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RESEARCH ARTICLE

Serological and molecular evidences of cucumber mosaic virus infection in *Valeriana jatamansi* in Himachal Pradesh, India

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Abstract

Indian valerian *(Valeriana jatamansi)* is a highly valuable perennial herb that grows at higher altitudes of the Indian Himalayan Region. Essential oil and plant extracts derived from roots and rhizomes are used in flavoring, aromatic and pharmaceutical industries. These underground parts contain bioactive compounds such as valepotriates, including valtrate, didrovaltrate, acevaltrate, valtroxal, valerenic acid and badrinol that are vital for formulation of sedative and anxiolytic drugs. However, several plants were found to exhibit symptoms that are typical of plant virus infection. Infection of virus on plants leads to a severe reduction in plant vigour and essential oil content. Characteristic symptoms include chlorotic or yellow mosaic and stunted plant growth. Foliar samples of diseased plants were taken, DAS-ELISA and reverse transcription polymerase chain reaction (RT-PCR) analyses were used to detect the viruses in the diseased plants. The results revealed that cucumber mosaic virus (CMV) was present in all the plants showing the symptoms of virus infection.

Keywords: CMV; Cucumovirus; DAS-ELISA; RT-PCR; serological; *Valeriana jatamansi*

Introduction

Valeriana jatamansi Jones Syn. *Valeriana wallichii* DC (English name- Indian Valerian, Trade name- Tagar) is a small herb with a short often strong-smelling rootstock. The plant belonging to the family Caprifoliaceae is widely distributed in the temperate regions of the Himalayas, from Kashmir to Bhutan, at altitudes ranging from 1200 to 4000 m (1). The herb mostly grows in moist, rocky, grassy slopes and on stones with coarse sandy loam soil (2). Its therapeutic effectiveness is attributed to a variety of biomolecules like essential oils and valepotriates synthesized and accumulated in its rhizomes and roots of the plant. Traditionally, *V. jatamansi* has been used to treat epilepsy, asthma, leprosy, cholera, mental disorders, insomnia, emotional problems, hysterical fits and skin conditions. The roots have aphrodisiac, antiperiodic, hypnotic and laxative properties. In cases of snakebite and scorpion sting, the rhizomes were administered in conjunction with other medications (3-6).

Renowned for its medicinal properties, it has been used for its sedative and tranquilizing, neurostimulant and antidepressant effects. The rhizomes and roots contain valepotriates like valtrate, didrovaltrate, acevaltrate, valtroxal, valerenic acid and badrinol (3, 7, 8). The sedative and tranquilizing activity of valerian plants are due to these valepotriates present in underground parts of *V. jatamansi*. The plant exhibits diverse therapeutic properties including anticancer, antifungal, antibacterial (9), antiprotozoal, anti-inflammatory and neuroprotective effects (10-12). Major compounds identified in essential oil include patchouli alcohol (0.4 %-63.7 %), maaliol (2.9 %-53.8 %), seychellene (4.1 %-27.4 %) and β-gurjunene (3.0 %-20.8 %) (13). Due to the presence of essential oils and valepotriates, the plant holds significant importance in the perfumery and pharmaceutical industries. The plant also demonstrates antimicrobial properties, inhibiting the growth of bacteria like *Staphylococcus aureus* and *Bacillus subtilis* (14).

These antimicrobial properties are closely linked to the composition of essential oils present in the rhizomes. Since *V. jatamansi* is a perennial plant and conventionally propagated by vegetative means, any virus in the planting material results in the spread of virus to a large extent. The composition and relative proportion of essential oil content in rhizomes is notably reduced by infection of virus. Cucumber mosaic virus (CMV), belonging to genus Cucumovirus and family Bromoviridae is one of the widely distributed plant viruses having a broad host range. CMV infection on valerian plant is responsible for causing the significant reduction in plant vigour which could further lead to reduction in yield of essential oil. Virus-infected *Valeriana* plants exhibited symptoms like interveinal chlorosis and mosaic symptoms (15, 16). CMV, alfalfa mosaic virus (AMV) and potato virus Y were found infecting essential oil-bearing and medicinal plants including *V. officinalis* in Bulgaria. AMV caused bright yellow spots on leaf lamina while CMV caused pale green mosaic spots on leaf lamina (17, 18). To identify the viruses infecting *V. jatamansi* plants, serological and PCR-based techniques were used because of their high sensitivity and high specificity for

detection (19-22).

