International Journal of Education and Science Research
Review
E-ISSN 2348-6457
Volume-3 Issue-6 December- 2016 P-ISSN 2348-1817
www.ijesrr.org Email- editor@ijesrr.org

# IDENTIFICATION AND DIAGNOSIS OF VIRAL DISEASE OF *ALSTROEMERIA* IN H. P REGION

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**ABSTRACT:** Floriculture is fast emerging money spinning component in agriculture sector, growing at a modest rate throughout world and has become the potential agricultural activity especially for the developing countries. Alstroemeria is mainly cultured to produce cut flower and it becomes one of the most important flowers in the international market. Alstroemeriaplant were surveyed for viruses in Himachal Pradesh, India from 2013-2014. Five different virus isolates were collected from 57 Alstroemeriaplants grown on localities of Solan, Kangra and Shimla districts that were infected with some virus species. Identification and characterization of different virus isolates has been done on the basis of symtomatolgy, host range and transmission. Results revealed that most of the sampled plants were infected singly with the Lily symptomless virus (LSV).

**KEYWORDS:** LSV, Alstroemeria, Identification, Transmission

## INTRODUCTION

Alstroemeria**,** also known as the Lily-of-the-Incas, Peruvian Lily, or Inca Lily, has been grown in the United States since the 1970'smainly as a cut flower crop. During the last two decades, Alstroemeria has been one of the most commercially successful ornamental cut flowers in India, Japan, the Netherlands, the U.K., and the USA. Especially, characteristics like long vase-life, large color variety and allow energy required during cultivation have stimulated this success. The production of Alstroemeria flowers has been rapidly increasing in Europe and other parts of the world (Spence et al. 2000). Up to now, a huge number of cultivars have been released on the commercial market mainly as cut flowers, however, Alstroemeriaplants are also known as potand garden plants on a small scale (Van Schaik 1998, Park et al. 2010).In the year 2004, Alstroemeriacut flowers ranked in the10th position of the sales volume (http://www.gardenguides.com/86199-top-10-flowers.html). Recently, it has ranked in the 10$^{th}$position at the auction in Aalsmeer, The Netherlands in2008 (http://www.floraholland.com).

Alstroemeria hybrids were first introduced into India from Italy and grown at three places; Srinagar (Jammu and Kashmir), Chail (Himachal Pradesh) and Ooty (Tamilnadu) during 1999–2000. Nine hybrids (cv. Alladin, Amor, Capri, Cindrella, Pluto, Rosita, Serena, Tiara and Variety No.14) were procured from these places by the Institute of Himalayan Bioresource Technology (IHBT) in2000–2001. During cultivation, most of the plants exhibited Vein clearing or chlorotic streaks, curl stripe, necrotic spots and mosaic on the leaves suggesting that the plants were infected with a virus.

Lily symptomless virus (LSV; family, Genus Carlavirus, species) is the most prevalent virus infecting Alstroemeria(Van Zaayen 1995), and it has been reported in USA, Europe, Australia and Asia (Allen, 1969; Hsu et al., 1995; Niimi et al., 1999; Zheng, 2003 and Singh, 2005). It is also one of the most harmful viruses of that causes severe losses in terms of quantity as well as quality of bulb and flower production (Asjer, 2000). The host range of LSV is mostly distributed in genus Lilium, however, in some reported in Alstroemeria (Nicolaisen and Nielsen, 2001). The observed abnormalities such as growth reduction, smaller flowers and lower bulb yield can be caused by combined infection with LSV (Asjer, 2000) which threatens the yield and commercial production of Alstroemeria plants.LSV contains a filamentous viral particle, 640 nm in length and 17-18 nm in diameter. The genomic RNA of LSV is constituted of 8,394

nucleotides (excluding the poly (A) tail) and contains six open reading frames (ORFs) coding for proteins of Mr 220 kDa (1,948 aa), 25 kDa (228 aa), 12 kDa (106 aa), 7 kDa (64 aa), 32 kDa (291 aa) and 16 kDa (140 aa) from the 5' to 3' end respectively, composed of monopartite, single-stranded, plus sense RNA molecules. The ORF5 (7140-8015 nts) encodes a CP of 291 AA and genomic RNA of LSV is encapsidated by the single type of CP with a Mr of 32 kDa (Meme link et al., 1990; Choi and Ryu, 2003). The 3' terminal of carlavius group is linked with a poly (A) tail (Henderson et al., 1992; Fuji et al., 2002).

