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Research Paper

Bio-efficacy of botanical extracts against Carnation Mottle Virus in carnations

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Abstract: Carnation Mottle Virus (CarMV) is an important viral agent infecting carnation and causing considerable loss to this crop. The research was conducted to study the effect of extracts of medicinal plants on CarMV. The infected plants were treated with extracts of *Asparagus sp., Ocimum spp.* and *Vitex sp.* prepared in different solvents by soxhlet apparatus. Laboratory bio-assay as well as studies under field conditions was done to see their effect on viral diseases. The presence of virus was evaluated using DAS-ELISA and scoring was done through microtitre plate reader at 405 nm wavelength. *Asparagus* extract in acetone (AA) & methanol (AM) appears to exhibit maximum inhibitory potential against CarMV, when added to the medium after autoclaving at 10 mg/l concentration. There was no loss of inhibitory potential due to heat when this extract was added before autoclaving the medium. *Asparagus* extract in methanol (AM) was also effective at 10 mg/l. in both cases i.e. addition before and after autoclaving. Extract of *Ocimum sanctum* in water (OSW) was also found effective; however, its effectiveness was confined only to, after autoclaving, additions of the extract to the medium. Field spray on the other hand could not eliminate the virus under test. However, inhibition to certain degree was recorded**.**
Keywords: Botanical extracts, Carnation Mottle Virus, carnation, *Asparagus sp., Ocimum spp.* And *Vitex sp.*

INTRODUCTION:

Carnation *(Dianthus caryophyllus)*, or clove pink, is one of the most important winter season flowers, excellent as a cut flower, highly suitable for bedding, pots & edging. Carnation Mottle Virus (CarMV), Carnation Latent Virus (CLV) & Carnation Necrotic Fleck Virus (CNFV) are most detrimental viruses affecting the crop, causing significant economic loss to the farmers. Carnation mottle virus (CarMV) is a plant pathogenic virus of the family *Tombusviridae*. CarMV leads from mild to severe infection in all types of carnations. This virus is responsible for poor quality of cut flowers in terms of size, split calyces and reduced vigor, in addition to lesser yield in terms of lateral

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shoots, total number of flowers and fresh weight (Lisa, 1995).

Virus diseases are mostly managed by controlling their vector by using insecticides. Indiscriminate use of chemical insecticides leads to negative impact on environment, human health and soil fertility as well as these are not economical. An alternative of these chemical pesticides is utilization of bio-control agents such as botanical extracts (Verma and Dwivedi 1983; Zhang *et al.* 1990; Manickam and Rajappan 1998; Madhusudhan *et al.* 2005; Hamidson *et al.* 2018). The purpose of this study was to determine inhibitory effect of botanical extracts prepared in water and other solvents against CarMV.

MATERIALS AND METHODS:

The research work was done in Laboratory of Department of Biotechnology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). Virus infected carnation plants were selected on the basis of visual symptoms that ranged from mild mottling to mosaic and curling symptoms. The plants were further preserved in the glasshouse for the research work. Identification of the virus before and after attempts of retrieving virus free plants was done through serological indexing method (DAS- ELISA) in the laboratory under control conditions. Scoring was done visually and in microtitre plate reader at 405 nm wavelength. The carnation plants found positive for CarMV were maintained for further experimental purpose. After serological testing, the plants found positive for CarMV were used for *in-vitro* multiplication. Nodal segments were surface sterilized using standard protocol & cultured on modified MS medium 1962**.**

Botanical extracts were prepared from leaves of *Asparagus adscendense* Roxb. *Vitex negundo* Linn. and *Ocimum spp. (O. cannum and O. sanctum* Linn.) by soxhlet extraction method starting from least polar to highly polar solvent i.e. acetone, methanol and finally water. The extracts so obtained were dried in oven at 25^0C ±1 and then used for further experiments. The infected plants maintained in field were sprayed at various concentrations of the extracts

The extracts were added to the medium at varying concentrations i.e. 3, 5, 10, 15 and 20 mg/l. These were added to medium at two levels of media preparation i.e. before and after autoclaving the medium. The extract was filtered through sintered glass crucible assembly under laminar air flow and then added in the media after autoclave.

RESULTS:

Effect of spray on virus elimination: *Asparagus acetone* extract (AA) when sprayed for one week gave significant inhibitory results (Mean OD values 0.992) at higher concentration of 500 mg/l (Table 1). On prolonging the spraying period to second week, reduction in virus concentration was observed even at lower concentrations of 100 mg/l and 300 mg/l (mean OD value 0.924 and 0.933). Similar trend of virus inhibition was observed with *Asparagus* water (AW) extract at lower concentrations of 3, 5 and 7 mg/l (mean OD values 0.940, 0.928 and 0.894 respectively), when sprayed for second week. Higher concentrations of some extracts had adverse effect on plant health in some cases.

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Table 1: Effect of field sprays on elimination/inhibition of CarMV in carnation

Treatment	Plant Extract	Concentrations R* mg/l	Mean OD Values at 405nm	
			After one Week	After two weeks
T1	*Asparagus* (Acetone)	100 300 500	1.080 1.225 0.992	0.924 0.933 0.921
T2	*Asparagus* (methanol)	100 300 500	1.225 1.221 1.026	0.840 0.820 0.812
T3	*Asparagus* (water)	3 5 7	1.385 1.383 1.323	0.940 0.928 0.894
T4	*Ocimum Cannum* (acetone)	100 300 500	1.085 0.945 0.925	1.084 0.934 0.833
T5	*Ocimum cannum* (methanol)	3 5 7	1.084 1.154 1.024	0.945 0.543 0.524
T6	*Vitex* (acetone)	3 5 7	0.833 0.822 0.564	0.820 0.784 0.525
T7	*Vitex* (methanol)	100 300 500	1.093 0.884 0.995	0.798 0.883 0.980
T8	*Vitex* (water)	100 300 500	0.834 0.724 0.593	0.622 0.612 0.581
T9	*Ocimum sanctum* (methanol)	100 300 500	1.124 0.842 0.921	1.024 0.740 0.884
T10	Control (+ve)	0	1.548	1.496
T11	Control (-ve)	0	0.213	0.235