Symptom observation is the common method to diagnose viral diseases in field. The various symptoms observed include chlorosis, yellow mosaic and stunted plant growth in valerian plants infected by CMV and AMV in Bulgaria (17). However, mixed infection or latent infection of some viruses in host show variable symptoms and make it difficult to detect the specific virus. Therefore, serological and molecular techniques are used as they are simple, fast, specific, cost effective and highly sensitive to detect plant viruses.

The presence of a virus on commercial plantation of medicinal plants demands regular monitoring and identification of virus, followed by the development of disease management strategies. During extensive surveys conducted at Medicinal and Aromatic Plants Research Farm, Shilly, Solan (HP) valerian plants were found to exhibit severe symptoms of mosaic, leaf deformation accompanied by stunted plant growth. Considering the uses and growing demand of *V. jatamansi* in industries, the present study was carried out with an objective to detect and identify the virus infecting *Valeriana* plants by using serological and PCR-based detection methods.

Materials and Methods

The present study was carried out in Plant Virology Laboratory, Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP). Survey was conducted at Medicinal and Aromatic Plants Research Farm, Shilly, Solan (HP) during the spring season (April–May) of 2024 and 2025 to identify plants showing the symptoms of virus infection. Around 30 symptomatic leaf samples representing two lines i.e. U/F/9 and U/B/45, along with the healthy-looking plants (U/F/47, D/F/19 and D/B/15), were collected in small plastic bags, immediately kept in an ice box, taken to the laboratory and kept at 4 °C until further processing.

Serological detection

The plants were initially diagnosed on the basis of visual symptoms developed on the diseased plants. The leaves from symptomatic and asymptomatic plants were subjected to alkaline phosphatase-based direct double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for serological detection with a few modifications in conventional procedure (23). The assay was performed by using Nunc Maxisorp F96 polystyrene microplate and antisera against CMV supplied by Bioreba, Switzerland. 200 µL of diluted primary antibody with coating buffer (1:1000) against CMV was added to each well with two replications for each sample. The plate was covered with a foil and incubated at 30 °C for 4 hr in a humid box. After completion of the incubation period, the wells were washed thrice with PBS-Tween. Test samples were crushed in extraction buffer and 200 µL of aliquot was added to each well along with the positive and negative controls and incubated overnight at 4 °C—6 °C. Following another washing, alkaline phosphatase conjugate antibody (1:1000) was added to each well and again incubated for 5 hr at 30 °C. After washing the plate, freshly prepared p-nitrophenyl phosphate (pNPP) substrate (200 µL per well) was added and the plate was incubated in a dark and humid box at room temperature. The reaction was stopped by adding 50 µL of 3 M NaOH per well once the yellow colour appeared after 60-90 min. The final OD values were obtained by using microplate reader (Bio-Rad, iMark Microplate Reader) at a wavelength of 405 nm. The samples having OD value more than twice of the negative control were considered positive for tested virus. Leaf samples showing positive results in DAS-ELISA were further subjected to molecular detection by RT-PCR.

RNA isolation and PCR amplification

For molecular diagnosis, total RNA of the symptomatic and healthy valerian plant was extracted by using TRIzol™ Reagent (Invitrogen, Thermo Fisher Scientific) following the manufacturer's protocol. Total RNA isolated from 500 mg of leaf sample was stored at -20 °C for further use. The isolated RNA was converted to cDNA by the process of reverse transcription using cDNA synthesis kit (Applied Biosciences) according to manufacturer's instructions. cDNA synthesis was performed in 25 µL reaction mixture containing 1 µg of RNA template, 5 µL of 10× RT buffer, 2 µL of 25× dNTP mix, 5 µL of 10× RT random primer, 2.5 µL of MultiScribe reverse transcriptase and 8 µL of nuclease-free water. The reaction was carried out in three cycles, each at 25 °C for 10 min, cDNA synthesis at 35 °C for 120 min and enzyme inactivation at 85 °C for 5 min using the Applied Biosystems PCR system (20, 22).

