virus infection than post-inoculation rubbing (Table 3).
- The most effective extract on virus infectivity causing complete inhibition (100%) and more reduction in virus infection were those of *C. sativus* followed by the extracts of *A. sativa* and *T. farfara* both *in vitro* and *in vivo.*
- The least effective extract on virus infectivity was that of *O. basilicum* both *in vitro* and *in vivo.*
- Complete virus inhibition (100%) was obtained by using *C. sativus* extract, incubated for 50 and 90 minutes storage with the virus *in vitro*, and at 90 minutes *in vivo* using pre-inoculation rubbing.
- 100% virus inhibition was also obtained on using extracts from *Avena sativa* incubated for only 90 minutes storage with the virus *in vitro.*
- Statistical analysis showed a significant results on using extracts of *C. sativus*, *T. farfara* and *O. basilicum* and insignificant results on using extracts of *A. sativa* at 15 min storage period.

These results agree with other studies on plant extracts as antiviral agents or inhibitors against different viruses (Schuster *et al.*, 1995; Sindelarova *et al.*, 1996; Hou *et al.*, 1998 and Bhamuprakash *et al.*, 2008).

**Effect of different dilutions of natural substances extracts on their antiviral activity on HRS of TMV *in vitro***

Obtained results presented in table (4) showed that:

- Antiviral activity of the used extracts was found to be highly affected by dilutions as shown by comparing the number of local lesions produced using diluted and undiluted extracts.
- When the dilutions rose from $10^{-1}$ to $10^{-2}$ to $10^{-3}$ and $10^{-4}$, the mean number of local lesions gradually increased recording {(15.6, 20.4, 31.7 and 81.2), (20.1, 27.4, 35.1 and 95.3), (35.6, 43.5, 52.8 and 115.3) and (43.5, 57.8, 68.5 and 119.3)} for *C. sativus, A. sativa, T. farfara* and *O. basilicum*, respectively.
- Antiviral activity of extract obtained from *O. basilicum* was found to be the highly affected one by dilutions, followed by extract from *T. farfara*, and then that from *A. sativa.* The least effective one by dilutions was the extract obtained from *C. sativus*, as shown from the number of local lesions formed using different extracts (43.5, 57.8, 68.5 and 119.3 for *O. basilicum*), (35.6, 43.5, 52.8 and 115.3 for *T. farfara*), (20.1, 27.4, 35.1 and 95.3 for *A. sativa*) and (15.6, 20.4, 31.7 and 81.2 for *C. sativus*) for different dilutions $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$, respectively.
- All the extracts were active and not completely inactivated up to a dilution of $10^{-4}$.

**Physical properties of the plant inhibitors**

Physical properties of the virus inhibitors were studied and the obtained results tabulated in table (5). The results showed that:

- Inhibitors in extracts of *C. sativus*, *A. sativa*, *T. farfara* and *O. basilicum* were completely inactivated when kept at 85, 81, 45 and 40 $^{0}$C for 10 minutes, respectively.
- The inhibitors were survived for more than 85, 45, 27 and 10 days at room temperature for the extracts of *C. sativus*, *A. sativa*, *T. farfara* and *O. basilicum*, respectively.
- The inhibitors were active at pH 2-11 for extracts of *C. sativus* and *A. sativa* and were active at pH 3-9 for extracts of *T. farfara* and *O. basilicum*, respectively.
- The inhibitors in extracts of *C. sativus*, *A. sativa* not precipitated by a saturated solution of ammonium sulfate, while that of *T. farfara* and *O. basilicum* were precipitated.

Studies revealed that all the tested extracts have

Table (1): Effect of undiluted extracts of the four tested plant species on the infectivity of heat resistant strain of TMV *in vitro* and *in vivo* at 15 minutes (using 10 *Datura metel* leaves for inoculation in each treatment)

| Name of plant extract | | *Crocus sativus* extract | | | | *Tussilago farfara* extract | | | | *Avena sativa* extract | | | | *Ocimum basilicum* extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type of treatment | | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition |
| In vitro (Storage period 15 min) | | 130.0 | 13 | 10 | 90 | 133.2 | 77.1 | 57.9 | 42.1 | 137.2 | 15.3 | 11.2 | 88.8 | 140.2 | 86.8 | 61.9 | 38.1 |
| *In vivo* | Pre-inoculation (15 min before inoculatic n) | 125.4 | 56.3 | 44.9 | 55.1 | 137.4 | 85.2 | 62 | 38 | 131.4 | 65.5 | 49.8 | 50.2 | 132.3 | 89.8 | 67.9 | 32.1 |
| | Post-inoculation (15 min after inoculation) | 135.1 | 69.4 | 51.9 | 48.6 | 131.5 | 92.1 | 70.1 | 29.9 | 134 | 76.2 | 56.9 | 43.1 | 129.5 | 107.1 | 82.7 | 17.3 |