Management of plant virus and viroid diseases is a matter of vital importance and concern to the farmer, horticulturist, forester and gardener. It is well established that the virus and viroid diseases in different crops cause enormous losses in terms of quantity and quality of products. Therefore, in this study, several isolates of virus from Alstroemeria were characterized using symtomatology, host range, transmission and bio-physical properties.

## MATERIALS AND METHODS

### *Collection of Plant material and virus isolates*

Leaves from Alstroemeriaplants with possible virus symptoms were collected during surveys of HP, India Alstroemerianurseries. For each sample, records were taken of location, cultivar or species and disease severity.

Leaf samples were stored in insulated cool bags and later refrigerated at 4–5 degC for further evaluation. Alstroemeriaplants that were subsequently found to be infected by LSV were dug up and potted up in glasshouses for future work.

### *Maintenance of virus isolates*

The isolates were maintained by mechanical sap inoculation on healthy plants of Oriental Alstroemeriacv. Marco Polo under insect proof glasshouse.

### *Raising and maintenance of test plants*

0.01 M phosphate buffer (pH 7.0) was used as an extracting medium (1:1 w/v) during the present studies. All experiments were conducted on young, healthy and vigorously growing plants of Alstroemeriacv. Marco Polo. The plants of same age and size, raised in earthen pots were inoculated at four to five leaf stages.

The virus cultures were maintained on Alstroemeriacv. Pluto and on tobacco (Nicotianatabacum var. White Burley) grown in pots and kept in cool place (25-30°C) during summers. During winter, though overall growth of plants was impaired but the plants remained alive throughout the winter season. To ensure insect free conditions in the glasshouse, Malathion (0.1 per cent) was sprayed at 10-15 days interval regularly.

### *Preparation of inoculums*

Young leaves from the infected plants showing prominent symptoms of virus served as source of inoculums throughout the course of the present investigations. The leaves were washed and then dried in two folds of blotting paper. They were cut into small pieces of 1-2 cm with sterilized scissors and crushed in a sterilized mortar with pestle. To every gram of leaf material, one ml of 0.01 M.phosphate buffer (pH 7.0) was added. The sap, thus obtained, was strained through double layer muslin cloth in sterilized petriplates and the filtrate was used for mechanical sap inoculations. Carborundum (600 mesh) was invariably used as an abrasive for inoculations.

### *Identification and characterization*

Selected isolates of virus from Alstroemeriawere characterized using symtomatology, host range, transmission and bio-physical properties

## TRANSMISSION

***Mechanical Transmission***

The leaves were inoculated either by rubbing the sap with forefinger or by the "cotton swab method". Before applying the inoculums, e minute quantity or fine carborundum powder was dusted on the leaves of the plants so as to create sub lethal injuries on leaf for easy entry of the virus. The leaves, soon after inoculation, were thoroughly washed with distilled water so that excess of inoculums or inhibitor(s), if present, be avoided.

***Transmission through Bulbs***

Bulbs of Alstroemeriacv. Pluto showing prominent symptoms under field conditions were collected after drying up of the plant and such bulbs were grown in insect proof cages in glasshouse. The development of symptoms was observed after the emergence of leaves in the following season.