Molecular detection

Molecular detection of CMV was performed by using RT-PCR with target RNA of CMV by using coat protein-specific primers CumvdpFor and CumvdpRev (Forward: GGCTGCAGTGGTCTCCTT; Reverse: GAGTCGAGTCATGGACAAATC) to amplify the expected fragment size of 950 bp. The synthesized cDNA was further subjected to PCR amplification in 25 µL of reaction volume containing 2.5 µL of 10× PCR reaction buffer, 1.5 µL of 0.2 mM dNTPs, 1 µL of $MgCl_2$, 1 µL of each forward and reverse primer, 0.3 U Taq polymerase, 2 µL cDNA as template and 10 µL of nuclease-free water. PCR conditions for amplification were set at 94 °C for 5 min (1 cycle); 94 °C for 1 min, 46 °C for 1 min, 72 °C for 1 min (35 cycles); final extension for 5 min at 72 °C (22). Amplified products were analysed by mixing PCR product with an equal volume of loading dye and analysed on 1.2 % agarose gel. A 100 bp DNA molecular marker was used to estimate the size of PCR amplicon.

Results and Discussion

Thirty samples of *V. jatamansi* from Shilly farm were analysed for the presence of virus infection. Out of five lines screened for virus presence, CMV was detected in plant samples belonging to the two lines, i.e., U/F/9 and U/B/45.

CMV infection established in the plants was responsible for mosaic, yellowing, deformation and curling of upper leaf and overall stunting of the infected plant (Fig. 1 & 2). Severe infection in plants leads to reduced vigour and growth, resulting in low yield of rhizomes.

The collected samples showing the typical symptoms of virus infection and the asymptomatic plants were subjected to serological detection by using DAS-ELISA to confirm the presence of virus in symptomatic plants. DAS-ELISA results showed that out of the five different lines, two samples i.e. S2 (OD = 1.139) and S4 (OD = 0.983) representing line U/F/9 and U/B/45 respectively were tested positive for the presence of CMV. However, three samples i.e. S1 (OD = 0.487), S3 (OD = 0.365) and S5 (OD = 0.432) representing lines U/F/47, D/F/19 and D/B/15 respectively were tested negative when compared to negative control showing OD value of 0.348 (Table 1). The colour reaction