Table (2): Effect of undiluted extracts of the four tested plant species on the infectivity of heat resistant strain of TMV *in vitro* and *in vivo* at 50 minutes (using 10 *Datura metel* leaves for inoculation in each treatment)

| Name of plant extract | | *Crocus sativus* extract | | | | *Tussilago farfara* extract | | | | *Avena sativa* extract | | | | *Ocimum basilicum* extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type of treatment | | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition |
| In vitro(Storage period 50 min) | | 125 | - | - | 100 | 131.1 | 61.7 | 47.1 | 52.9 | 135 | 65 | 4.8 | 95.2 | 127.1 | 74.2 | 58.4 | 41.6 |
| *In vivo* | Pre-inoculation (50 min before inoculation) | 128.3 | 38.6 | 30.1 | 69.9 | 137.4 | 81.4 | 59.2 | 40.8 | 131.4 | 45.6 | 34.7 | 65.3 | 129.4 | 83.2 | 64.3 | 35.7 |
| | Post-inoculation (50 min after inoculation) | 130.1 | 44.9 | 34.5 | 65.5 | 136.9 | 91.3 | 66.7 | 33.3 | 130.1 | 58.7 | 45.1 | 54.9 | 132.3 | 107.6 | 81.3 | 18.7 |

Table (3): Effect of undiluted extracts of the four tested plant species on the infectivity of heat resistant strain of TMV *in vitro* and *in vivo* at 90 minutes (using 10 *Datura metel* leaves for inoculation in each treatment)

| Name of plant extract | | *Crocus sativus* extract | | | | *Tussilago farfara* extract | | | | *Avena sativa* extract | | | | *Ocimum basilicum* extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of treatment | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition | Mean No of lesions per leaf in control plants | Mean No of lesions per leaf in treated plants | % of virus infection | % of virus inhibition |
| | *In vitro* (Storage period 90 min) | 132 | - | - | 100 | 127.3 | 47.7 | 37.5 | 62.5 | 129 | - | - | 100 | 126.7 | 69.3 | 54.7 | 45.3 |
| *In vivo* | Pre-inoculation (90 min before inoculation) | 126.3 | - | - | 100 | 129 | 66.3 | 51.4 | 48.6 | 124.5 | 13.1 | 10.5 | 89.5 | 125.5 | 78.9 | 62.9 | 37.1 |
| | Post-inoculation (90 min after inoculation) | 125 | 12.3 | 9.8 | 90.2 | 121.5 | 79.3 | 62.3 | 34.7 | 121 | 23.8 | 19.7 | 80.3 | 129.3 | 103.4 | 80 | 20 |

Table (4): Effect of different dilutions of natural substances extracts of the four plant species on their antiviral activity *in vitro* at 15 minutes storage period (using 10 *Datura metel* leaves for inoculation in each treatment)

| Dilutions of extract | Mean No. of lesions per leaf | | | |
|---|---|---|---|---|
| | *Crocus sativus* infection | *Tussilago farfara* infection | *Avena sativa*infection | Ocimum basilicuminfection |
| [a] Control | 7.3 | 27.1 | 13.4 | 35.1 |
| $10^{-1}$ | 15.6 | 35.6 | 20.1 | 43.5 |
| $10^{-2}$ | 20.4 | 43.5 | 27.4 | 57.8 |
| $10^{-3}$ | 31.7 | 52.8 | 35.1 | 68.5 |
| $10^{-4}$ | 81.2 | 115.3 | 95.3 | 119.3 |
| Virus + d. w [b] | 120.3 | 123.5 | 119.7 | 122.1 |

a- virus + undiluted extract b- virus + distilled

Table (5): Summary of the physical properties of the extracts of the four tested plant species (using 10 *Datura metel* leaves for inoculation in each treatment)

| | Plant species | *Crocus sativus* | *Avena sativa* | *Tussilago farfara* | *Ocimum basilicum* |
|---|---|---|---|---|---|
| Physical properties | Thermal inactivation point (°C ) | 85 | 81 | 45 | 40 |
| | pH of activity | 2-11 | 2-11 | 3-9 | 3-9 |
| | Storage time (days) | > 85 | 45 | 27 | 10 |
| | Precipitation by ammonium sulphate | not precipitated | not precipitated | precipitated | Precipitated |

inhibitory effect both *in vitro* and *in vivo* on heat resistant strain of TMV, but in varying degrees depending on the incubation period with the virus *in vitro* and the period at which the extract was rubbed pre-inoculation or post-inoculation with the virus, suggesting that virus inhibitor components are present in the tested extracts but in varying degrees.

