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# Occurrence of *Rose Colour Break Tobamovirus* in Egypt

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*Rose colour break virus* was detected in naturally infected rose growing in Orman Garden, for ornamental plants,Giza Governorate and it was found to be widely spread in rose fields. The virus was transmitted mechanically and by grafting (chipbudding) but not by aphids. It was successfully purified from infected *N tabacum* L. cv. White Burley leaves. Only one band of purified virus preparation was observed 3.5 cm bellow the meniscus of the density – gradient column. Infectivity test of the viral zone was found positive. The absorption spectrum of the purified virus isolate had a maximum at 262 nm and a minimum at 251 nm. The max/min and A260/280 ratios of the virus isolate were 1.83 and 2.81 respectively. The yield of purified -virus was 6.1 mg/100g of tobacco leaves. Electron micrographs of the purified virus isolate revealed the presence of rod – shaped particles of 310-320 nm. for length and 15 nm. for width. The polyclonal antibody raised against the virus under study had a virus –specific titer of 1:2000.

## INTRODUCTION

Rose (*Rosa hybrida)* is the most important crop in the floriculture industry and cut flower roses growing in Egypt. It is cultivated in many Governorates specially El-Giza. El-Kalubia and El-Minofia (the cultivated areas aren't devoted).

Viruses that infect rose belonging mainly to the *genera Ilarvirus* and *Nepovirus*. Among *Ilarviruses*. *Prunus* necrotic *ring sopt virus* (PNRSV) (Fulton, 1970), *Apple mosaic virus* (ApMV) (Fulton, 1952 and 1967). *Rose mosaic virus* (Fulton, 1967). *Tobacco streak virus* (TSV) (Converse and Bartlett, 1979). Among *Nepovirus*. *Arabis mosaic virus* (ArMV) and *Strawberry latent ring spot virus* (SLRSV). alone or in complexes with *Ilarviruses* infecting garden and greenhouse roses (Johnstone *et al.* 1995).

*Rose colour break virus* (RCBV) as well as other virus symptoms persist flower colour break in *Rosa spp.*- deformed flecked and streaked petals. First report in *Rosa spp.* from Essex, U.K. was reported by Hicks and Frost (1984). In Egypt, the present work is the first report on the occurrence of this disease, thus it was of great importance to have some details about this disease and its causal virus. In view of the importance of rose viruses, this work was conducted to study in details of RCBV that was identified after isolation from rose plants grown under local condition. Modified method of purification of the local isolate was applied and antiserum was produced. The induced antiserum against the virus isolate has also used to test the degree of susceptibility of the local and some imported cultivars to RCBV infection as well as the virus effect on some flower characters. Such obtained results would be useful in breeding for resistance against such viruses in the future.

In order to have some possible sources of resistance to RCBV able to used in breeding program, several cultivars of rose were tested. Enzyme linked immunosorbent assay (ELISA) technique was used successfully by several authors (Adams, 1978) and Clark *et al.*, 1976).

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## MATERIALS AND METHODS

### 1-Source of the virus isolate

Samples from naturally infected rose plants exhibiting characteristic rose colour break virus symptoms consisted of deformed flecked and streaked petals, and systemic mosaic or leaf mottle symptoms were collected from Orman Garden (Giza Governorate). Each sample was treated separately in the subsequent experiments.

### 2- Virus isolation

Infected leaves and petals of rose (Rosa multiflora Thumb. cv. Charlesdemills and Rosa setigera michx cv. Queen bee) were ground in a sterilized mortar. Phosphate buffer solution,0.1 M, pH 7 was used in preparing the crude sap (Fulton, 1952). The obtained sap was used in mechanical inoculation of healthy test plants,i.e. *Chenopodium amaranticolor*, *Ch. quinoa*, and *N. glutinosa*.

The inoculated seedlings were kept under observation in an insect proof greenhouse at 25°C. The single local lesion technique described by Kuhn (1964) was followed to obtain pure virus isolate from the local lesions developed on inoculated leaves of *Ch. amaranticolor*. The virus isolate was propagated *in N. tabacum* cv. White Burley which served as the source of virus infection for the subsequent experiments.