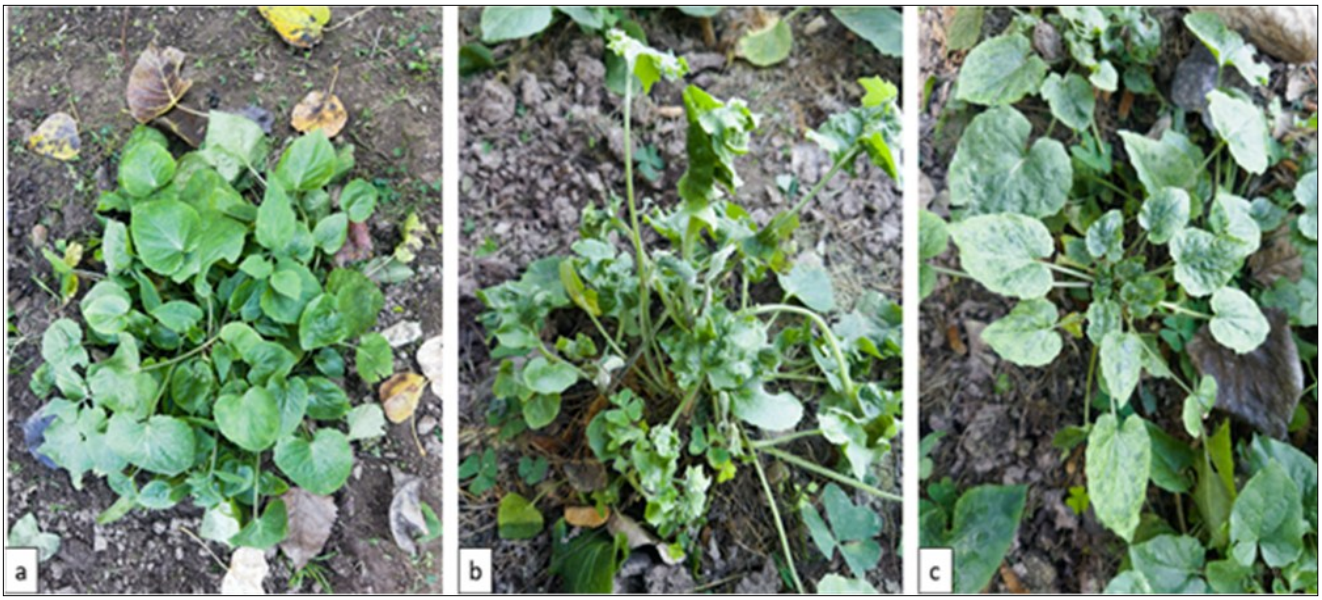

Fig. 1. CMV infected plants showing leaf deformation and yellowing. (a) Healthy plant; (b, c) infected plants with leaf deformation and yellowing symptoms.