Extracts from *C. sativus* had the inhibitoriest effect (100% virus inhibition) on virus infectivity when incubated with the virus *in vitro* at 50 and 90 minutes and *in vivo* at only 90 minutes pre-inoculation. It was followed by extracts of *A. sativa* when incubated for 90 minutes with the virus *in vitro* (causing 100% virus inhibition). The least effective extracts were those of *O. basilicum* followed by extracts of *T. farfara* both *in vitro* and *in vivo* which showed more or less few invaluable antiviral effect, suggesting the presence of virus inhibitor components either poor, and this may be due to the presence of the inhibitors in low concentrations, or their effect is reduced as a result of the antagonistic effects of enhancing compounds Joao, *et al*,. 1988.

The inhibitory effect of the used extract *in vitro* and *in vivo* increased with the increment of the storage period of the virus with the extract. The use of *in vitro* technique showed higher inhibitory effect than that induced by *in vivo*. This may be due to the fact that the used extracts affected the virus directly *in vitro*.

In the present study, pre-inoculation rubbing with the different plant extracts was inhibitorier to HRS of TMV infection than post-inoculation rubbing. This is in agreement with Barakat and Stevens (1986), Stoimenova and Angelov (1995), Al-Khazindar(1999), Tarus *et al.* 2006 and Chen *et al.* 2009).

The inhibitory properties of the plant extracts decreased with progressive dilutions as proved by Verma and Dwivedi (1984), Verma and Khan (1985), Alexandre *et al.* (1997) and De Clerc, (2007). They stated that dilution is effective in removing the inhibitory activity which agrees with the obtained results, as the undiluted extract showed the highest antiviral activity.

*C. sativus* and *A. sativa* extracts were found to be heat resistant or thermo stable and were either polysaccharides (Hodgson *et al.*, 1969) or other non-proteinaceous inhibitors (Apablaza and Bernier, 1972). *T. farfara* and *O. basilicum* extracts were found to be heat-sensitive or theromlabile and may be proteins or glycoproteins (Frötschl *et al.* 1990; Shen *et al.*, 2009 and Yan *et al.*, 2010).

Concerning the subjection of the four plant extracts to a wide range of pH values, our results showed that the antiviral property of the extracts decreased gradually by increasing the extract acidity towards pH 2 and the alkalinity towards pH11). The most potent antiviral activity of the extracts ranged between the pH values of 4 to 8. All the tested extracts have a wide pH range. Extracts from *C. sativus* and *A. sativa* remained active between pH range of 2.0 and 11.0, while extracts from *T. farfara* and *O. basilicum* remained active between pH range values of 3.0 and 9.0. These results are in general agreement with Choi and Jung (1984), Verma and Dwivedi (1984), Singh *et al.* (1988), Alexandre *et al.* (1997), and Verma *et al.*, (2008). They found that most of the inhibitory activity of plant extracts was lowered in strong acidic and basic solutions.

Obtained results showed also that *C. sativus* extract retained full inhibitory activity up to more than 85 days at room temperature (25-30°C). *A. sativa*, *T. farfara* and *O. basilicum* extracts can retain different amounts of their antiviral activity and resist aging for 45, 27 and 10 days, respectively. These results are in accordance with many workers, since the storage period of some plant inhibitors ranged from few hours (Choi and Jung 1984) to few days (Singh *et al.* 1988) and to months (Noronha *et al.* 1995 and Verma *et al.* 2008). Longevity for several weeks suggests the presence of compounds other than proteins to be the active inhibitory ingredients. The presence of proteinaceous inhibitors in the extracts might be inferred if the extracts have a short "shelf life" (Barakat and Stevens 1986).

By studying the effect of ammonium sulfate as a protein precipitant on the different extracts, obtained results showed that extracts from *C. sativus* and *A. sativa* plants were not precipitated by ammonium sulfate, while *T. farfara* and *O. basilicum* inhibitors showed complete precipitation by ammonium sulfate, confirming the suggestion that these last two inhibitors might be proteinaceous in nature (Choi and Jung 1984, Singh *et al.* 1988, Stoimenova and Angelov 1995; Alexandre *et al.* 1997; Vijayan *et al.*, 2004; Bawm *et al.*, 2008 and Su-A Lee *et al.*, 2010).

Evidences collected from experiments designed in this section (physical properties) showed that *C. sativus* extract possesses the major antiviral effect; it is thermo stable, it has a wide pH range and it can resist storage for more than 85 days and its inhibitors are not precipitated by ammonium sulfate. This is in addition to its ability in giving a complete virus inhibition (100% inhibition) using the extracts incubated for 50 and 90 minutes storage with the virus *in vitro*, and at 90 minutes *in vivo* using pre-inoculation rubbing and its inhibitors not completely inactivated up to a dilution of $10^{-4}$. This is besides its nutritional and medicinal value. It has an antibacterial and antifungal effect (Luis and Rafael, 2011).

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