### 3-Virus identification

#### *1.Host range and diagnostic plants*

Ten seedlings from each of 24 plant species belonging to 6 families were mechanically inoculated, with the virus isolate. An equal number of healthy seedlings of the same age and cultivar were rubbed with buffer and kept to serve as control. The plants were examined daily up to 30 days for symptoms development. Plants showed no symptoms were checked by back inoculation to the indicator hosts (*Ch. amaranticolor* and/or *Ch. quinoa*).

#### *2-Variatal susceptibility*

Ten virus- free seedlings as indicated by ELISA test for each rose cultivar were inoculated by chip budding (3 chips/ seedling). Inoculated seedlings kept in the green- house one to three months after inoculation were checked for external symptoms and by indirect ELISA test for virus presence.

#### *3-Virus stability in crude sap*

Dilution end point (DEP), thermal inactivation point (TIP)and longevity *in vitro* (LIV) of the virus isolate were determined, according to the techniques described by Noordam (1973). *N. tabacum* cv. White Burley, was used as a source plant for the virus isolate, while *Ch. amaranticolor* and/or *Ch. quinoa* were used as indicator hosts. Each experiment was repeated twice.

#### *4-.Modes of transmission*

*a. Insect transmission*

The green peach aphid, *Myzus persicae* Sulz. was used for transmission of the virus isolate. Insects were reared

on cabbage seedlings under insect-proof cages. Virus- free aphids were starved for one hour, before being allowed to feed on virus infected tobacco leaves (*N. tabacum* cv. White Burley) for 5 min and then transferred to 10 healthy seedlings of tobacco plants (five aphids per seedling). After 24 hours, aphids were killed by spraying with a systemic insecticide (Basudin 600) with rate of 1ml/l.

For control, the same procedure was used except that the aphids were fed on virus free seedlings. Plants were examined daily for four weeks and the percentage of transmission was recorded.

*b. Graft transmission*

Bark tissue from young shoots of the infected rose (cv. Red moss) tree was side grafted on potted rose (cvs. Don Juan, James Mason and Queen bee) seedlings of free virus symptoms after testing by ELISA using RCBV, PNRSV, ArMV, ApMV and TMV antisera supplied by SANOFI (Sante Animale, Paris, France) for routine testing in Virus & Phytoplasma Res. Dept., In each trail, at least 10 seedling were used, inoculated rootstocks and scions were tied together with plastic strips. Three to four months after inoculation plant showing typical symptoms were checked by back inoculation into the indicator host plant and/or by ELISA.

**5-Serological reaction**

An antiserum against RCBV used for serological detection of the viral isolate, with ELISA test (Clark and Adams, 1977) was applied. The absorbance was measured at 405 nm. by ELISA reader (Dynatech MR 7000). Reading twice those of healthy controls averages were considered positive.

**6-Presence of RCBV in flower parts**

Flowers from infected rose cultivar Charlessdemills were collected during the growing season (March and April)) aseptically separated to their morphological organs (sepals, petals and sexual organs).Each organ was used to inoculate the indicator hosts, and also was tested by ELISA.

**7- Virus purification**

A combination of methods adopted for purification of *Rose mosaic Tobamovirus* by Gooding and Hebert (1967); Bruening *et al* (1976) and Hicks and Forst (1984) were used. One hundred grams of frozen (–80 °C.) systemically infected tobacco leaves (showing distinct symptoms harvested 4 weeks after virus inoculation) were homogenized for 5 min in Braun blender in 300 ml of 0.5 M potassium phosphate buffer, containing 0.005 ml ethylenediamine tetraacetic acid (EDTA), sodium sulfite (Na2 So 3) and 0.01 M diethyldithiocarbamate (DIECA), pH 8.5. The homogenate was filtered through two layers of cheesecloth and subjected to low speed centrifugation (LSC) at 8000 rpm. for 10 min. The supernatant was stirred with 25% chloroform plus 25% carbon tetrachloride for 30min. and the aqueous phase was separated by LSC at 8000 rpm for 10 min. at 4 °C. in a Beckman J-21C centrifuge using JA-20 rotor. The virus was precipitated from the supernatant with 6% polyethylene glycol (PEG 6000 ) and 0.3 M sodium chloride (NaCl) by stirring overnight at 4 °C. The pellets were collected by 20 min. centrifugation at 10.000 rpm at 4 °C. and suspended in 70 ml 0.01 M borate