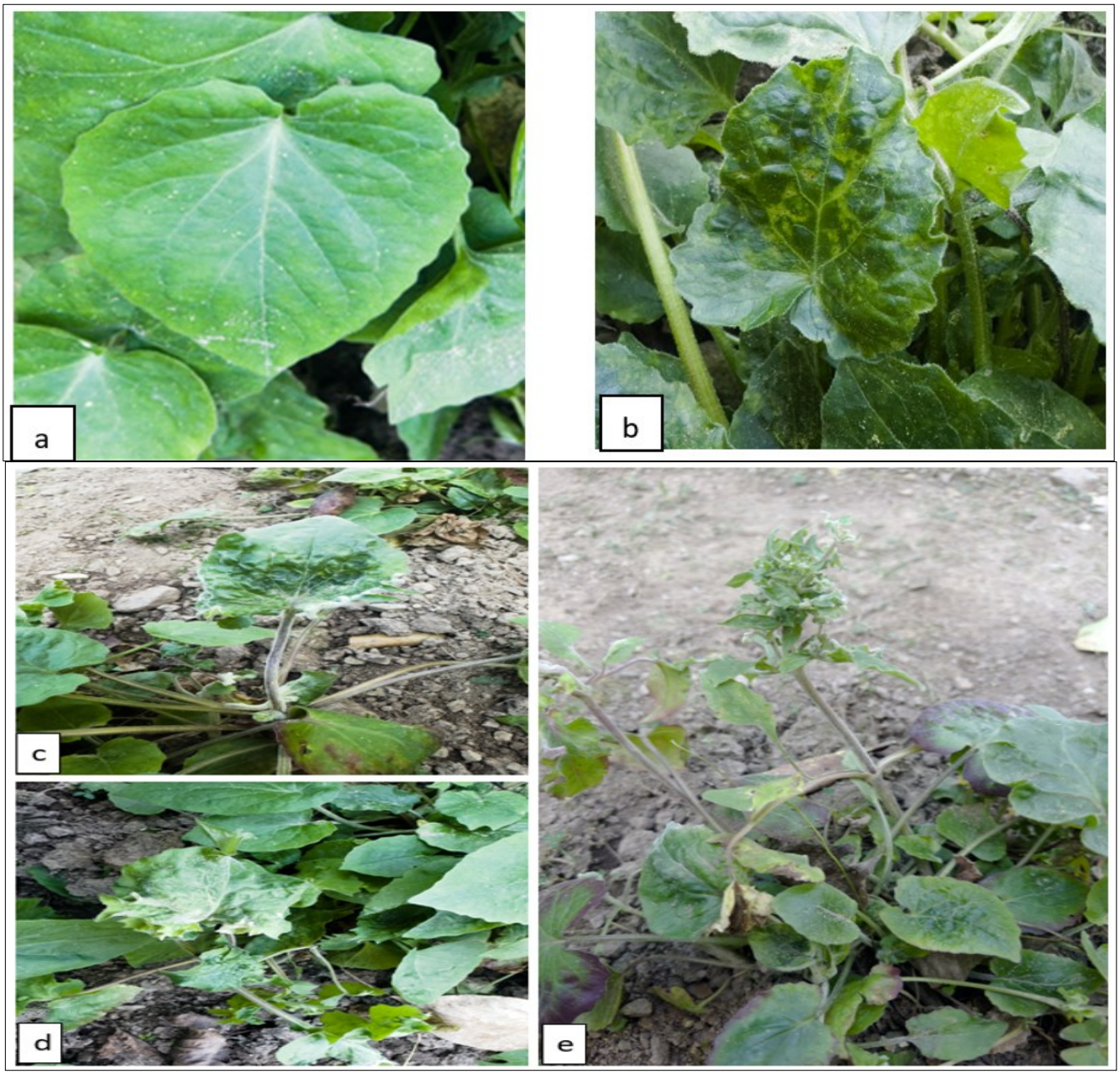

Fig. 2. Symptoms on leaves of infected plant showing mosaic and curling. (a) Leaf of healthy plant ;(b) mosaic and yellowing on leaves; (c, d) curling of leaves (e) leaf distortion.

in S2 and S4, confirmed the presence of CMV infection in tested samples, while the colourless reaction in samples S1, S3 and S5 confirmed the absence of CMV in asymptomatic plants tested (Fig. 3).

The sap extracted from leaves of samples that tested positive in the ELISA assay was mechanically inoculated onto *Nicotiana tabacum* plants to confirm the presence of the pathogen. The symptoms on inoculated plants started appearing after 5-7 days post inoculation. The mechanically infected plants of *Nicotiana* were maintained in glass house.

Molecular detection by using RT-PCR based detection of virus using total RNA from infected plant samples and CMV coat protein gene-specific primer resulted in expected amplicon of 950 bp from all the symptomatic leaves collected from field along with the mechanically inoculated tobacco plant (Fig. 4). However, no band was present in healthy plant samples. The positive amplification of expected size band indicated the presence of CMV in symptomatic valerian plants of both the lines (U/F/9 and U/B/45).

CMV is undoubtedly a serious and economically very important virus infecting plants. The severe infection in the plants ultimately results in the low yield of rhizomes. Also, due to the vegetative propagation of valerian plants, presence of any virus in mother plants could result in the widespread transmission of virus to wider geographical areas. Due to its largest host range and non-persistent transmission by more than 86 aphid species, CMV has become a serious threat to various crops (24, 25).

Serological detection using DAS-ELISA revealed the presence of the virus in several essential oil-bearing and medicinal plants, including *Valeriana officinalis*, in Bulgaria (17). For molecular detection, reverse transcription and PCR-based techniques were employed and identification of three cucumoviruses infecting various plants by using the gene-specific primer (CPTALL-3 and CPTALL-5) that flank the coat protein gene and amplify a DNA fragment of approximately 938-966 bp was made (26). Therefore, this set of gene-specific primer can be useful for amplification of virus coat protein gene of members of cucumoviruses. The characterization of CMV isolate from *V. jatamansi* in Himachal Pradesh was first time made during the year 2012-2013 from three different locations (27). Serological detection confirmed the presence of CMV in valerian plants and RT-PCR based molecular detection further supported the findings. The comparative evaluation of serological and molecular detection revealed that RT-PCR is more specific and sensitive technique for detection, because the technique can also detect the presence of virus in latent state or if present in a very low concentration. On the other hand, serological detection can be useful for large scale detection of planting material for mass propagation as more number of samples can be processed in lesser time. Hence, based on the serological and molecular analyses, the present investigation confirms the presence of CMV infection in *V. jatamansi* plants at Shilly Nursery, Solan.