**Transmission by Aphids**

For aphid transmission tests, virus free colonies of most commonly encountered aphid species (MyzuspersicaeSulz, Aphis craccivora Koch, and Brevicorynebrassicae Linn.) in and around Alstroemeria experimental fields were examined for their possible role to act as vectors. A few adults of these species were collected from their healthy host plants and maintained on Alstroemeriacv. Pluto in isolation chambers of 3' x 3' x 3' size covered with nylon net of 80 mesh.

Non-viruliferousapterous forms of each aphid species were removed from their colonies with gentle tapping and by moist camel hairbrush to a separate petridish. These were then given one hour pre-acquisition Tasting. Sections of leaf tissues infested with 6-10 aphids were placed on the leaf of Alstroemeriaplants. For each isolate, ten plants were inoculated and kept in separate insect proof cages. After 24 hours of inoculation access, the plants were sprayed with 0.1 per cent Malathion to kill the aphids. These plants were observed for 2-3 weeks for symptom development under glasshouse conditions.

***Host range***

For studying the host range of the virus, plants of different species were grown under insect proof glasshouse conditions as described earlier. The plants of each species were inoculated with standard extract of the virus and were kept under observation for 5-6 weeks for development of symptoms. The plants, which did not show any apparent symptoms, were back inoculated onto original host (Alstroemeriacv. Pluto) to check for the symptomless infections.

***Biophysical properties***

Biophysical properties of the isolate under investigation such as thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (IN) were determined as per standard methods described by Noordam (1973). However, details of the procedures adopted are given here under:

***Thermal Inactivation Point (TIP)***

Studies on thermal inactivation point were conducted at different temperatures ranging from 30-70°C with 5°C interval. The sap was expressed in 0.01 M phosphate buffer (pH 7.0) by crushing about 100 g infected leaves (w/v 1:1). Homogenate was filtered and pipetted out in several thin walled test tubes each containing 2 ml sap. First tube in the series was heated for 10 min in thermostatically controlled water bath at the lowest temperature treatment. The temperature of sap was recorded by inserting a thermometer directly into the test tube and after treatment the tube was removed and cooled in running tap water. Thereafter, the water bath was maintained at the next higher temperature treatment. The process was continued till the last test tube was heated at the required temperature. Untreated infected sap served as control.

***Dilution End Point (DEP)***

To determine the dilution end point of the virus, infectious crude sap was prepared. One ml of crude sap was used to prepare a series of dilution in 0.01M phosphate buffer (pH 7.0). The dilutions tested were

1:10, 1:100, 1:1000 and 1:10,000. Inoculations were made starting from highest dilution to the lowest dilution and undiluted crude sap served as control. The inoculated plants were kept under observation for the development of symptoms.

***Longevity in vitro***

Crude sap of the virus infected leaves was prepared and distributed in two sterilized conical flasks. They were plugged with non-absorbent cotton and covered with butter paper to prevent any evaporation.

***i. At room temperature (22-26°C):*** One of the flasks was kept at room temperature (22-26°C) for subsequent daily inoculations. However, the first inoculation was made soon after the extraction of infectious sap.

***ii. At refrigeration temperature (4±1°C):*** Infectious sap far daily inoculations were stored in refrigerator at 4±1°C and all other steps were same as described above for room temperature.

## RESULT AND DISCUSSION

The viral nature of the incitation was further substantiated by serial sap transmission tests. Hamilton et al. (1981) has suggested certain guidelines for the identification and characterization of plant viruses. Thus, efforts were made during the course of present investigations to identify the isolate by studying as many parameters as could be possible, as suggested by the above mentioned workers. The criteria included symtomatology, host range, transmission and biophysical properties

## SYMTOMATOLOGY

A detailed account of symtomatology evoked by the isolates under glasshouse conditions are clearly shown in figure 1 and figure 2, infected plants showed mottling, mosaic, color breaking and leaf deformation.

Symptoms differed from cultivar to cultivar though some showed milder or fewer symptoms in comparison to the others. Symptoms varied from mild mottling of leaves to severe deformations in flowers. Initial symptoms appear in the form of mottling and interveinal chlorosis of leaves (Fig 1), and newly emerged leaves showing curling along with the mottle symptoms. Necrosis of the leaves is also of common occurrence (figure 2).