and suspended in 70 ml 0.01 M borate buffer, pH 7.5,containing 0.1 % 2-merabtoethanol (2-ME) and left overnight at 4 °C. with slowly stirring, then centrifuged for 10 min. at 8000 rpm at 4 °C to eliminate any non-soluble materials followed by centrifugation for 90 min. at 30.000 rpm at 4 °C. in a Beckman L8-80 M Ultracentrifuge using rotor 80 Ti. Pellets were resuspened in 0.01 M borate buffer, pH 7.5, and then layered on top of 10-40 % sucrose gradient prepared in 0.01 M borate buffer, pH 7.5, and centrifuged for 2 hours at 28.000 rpm in a Beckman SW 60 rotor at 4 °C. Gradient columns were stored over night at 8 °C prior to use. Virus zones were collected with a bent tip hypodermal needle and syringe, diluted 1:1 with 0.001 M borate buffer, pH 7.5, without additives, then concentrated by centrifugation for 90 min. at 36.000 rpm. Final pellets were suspended in one ml of 0.001 M borate buffer and centrifuged at 3000 rpm for 10 min to remove insoluble material. Infectivity was tested on leaves of *Ch. quinoa* and/or *Ch. amaranticolor*.

### *1. U.V.absorption spectrum of the purified virus isolate*

The absorption spectrum of RBCV purified preparation was determined spectrophotometrically using Spectronic 2000. Virus concentration was calculated using the extension coefficient of 1.7 (Noordam, 1973).

### *2. Electoron microscopy*

Purified virus preparations were negatively stained with 2% uranyl acetate, mounted on formvar-coated grids and examined with a Philips 301 EM (Electon Microscope Unit, Specialized Hospital, Ain Shams University, Cairo, Egypt).

### 8-Production of antiserum specific to RCBV

Polyclonal antibodies, raised against RCBV were prepared according to the method described by Hampton *et al.* (1992). A New Zealand white rabbit was injected six times at weekly interval, with one mg of virus in 0.5 ml of buffer emulsified with an equal volume of Freund's complete adjuvant for the first and incomplete adjuvant for the five subsequent intramuscular and / or subcutaneous injections. Intravenous injections without adjuvant were given two times with one ml of purified virus preparation (1mg/ml) into the external marginal ear vein of the rabbit. Bleeding was collected 10 days after the last injection from the ear that was not used for injection. For titration of antibodies, the indirect ELISA test described by Lommel *et al.* (1982) was used.

## RESULTS AND DISCUSSION

### Isolation and Identification

Virus isolate was obtained from naturally infected rose plants grown at the Orman Garden in Giza Governorate. The isolate was obtained from a rose plant cvs. Don Juan, James mason and Queen bee with symptoms of rose color break, deformed fleck and streaked petals (Fig.1b and c). Symptoms appeared in leaves (Fig. 1a) were obtained from naturally infected rose plants grown at the Orman Garden in Giza showing mosaic and chlorotic lines. The symptoms were very similar to those illustrated by Farrar and Forst (1972). Subsequent work clearly proved that the virus under study is *Rose color break Tobamovirus*. These results were based mainly on symptomatology, host range, stability in sap, modes of

transmission, serology and electron microscopy.

**Host range and diagnostic plants**

Reactions of 20-plant species belnging to seven different families to virus infection are shown in Table (1) and Fig (1). The presence of the virus isolate in all tested plants was further confirmed biologically using the indicator host plant or serologically by ELISA. Such results were similar to those reported by Farrar and Forst (1972); Horst (1983); Hicks and Forst (1984) and Moury *et al.* (2001) .In earlier work on plant viruses, host range was used as an important criterion in diagnosis. Such information may still be important, or even crucial in certain circumstances (Matthews, 1991, 1993).