Table 1. DAS-ELISA results of five lines tested against CMV antisera

Sl. no.	Variety/Line	Symptoms	OD values (405 nm)
1.	D/B/15	-	0.487
2.	U/F/9	Mosaic, yellowing, leaf curling	1.139
3.	D/F/19	-	0.365
4.	U/B/45	Yellowing, leaf curling and deformation	0.983
5.	U/F/47	-	0.432
6.	Negative control	-	0.348
7.	Positive control	-	1.953

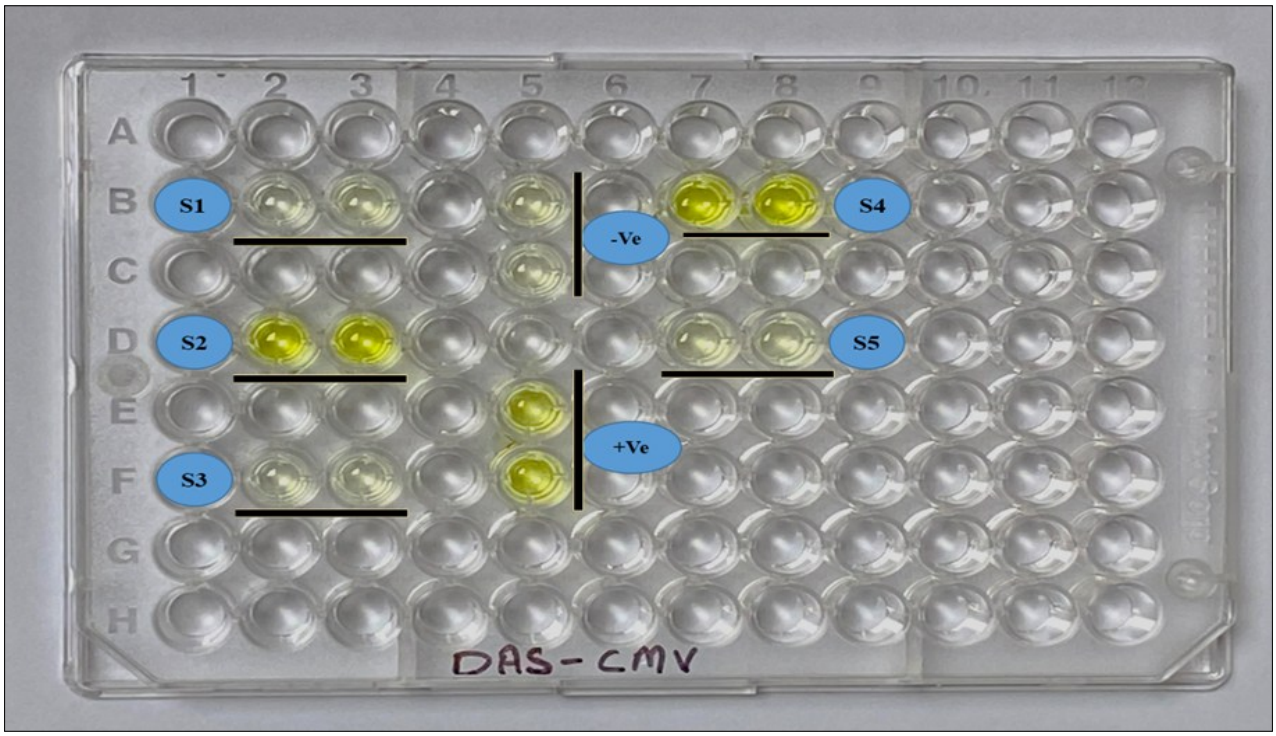

Fig. 3. Serological detection of CMV in *V. jatamansi* by DAS-ELISA.