**Figure 1 Symptomatolgy of Lily symptomless carlavirus in Alstroemeria plant: (a) Severe mottling of leaves and (b) Interveinal chlorosis in severely infected plants**

**Figure 2 Necrotic leaves of Alstroemeria due to LSV infection**

Several infected plants exhibited early yellowing of the lower stem leaves resulting in early senescence of the entire plant. The virus was also observed to be reducing the bulb size significantly. Allen (1975) and Boontjes (1978) also observed similar symptoms in Lilium infected with LSV. Blake and Wilson (1996) also reported that flowers produced in the axils of lower stem leaves turned yellow in LSV infected plants.

## HOST RANGE

40 plant species belonging to ten different families were raised under insect proof glasshouse conditions. Ten plants of each species were inoculated mechanically with the standard inocula of isolate. Inoculated plants were observed for 4-6 weeks for the appearance of symptoms. The plants of the familiescucurbitaceae and leguminosae were inoculated at the first true leaf and cotyledonary stages, respectively. In case of other families, the plants were inoculated at 4-5 leaf stage.

Results of the host range experiments are tabulated in Table 1. A perusal of the data revealed that the isolate infected members of the families Liliaceae, Chenopodiaceae and Solanaceae only.

**Table 1: Host range of Alstroemeriavirus isolate**

| Family/ Host | Symptoms |
|---|---|
| **Amaranthaceae** | |
| AmaranthuscaudatusL | - |
| GomphrenaglobosaL | - |
| **Chenopodiaceae** | - |
| Chenopodium album L | LL.CR |
| C. amaranticolor(Plate 18, 19 and 20) | - |
| Coaste and Reyn | - |
| C. quinoa Wild | - |
| C. muraleL | - |
| **Compositae** | |
| Zinnia elegansJacq. | - |
| Callistephuschinensis | - |
| Calendula officinalis | - |
| **Cruciferae** | |
| Brassica oleracea var. capitataB. oleracea var. botrytis | |
| **Cucurbitacae** | |
| CucumissativusL. | |
| LuffaactangulaL. | |
| **Leguminosae** | |
| ViciafabaL. | |
| VignasinensisSavi. | |
| PisumsativumL. | |
| LathyrusodoratusL. | |
| Phaseolus vulgaris | |
| Medicago sativa L. | |
| **Liliaceae** | |

| | |
|---|---|
| Lilium sp.<br>Tulipa sp. (Plate 21, 22 and 23)<br>Alstroemeriasp. (Plate 24) | Mo.FD<br>PD<br>M |

| | |
|---|---|
| **Polemoniaceae**<br>Phlox drummondi | **-** |
| **Ranunculaceae**<br>Delphinium ajacis | |
| **Solanaceae**<br>Capsicum annuum<br>Daturastramonium L.<br>D. metel L.<br>D. metelvarfestuosa<br>Nicotianaglutinosa L.<br>N. tabacum var. White Burley (Plate 25)<br>N. xanthi (Plate 26)<br>N. rustica L.<br>N. occidentalis (Plate 27)<br>N.clevelandii (Plate 28)<br>Lycopersiconesculentum<br>PhysalisfloridanaRydb.<br>Solanumnigrum L.<br>S. khasianum Clarke<br>NicandraphysaloidesGaerth | <br>**-**<br>**-**<br>**-**<br>**-**<br>**-**<br>**NS**<br>**LNS**<br>**-**<br>**NL**<br>**LNS**<br>**-**<br>**-**<br>**-**<br>**-** |

**Acronyms**

LL = Local lesions
CR = Chlorotic rings
FD = Flower deformation
Mo = Mottling
LNS = Local necrotic spots
PD = Petal deformation
NL = Necrotic lesion
NS = Necrotic streak
M = Mosaic

After careful comparison of the reaction of these isolates on certain indicator plants, these were considered to be the same isolates as all the isolates infected similar indicator plants and also produced similar type of symptoms in the indicator plants used for studies. These studies indicate that Alstroemeriais infected by LSV in Himachal Pradesh**,** Fuji et al 2007 also shows that carlavirus can infect Alstroemeria.