**3-2-Variatal susceptibility**

In order to have some possible sources of resistance to RCBV infection able to be used in breeding program, several rose cultivars were tested. Data in Table (4) show that the six rose cultivars tested were found susceptible to RCBV infection. The severity of symptoms was variable. Crimson Floorshow and Red Moss cultivars which have limited or medium resistance were showed mild symptoms and sometimes were symptomless. Whereas Charlesdemills and Queen Bee cultivars highly susceptible to virus infection and developed severe symptoms. A combination of factors leads to difference in severity of symptoms. In some cases, difference, in symptom severity has been attributable to individual viral gene (Fraser, 1992). Most resistant cultivars were developed in breeding program aimed at the introduction of a single resistance gene. Further-more, incomplete dominance may be a reflection of gene dosage or be due to environmental factors (Fisher and Rufty, 1993).

In this studied the susceptibility of Rose cultivars to RCBV infection was studied in artificial transmission by grafting (6 cultivars) with the virus. Obtained results indicated that, most cultivars expressed severe disease symptoms. Similar results were also recorded by (Farrar and Forst, 1979; Fulton, 1952; Hicks and Forst, 1984; Horst, 1983 and Ikin and Forst 1976).

**Virus transmission**

The different methods of virus transmission may be useful diagnostic criteria. Their usefulness may depend on the particular circumstances. Vegetative propagation which grafting is essentially a form of vegetative is essentially a form of vegetative propagation is an important horticultural practice, but it is unfortunately a very effective method for perpetuating and spreading viruses.

**Virus stability in crude sap**

Data in Table (2) indicated that RCBV isolate is inactivated by 10 min exposure to 95 ° C, at dilution $10^{-7}$ and by storage in crude sap for more than 700 days. Most data on persistence of infectivity in sap of the virus isolate are in line with RCBV (Hicks and Forst 1984).

Historically, stability of the virus as measured by infectivity (often in crude extracts) was an important criterion in attempting to establish groups of viruses.It is unfortunate that they are still sometimes invoked, since they have been shown to be far too variable to provide a sound basis for the identification of viruses or the placing of viruses into groups ( Francki, 1980 ).

**Table (1) : Host range and diagnostic host reaction of RBCV .**

| Host plant | % infection | Inculation (days) | Symptoms |
|---|---|---|---|
| **Family: Amaranthaceae** | | | |
| *Gompherena globosa* L. | 50 | 15 | L.C.L.&L.M. |
| **Familiy : Chenopodiaceae** | 100 | 6 | L.L. |
| *Chenopodium album* L. | 100 | 7 | C.S. |
| *C. amaranticolor* Cost & Reyn | 100 | 7 | C.S. |
| *C. quinoa* Willd. | | | |
| **Family: Compositeae** | 50 | 21 | L.M.&FCB |
| *Zinia elegans* | | | |
| **Family: Cucurbitaceae** | | | |
| *Cucumis sativus* L. | 40 | 12 | C.S.(in coty ledons) |
| cv. Beta Alfa. | | | |
| **Family: Fabaceae** | | | |
| *Pisum sativum* L. | - | - | - |
| cv. Little marvel | | | |
| *Vicia faba* L. | - | - | - |
| cv. Rebaya 40 | | | |
| **Family: Rosacae** | | | |
| *Rosa multiflora* Thumb | 80 | 60-70 | M.&D.P. |
| cvs. Charlesdemills | 70 | 60-70 | M.&FCB. |
| Baronneprevast | | | |
| *Rosa setigera* Michx. | 90 | 45 | S.M&D.P. |
| cv . Queen bee | | | |
| **Family: Solanaceae** | 60 | 12 | L.C.L. |
| *Datura stramonium* L. | | | |
| *Lycopersicon esculentum* Mill. | 40 | 15 | L.M. |
| cv. Ice. | | | |
| *Nicotiana. tabacum* L. | 100 | 8 | S.M. |
| cv. White Burley | 90 | 10 | M. |
| *N. debeneyi* Domin. | 100 | 7 | L.C.L.&M. |
| *N. gutlinoas* L. | 80 | 12-14 | M. |
| *N. rustica* L. | 70 | 15 | M. |
| *N. clevelandii.* | 100 | 8 | Cr. & B. |
| *N. tabacum* L. cv. samsun | | | |
| *Petunia hybrida* Vilm. | 60 | 18-21 | L.C.L.. Mo.& FCB |
| cv.Rose of Heaven | | | |

B.= Blisters, CS.= Chlorotic Spots, Cr. = Crinkling , D.P.= Deformed Petals, FCB.= Flower Color Break - ,= not react, L.C.L.= Local Chlorotic Lesion, L.M. = Light Mosaic, Mo.= Mottle, M.M. = Moderate Mosaic, N.= Necrosis, S.M. Severe Mosaic, V.B. = Vein Banding .