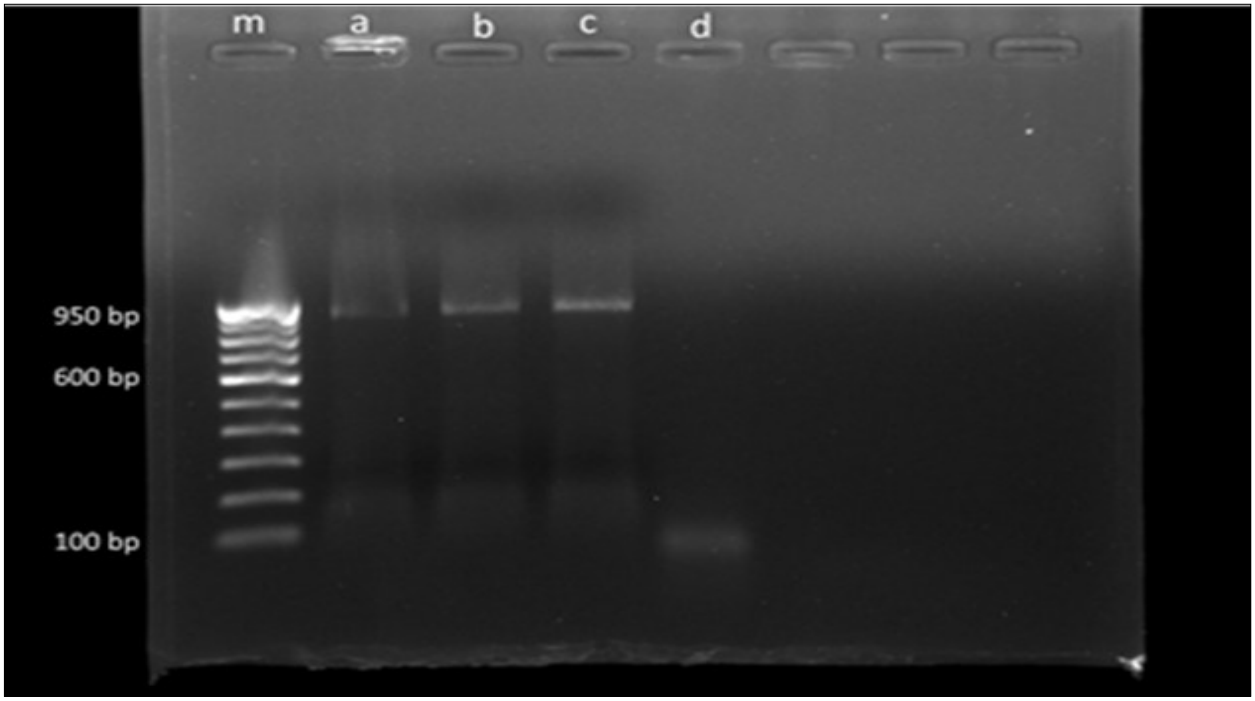

Fig. 4. Amplification of PCR product on 1 % agarose gel (m: 100 bp ladder; a: *N. tabacum*; b: U/F/9; c: U/B/45; d: healthy *Valeriana* plant).

Conclusion

The present study confirmed the occurrence of CMV infecting *V. jatamansi* in the Shilly region of Solan, Himachal Pradesh, using both serological (DAS-ELISA) and molecular (RT-PCR) assays. Infected plants exhibited characteristic symptoms such as mosaic, yellowing and leaf deformation, which contributed to reduced vigour and rhizome yield. CMV detection in lines U/F/9 and U/B/45 emphasizes the need for regular virus monitoring in medicinal plant cultivation, particularly in species propagated vegetatively.

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Authors' contributions

AC has conducted all the lab experiment including immunoassays, RT-PCR based molecular detection and drafted the manuscript. AH, AB and YPS analysed and interpreted the results and checked the manuscript. T participated in conducting the survey and writing the manuscript. AT and MT helped in conducting the field surveys. All the authors have read and approved the final version of manuscript.

Compliance with ethical standards

Conflict of interest: On the behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical issues: None

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