## TRANSMISSION

***Mechanical transmission:*** Standard extract of the virus prepared from infected leaves of Alstroemeriacv Pluto showing prominent symptoms were inoculated on young healthy test plants. The inoculated plants were kept under observation for six weeks for the development of symptoms.

The results of the mechanical sap inoculation experiments (Table 2) revealed that the isolates were easily sap transmissible. Symptoms produced on the inoculated plants were similar to those observed under natural conditions.

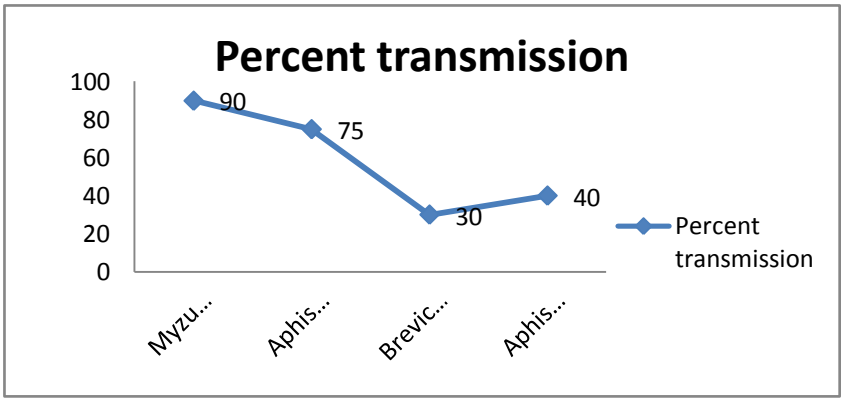
**Table: 2 Different Modes of Transmission of Alstroemeria Virus Isolate**

| Mode of transmission | Reaction |
|---|---|
| Bulbs | + |
| Sap | + |
| APHID SPECIES | |
| Myzuspersicae | + |
| Aphis craccivora | + |
| Brevicorynebrassicae | + |
| Aphis neri | + |

***Transmission through bulbs:*** Bulbs collected from the infected plants were planted under glasshouse to study the transmission of the causal virus through bulbs. The results (Table 2) revealed that the virus was transmissible through sap as the plants raised from the infected bulbs produced symptoms similar to those observed on the infected plants under field conditions.

***Transmission by aphids:*** Three aphid species viz. MyzuspersicaeSulz, Brevicorynebrassicae L., Aphis craccivora Koch. And Aphis nerithe mostly encountered aphid species in and around Alstroemeria crop under field conditions were tested for their transmission of the virus isolate.

A perusal of the data in graph 1 indicates that all three aphid species tested namely M. persicae, A. craccivora, B. brassicae A. neritransmitted the virus isolate. However, M. persicae was found to be the most efficient aphid vector as it resulted in a high rate of virus transmission (90 percent). The other two aphid species tested transmitted the virus but their rate of transmission was comparatively less.