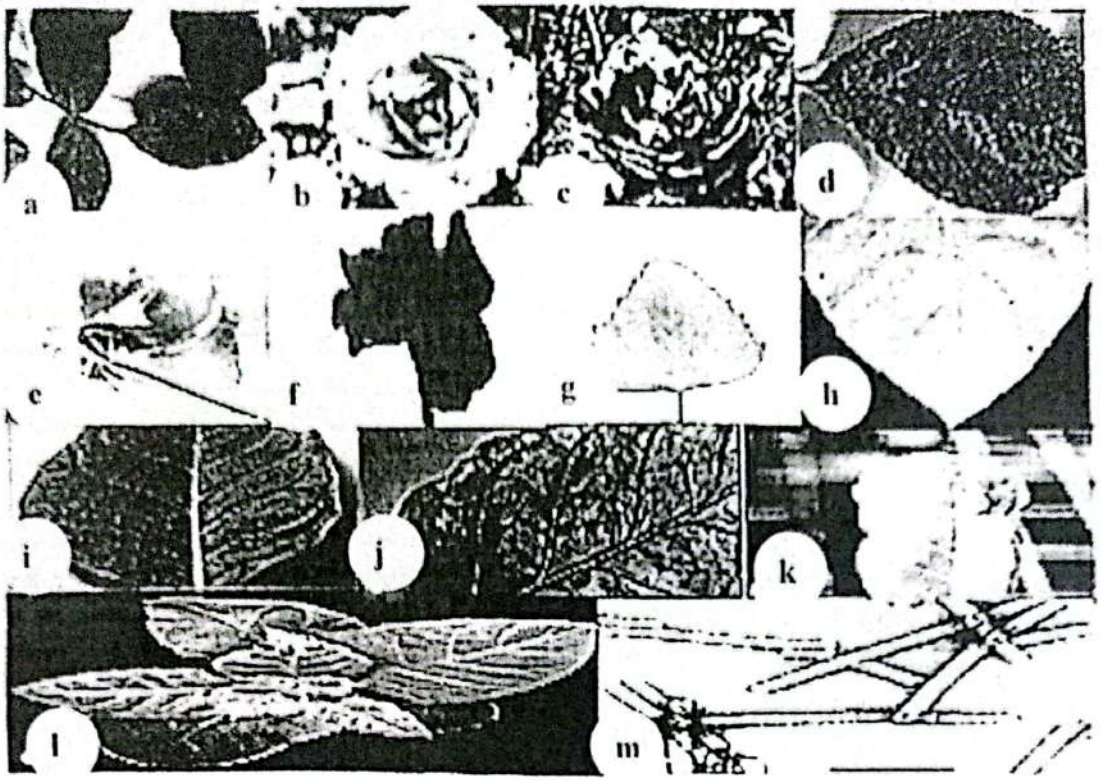


Fig (1): Symptoms of *Rose colour break virus* on naturally and artificially infected host plants:
a) Moderate mosaic symptoms appeared on rose leaves (*Rosa multiflora* Thumb. cv.Charesdemills) naturally infected with RCBV.
b) Severe colour break on petals of rose (*Rosa multiflora* Thumb. cv.Charesdemills) naturally infected by RCBV
c) Colour break symptom infected naturally by RCBV was appeared on petals of Queen bee rose (*Rosa setigera* Michx).
d) Severe mosaic on leaves of *Rosa setigera* Michx cv. Queen bee infected by grafting.
e) Light colour breaks on petals of pink rose (Rosa muliflora Thumb cv. Charesdemills). artificial infected by grafting.
f) Symptoms on leaves of *Rosa setigera* Michx cv. Queen bee showing deformation on petals (artificial infection by grafting).
g) Chlorotic local lesions appeared on *Ch. quinoa*.
h) *N. glutinosa* showing necrotic local lesion after 7 days of mechanically inoculation
i) Leaves of infected *N. debeneyi* developed chlorotic mottle.
j) Severe mosaic and vein banding appeared on *N. rustica* mechanically inoculated by RCBV.
k) Infected *N. tabacum* cv. White Burley showing mosaic.
l) Malformation on leaves of *N. Sumsun* mechanically inoculated by RCBV.
m) Electron micrographs of purified RCBV rod shaped virus particles stained with 2% uranyle acetate, X 80,000.