* R is residue after drying

O. cannum in acetone (OCA) and Methanol (OCM) at 3, 5, 7 and 100, 300, 500 mg/l concentrations respectively, exhibited inhibitory potential that increased on prolonging the applications for second week. Similarly in case of *Vitex* water (VW) and *Vitex* methanol (VM) as well as *O. sanctum* methanol (OSM), when the extract was applied for second week, results were very encouraging as enhanced inhibitory potential.

Effect of in *vitro* addition of extract on virus elimination:-

The *in-vitro* multiplications medium was first standardized for carnations micro propagation in a separate experiment. MS medium supplemented with BA 1.0mg/l and

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NAA 0.5 mg/l was found to be the best for establishment and rapid multiplication of carnations. The plant extract was added to this medium before as well as after autoclaving it. The data presented in Table-2, showed that Complete virus inhibition was obtained with AA extract at both the levels of extract addition. The mean O.D values were less than twice the mean OD values for negative control (0.277 and 0.285 respectively). Similar observations were recorded with AM extract at 3,5 and 7 mg/l concentration (mean OD 0.689, 0.649, 0.639), where reduction in virus concentration was observed and complete inhibition was recorded at 10 mg/l when extract was added after autoclaving (mean OD 0.488).

Vitex extract in methanol gave inhibitory response at all the four concentrations used in the experiment as observed through ELISA plate reader (mean OD value 0.721, 0.721, 0.710, 0.692 and 0.642, 0.632, 0.592, 0.598). OSA (*Ocimum sanctum* acetone) as well as OSW (*Ocimum sanctum* water) at 3, 5, 7 and 5, 10, 15, 20mg/l also exhibited virus inhibition. The results were almost same when OSA extract was added before autoclaving (mean OD values 0.712, 0.594, 0.586 and 0.576) and complete virus inhibitions was also achieved with acetone extract (OSA) only at concentrations of 10 mg/L, when added after autoclaving.

DISCUSSIONS:

The endogenously occurring substances of plant origin have been reported to induce systemic/localized resistance in several susceptible hosts and have been used for protecting the crops against virus infection (Verma and Barnwal 1989; Bhardwaj, S.V., S.J.Roy., Manisha and Anil Handa 2006). Many of these virus inhibitors are ribosome inactivating proteins (RIPs) (Barbieri and Stripe 1982). The resistance induced by such biotic moieties have been termed as acquired/induced resistance that may be localized or systemic in nature. Keeping in view both the aspects of induced and acquired resistance through plant abstracts, present studies were undertaken.

The inhibitory substances are present in different parts of the plants (Verma, 1986) and get released during extraction. In present studies AA (*Asparagus* acetone) and AM (*Asparagus* methanol) extracts at 300 and 500 mg/l were found to have substantial amount of inhibitory substances when sprayed for one week. Inhibition was also recorded with spray of AW (*Asparagus* water) but only when spray schedule was extended to second week. During present studies, it was observed that increase in the time of exposure to plant extracts caused decrease in virus concentration of infected plants.

Similarly, AM extract was also found effective in inhibiting CarMV. Other extracts that could inhibit CarMV were OSA (*Ocimum sanctum* acetone) and VM (*Vitex* methanol), when added to medium before autoclaving indicating loss of inhibitory potential at higher temperature. However, OSA and VM virus inactivation may be a consequence of virus precipitation hydrolysis, completing or chemical modifications (Verma and Parsad 1992). The virus inhibition may also take place due to altered metabolic environment within a cell leading to repressed viral expression or creation of secondary environment to indirectly influence virus infection and/or multiplications and/or spread in resistant tissues (Verma 1982).

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Table 2: Effect of *in-vitro* addition of extracts on CarMV elimination/inhibition in carnations

Treatment	Plant Extract	Concentrations R* mg/l	Mean OD Values at 405nm	
			After autoclaving	Before autoclaving
T1	*Asparagus* (Acetone)	3	0.798	0.810
		5	0.718	0.785
		7	0.697	0.745
		10	0.413	0.520
T2	*Asparagus* (methanol)	3	0.689	0.630
		5	0.649	0.625
		7	0.639	0.587
		10	0.488	0.507
T3	*Vitex* (methanol)	3	0.721	0.642
		5	0.721	0.632
		7	0.710	0.592
		10	0.692	0.598
T4	*Ocimum sanctum* (acetone)	3	0.710	0.712
		5	0.698	0.594
		7	0.597	0.586
		10	0.423	0.576
T5	*Ocimum sanctum* (water)	5	0.618	0.684
		10	0.576	0.740
		15	0.566	0.620
		20	0.564	0.597
T6	Control (+ve	0	0.833	0.843
T7	Control (-ve		0.277	0.285

*R is residue after drying

CONCLUSIONS:

Results of the study showed that application of medicinal plants extracts effectively reduced CarMV concentration in Carnations. Field sprays could only inhibit the virus to certain degree, whereas *in vitro* studies, complete virus inhibition was recorded when the extracts were added to medium before and after autoclaving under laboratory conditions. AA extract at 10 mg/l was most effective in inhibiting virus at both the levels of extract. Similar inhibitory potential was recorded with AM at 10 mg/l. The extracts of OSW and VM were also found effective. However, their effectiveness was confined only to after autoclaving additions to medium.

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