**Graph 1: Transmission of Alstroemeria virus isolate by aphids**

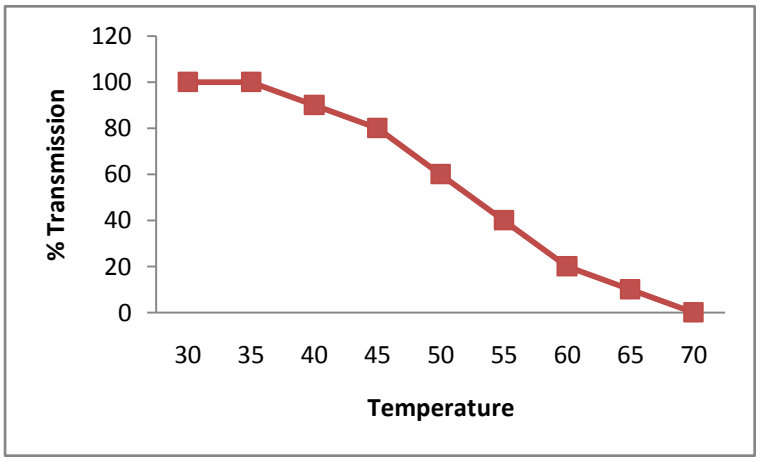
A detailed study of the transmission characteristics of the virus isolate revealed that the virus was transmissible through sap, bulb and aphid vectors (MyzuspersicaeSulz., Aphis craccivora Koch, Aphis neri and Brevicorynebrassicae L.). These results are in line with the findings of Brierley and Smith (1944a) and

Allen (1972) who reported LSV to be transmissible through sap. Transmission of LSV in a non-persistent manner by aphid vectors has been reported by a number of workers (Mowat and Stefanac, 1974; Derks and Asjes, 1975; Asjes et al, 1996)

## BIOPHYSICAL PROPERTIES

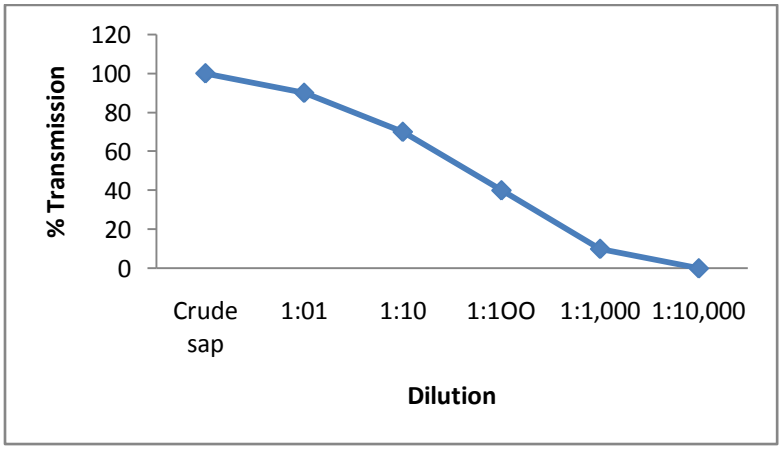
The biophysical properties such as thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) of the isolates were determined according to the standard procedures described. The results thus obtained are given hereunder:

***Thermal inactivation point (TIP):*** Data set out in graph 2 revealed that a temperature between 65°C and 70°C rendered the virus innocuous thereby indicating that the thermal inactivation point of the virus isolate ranged between 65°C and 70°C.




**Graph 2: Thermal inactivation point of Alstroemeriavirus isolate**

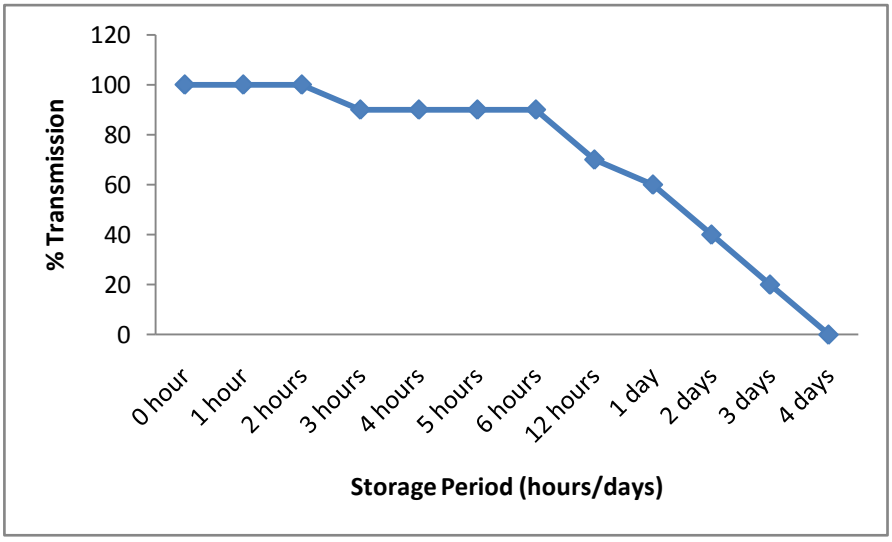
***Dilution end point (DEP):*** The dilution end point of the virus was determined by serial dilution of the infectious sap which was inoculated on the test plants. The results of the experiments are presented in graph 3. It is evident from the data that the dilution end point of the isolate ranged between 1:1,000 to 1:10,000.