**Table (2): Stability of *Rose color break virus*.**

| Treatment | Average No. of L.L. on *C. amaranticolor* leaves | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TIP | Unheated | 75° C | 80° C | 85° C | 90° C | 92° C | 95° C | 96° C | 97° C |
| | 58 | 36 | 31 | 24 | 9 | 6 | 4 | 0 | 0 |
| DEP | Undiluted | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
| | 56 | 47 | 45 | 40 | 32 | 17 | 15 | 3 | 0 |
| LIV | 0 d. | 2 d. | 4 d. | 6 d. | 10 d. | 30 d. | 60 d. | 300d. | 700d. |
| | 116 | 78 | 69 | 54 | 42 | 36 | 24 | 20 | 12 |

**Table (3): Determination of RCBV antiserum titer.**

| ELISA reading at 405 nm. | | | | |
|---|---|---|---|---|
| Antiserum dilution | Dilution of tissue extract 1/5 | | Dilution of tissue extract 1/10 | |
| | Infected | Healthy | Infected | Healthy |
| 1/1 | Over | 0.247 | 1.524 | 0.214 |
| 1/2 | Over | 0.195 | 1.369 | 0.110 |
| 1/4 | 2.245 | 0.181 | 1.126 | 0.102 |
| 1/8 | 1.523 | 0.140 | 1.112 | 0.094 |
| 1/16 | 1.227 | 0.122 | 0.940 | 0.090 |
| 1/32 | 1.012 | 0.100 | 0.812 | 0.085 |
| 1/64 | 0.859 | 0.082 | 0.760 | 0.076 |
| 1/128 | 0.844 | 0.080 | 0.618 | 0.070 |
| 1/256 | 0.826 | 0.074 | 0.590 | 0.061 |
| 1/512 | 0.712 | 0.068 | 0.468 | 0.058 |
| 1/1024 | 0.692 | 0.065 | 0.348 | 0.050 |
| 1/2048 | 0.456 | 0.066 | 0.192 | 0.038 |
| 1/4096 | 0.110 | 0.052 | 0.090 | 0.033 |

**Table (4): Susceptibility of different Rose cultivars against artificial infection with RCBV.**

| Cultivars | I/T | % of transmission | D.S. |
|---|---|---|---|
| *Baronne Prevast | 7/10 | 70 | ++ |
| *Caresdemills | 8/10 | 80 | +++ |
| *Crimson Floorshow | 4/10 | 40 | + |
| *Don Juan | 8/10 | 80 | ++ |
| *Queen Bee | 9/10 | 90 | +++ |
| *Red Moss | 5/10 | 50 | + |

** I/T = No. of infected / No. of inoculated (tested) seedlings.
** D.S. = Disease severity (+,++ and +++).

***Virus transmission***

***a-Insect transmission***

In this work trails have failed to transmit RCBV isolate by *Myzus persicae* Sulz.This result also agree with those of Fulton (1967); Farrar and Forst (1972); Ikin and Forst (1976)and Converse and Bartlett (1979).

***b-Graft transmission***

Successful virus transmission obtained when side grafting was used. All viruses which are systemic in their hosts can be transmitted by grafting between susceptible and compatible plants (Matthews. 1993).

**Serological reaction**

Positive reaction obtained using specific antiserum against RCBV at a dilution of 1:1000 confirmed the identity of the virus under study. Serological tests. such as ELISA provide rapid and convenient methods for the identification and estimation of plant viruses (Matthews. 1991.1993)

**Presence of RCBV in flower parts**

Data in Table (5) indicate that RCBV is present in petals and sepals. Whereas. the sexual organs revealed negative reactions. It was found that petals contained high concentration of the virus than sepals during March and April. Awed. Maisa (1988) found that BCMV in Suisse Blanc bean cultivar and CPAMV in cowpea cv. Cream No.7 were present in all flower parts. Petals and pistils contaied higher concentrations of BCMV and CPAMV than sepals.

**Virus purification**

Data herein show that, the purified preparation was sedimented as a single opalescent band 3.5 cm below the meniscus of the sucrose density gradient column. Virus collected from the band had a maximum absorbance at 262 nm and a minimum at 251nm. (Fig.2). This band was found infectious when tested on *Ch. quinoa* leaves, numerous chlorotic local lesions were observed six days after inoculation. The max/min and A260/280 ratios of the virus isolate were 1.83 and 2.81 respectively. The virus obtained from the light-scattering zone was highly infectious to *Ch. quinoa* plants indicating that this method of purification did not affect the infectivity of the virus isolate. The yield of purified virus was 6.1 mg/100g of tobacco leaves. These results were similar to those reported by other investigators (Noordam, 1973 and Kicks & Forst 1984). Electron microscopy of purified virus preparation (Fig. 1m), indicated that relatively little extraneous material was present in these preparations. Full-length virus particles showed an average length of 310-320 nm. and 15 nm for width. This result suggested that the method of purification used was quite successful in purifying the virus under study. The previous results are in agreement with Hicks and Forst (1984).

**Determination of antiserum titer**

In the present work, polyclonal antibodies raised against RCBV had a titer 2048 as determined by indirect ELISA test (Table 3). However, one of the major goal, in this study is to produce an antiserum against RCBV used in ELISA to cover the continuous increasing needs for accurate and fast virus detection and for production of virus-free materials. When plant pathologists become involved in immunology, the goal, generally, is to generate an antibody probe, which will specifically identify a target antigen in any assay (Hampton *et al*., 1990)

**Table (5): Presence and concentration of RCBV in Rose flowers parts during the growing season (March and April) as determined by ELISA and expressed by O.D. 405 A°**

| Source of | DAS-ELISA (A° 405) | | |
|---|---|---|---|
| | Reaction | In March | In April |
| Sepals | + | 0.295 | 0.281 |
| Petals | + | 0.490 | 0.365 |
| Sexual organs | - | 0.063 | 0.047 |

+ = Positive reaction.
- = Negative reaction.
Negative control = 0.122.

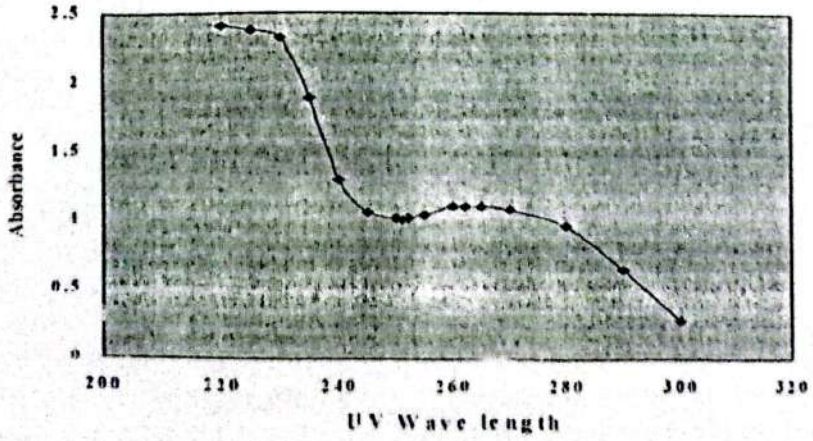


Fig (2): Ultra-Violet absorbance spectrum of the purified RCBV isolate .

## REFERENCES


Abo El-Ella, Amal, A. (1994). Studies on viral diseases of stone – fruits. MSc. Theses, Fac. Of Agric. Ain Shams University.

Awad , Maisa, A.(1988). Studies on some seed transmitted viruses in Leguminous plants . Ph.D. Thesis. Fac. Of Agric. Cairo University.