**Graph 3: Dilution End Point Of Alstroemeriavirus Isolate**

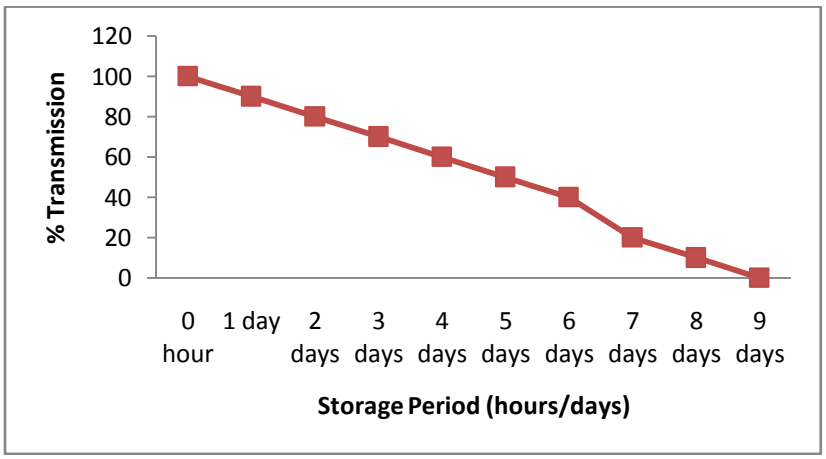
***Longevity in vitro (LIV):*** The ageing in vitro of the isolate was determined by keeping the infectious sap at room temperature (22-26°C) and under refrigeration (4±1°C) as per the procedure given in the chapter "Materials and Methods". The results of the experiments are presented in Graph 4 and 5.

The data presented in graph 4 revealed that the longevity in vitro of the isolate ranged from 3-4 days when stored at room temperature (22-26°C). The data also indicated that the virus started losing its infectivity merely two hours after storage at room temperature.




**Graph 4: Longevity In Vitro Of Alstroemeriavirus Isolate At Room Temperature**

The data set out in graph 5 indicates that the ageing of the virus isolate was delayed when infected sap was stored at freezing temperature (4±1°C). The virus isolate could retain its infectivity up to 8 days.




**Graph 5: Longevity In Vitro Of Alstroemeriavirus Isolate Under Refrigeration**

The virus isolate under present investigations had a thermal inactivation point of 65-70°C, dilution end point of 1:1,000: 10,000 and longevity in vitro of 3-4 days and 8-9 days at room temperature and under refrigeration, respectively. The biophysical properties recorded under present investigations are in argument with those reported for LSV by Allen (1972). Recently, European and Mediterranean Plant Protection Organization, Paris has also described similar biophysical properties of LSV in its Plant Quarahtine Database (EPPO, 2002).

## CONCLUSION

Results of the experiment conducted to study the host range of virus isolate revealed that the virus had a narrow host range restricted largely. These findings are in accordance with the report suggesting that LSV has a narrow host range often restricted (Mcwhorter and Allen 1964; Allen, 1972). Thus, this is concluded that the Alstroemeriais infected by LSV (Lily symptomless virus).

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