Bruening, G.; Beachy,R.N.; Scalla,R. and. Zailtlin,M. (1976). *In Vitro* and *in vivo* translation of the ribonucleic acid of a cowpea strain of *Tobacco mosaic virus*. Virology 71:498-517.

Clark, M. F. and Adams, A. N. (1977). Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. gen. Virology 34:475-483.

Converse, R. H., and Bartlett, A. B. (1979). Occurrence of viruses in some wild *Rubus* and *Rosa* species in Oregon. Plant Dis. Rep. 63: 441-444.

Farrar, E. H. and Forst. R.R. (1972). Isolation and Identification of *Rose colour break virus*. Plant Pathology 21: 97-101.

Fasr. R.S.S.(1992). The genetics of plant-virus interaction implications for plant breeding .Euphytica 63:175-185.

Fischer, D.B. and Rufty. R.c.(1993). Inheritance partial resistance to tobacco etch virus and tobacco vein mottling virus in burly tobacco cultivar sota 6505. Plant Dis. 77:662-666.

Fraser, R.S.S.(1992). The genetics of plant-virus interaction: implications for plant breeding .Euphytica 63:175-185.

Fischer. D.B. and Rufty. R.c.(1993). Inheritance partial resistance to tobacco etch virus and *Tobacco vein mottling virus* in burly Tobacco cultivar sota 6505. Plant Dis. 77:662-666.

Franki, R. I. B. (1980). Limited value of the thermal inactivation point, longevity *in vitro* and dilution end point as criteria for characterization, identification and classification of plant viruses. Intervirology 13: 91-98.

Fulton, W. R. (1952). Mechanical transmission and properties of *Rose mosaic virus*. Phytopathology 42: 413-416.

Fulton, W. R. (1967). Purification and serology of *Rose mosaic virus*. Phytopathology 57: 1197-1201.

Fulton, W. R. (1970). Prunus necrotic ring spot virus. No. 5 in: CMI/ AAB Descriptions of Plant Viruses. Commoaw. Mycol. Inst./ Assoc. Appl. Biol., Kew, Surrey, England.

Gooding, G. V. and Hebert,T.T. (1967). A simple technique for purification of *Tobacco mosaic virus* in large quantities. Phytopathology 57: 1285.

Hampton, R.O., Shukla, D.D.and Jordan , R.L. (1992). Comparative potyvirus host range , serology, and coat protein peptide profiles of white *Lupin mosaic virus*. Phytopathology 82: 566-571.

Hicks, R.G.T. and Forst, R. R. (1984). *Rose color break virus* in U.K. Plant pathology 33:581.

Horst,R.K..(1983). Compendium of Rose Diseases .9pp.

Ikin, R., and Forst, R. R. (1976). Virus diseases of roses. Vol. 2. Strawberry *Latent ringspot virus* R/ 1:2.6 or2* 1.6/38:S/s:Ne. Phytopathology 87:205-223.

Johnstone, G.R., Munro,D., Brown, G. S., and Skotland, C. B. (1995). Serological detection, occurrence and spread of ilarviruses in temperate fruit crops, hops and roses in Tasmania. Acta Hortic. 386:132-135.

Kuhn, C. W. (1964). Separation of cowpea virus mixture.. Phytopathology 54: 739-740.

Lommel, S.A., McCain, A. H. and Morris, T. J. (1982). Evaluation of indirect Enzyme –linked immunosorbent assay for the detection of plant viruses. Phytopathology 72:1018-1022.

Matthews,R.E.F. (1991).Plant Virology, 3 rd ed . Academic Press. Inc. 835 pp.

Matthews,R.E.F. (1993). Diagnosis of Plant Virus Diseases. chapter 6. CRC Press, Boca Raton Ann Arbor London Tokyo.374 pp.

Moury,B., L. Cardin, J.P. Onesto, T. Candresse ,and A. Poupet (2001). Survey of Prunus necrotic ring spot virus in rose and its variability in rose and Prunus spp. Phtopathology 91:84-91.

Noordam, D. ((1973). Identification of Plant Viruses Methods and Experiments. Centre Agricultural Publishing and Documentation, Wageningen. Netherlands 207pp.