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Prevention and Control of Viral Diseases of Crops through Phytoproteins along with Bioenhancers

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Abstract

Treatment of *Lycopersicon esculentum* and *Lagenaria siceraria* plants, with phytoproteins, isolated from roots of *Boerhaavia diffusa* and leaves of *Clerodendrum aculeatum,* mixed separately with bio enhancer, could significantly prevent the infection and spread of tomato mosaic/tomato leaf curl virus. It was revealed that the potential of CA phytoprotein mediated induction of resistance was increased, in plants treated with CA phytoprotein along with bio enhancer M9 and M3. The CA phytoprotein mixed together with M9 was found to be more efficacious in protecting crops against viruses than *Clerodendrum aculeatum* alone. On the other hand, potential of *Boerhaavia diffusa* phytoprotein was also enhanced by the addition of bio enhancers M9 and M3, in inducing systemic resistance in *Lycopersicon esculentum* and *Lagenaria siceraria* plants, against natural infection of tomato mosaic/tomato leaf curl or cucumber mosaic virus respectively. Higher degree of modified systemic resistance was induced by BD phytoprotein, mixed with bio enhancers as compared to BD phytoprotein alone. However, BD phytoprotein in combination with M9 was found to be more effective than BD alone or BD with M3.

Keywords: Phytoproteins; Antiviral; Induced resistance; Modifier; Bio enhancer

Introduction

Plant viruses are a major challenge in the production of vegetables, as a large number of diseases are caused by different viruses and the yield and quality is badly affected. They may do so either directly or by increasing plant vulnerability to other pathogens or adverse growing conditions [1-3].

A large number of viruses viz. Cucumber Mosaic Virus and its strain, Squash Mosaic Virus, Melon Mosaic Virus and Bottle Gourd Mosaic Virus are reported to cause heavy losses in cultivated cucurbits [4,5]. The important viruses affecting bottle gourd are Cucumber Green Mottle Mosaic Virus [6], Cucumber Mosaic Virus [7], Watermelon Mosaic Virus [8] and Water Melon Bud Necrosis Virus [9,10]. Apart from these viruses, begomovirusdiseases are also emerging as serious problems in many cucurbit crops in India [10,11], have been reported to infect *Lagenaria siceraria* L. Standle. Among these viruses, Tomato (*Lycopersicon esculentum* Mill) is an important vegetable crop, being grown across the globe in a wide range of climatic conditions. The cultivation and production of this crop has been affected badly due to a number of diseases incited by fungi, bacteria, phytoplasma and viruses. Tomato is prone to a large number of viral diseases like tomato mosaic virus [12], Tomato Leaf Curl Virus (TLCV) [13], Cucumber Mosaic Virus (CMV) [14], Tomato Spotted Wilt Virus (TSWV) [15], Tomato Aspermy Virus [16].

So far, no direct control measure is available for all these viruses. However, through insecticides, further spread of disease may be checked up to some extent by killing their insect vectors but due to broad spectrum toxicity, environmental pollution and expensive nature of chemical insecticides, currently pest/insect control through botanical biopesticides is gaining stature. Such botanicals, mostly of protein nature and eco-friendly, have no phytotoxicity, easily biodegradable, did not leave any residue and may be obtained in large quantities [17,18]. Recently we have isolated an antiviral glycoprotein from the roots of *Boerhaavia diffusa* and a protein from the leaves of *Clerodendrum aculeatum* plants which have shown broad spectrum and very high antiviral activity against majority of phytopathogenic viruses [19-25].

The present investigations were carried out to study the effect of antiviral resistance inducing activity, of phytoproteins isolated from *B. diffusa* and *C. aculeatum*, and the enhancement in their resistance inducing efficacy so as to prolong the antiviral state/systemic induced resistance in the host plants, treated with phytoproteins mixed with a few modifiers/bio enhancers of biological origin.

Methods

Preparation of inhibitor

Extraction of phytoproteins from the leaves of *Clerodendrum aculeatum L* and roots of *Boerhaavia diffusa L.* was carried out, essentially according to the procedure as described earlier with slight modification [24-27]. The lust green and fresh leaves were harvested from healthy and vigorously growing *Clerodendrum aculeatum* plants and ground in a freshly prepared 0.2 M phosphate buffer (PB) of pH 6.6 containing 0.1% β mercaptoethanol in the ratio of 1:2. It was then squeezed through double-layered muslin cloth. The extracted sap was centrifuged at 8000 g for 10 min to remove the cell debris. The pellet was discarded and supernatant obtained was used for further

experimental work. The 1:2 extract was diluted to 1:5, 1:10, and 1:20. Each experiment was replicated at least thrice to confirm its validity.

The roots of *Boerhaavia diffusa* were collected from the profusely growing robust plants in the field, washed with distilled water, cut into small pieces, dried under shade and ground to fine powder in a grinder. The root powder was then mixed/soaked (1 g/10 ml) in 0.2 M phosphate buffer, pH 6.6 containing 0.1% β mercaptoethanol and left overnight at 40°C. It was then filtered through two folds of muslin cloth and the filtrate so obtained was centrifuged at 8,000 g for 15 min. A saturated solution of ammonium sulphate (60%) was added to the supernatant with continuous stirring and left overnight at 4°C. Thick precipitate appeared within one hour. The mixture was then centrifuged at 5,000 g for 15 min so as to separate the precipitate. The precipitate was collected and the supernatant was discarded. The precipitate obtained was suspended in small amount of buffer [20 g fresh weight/ml of 0.2 M PB (pH 6.6)] and then dialyzed against running water, in a dialysis bag, to remove ammonium sulphate, if any, and to obtain total protein fraction. The dialyzed protein fraction was either diluted as per requirement or was concentrated through freeze-drying. Lyophilized protein was stored at –20°C. A similar protocol was adopted for separating a protein fraction from the leaves of *Clerodendrum aculeatum* also. For VIA work buffer used was sodium acetate, instead of phosphate buffer.

Raising of test plants

Test plants were grown in earthen pots filled with compost and soil (1:2) and kept in an insect free glass house under natural light conditions. Plants of same age group with same height and vigour having at least 4 inoculable leaves in each were used for experimental purposes.

Maintenance of virus culture

The cultures of cucumber mosaic, tomato mosaic/tomato leaf curl virus(es) were maintained separately, in their respective systemic hosts (CMV in *Lagenaria siceraria L.* Standle and tomato mosaic/tomato leaf curl virus in *Lycopersicon esculentum* Mill. by successive inoculations from severely infected plants.

Preparation of virus inoculum

Virus inoculum, in each case, was prepared separately by crushing virus infected leaves, showing severe disease symptoms in distilled water (1:2 proportion), in a mortar. The homogenized tissue was squeezed through two folds of muslin cloth and the filtrate was centrifuged at 8,000 rpm for 10 min. The clear supernatant was used as virus inoculum after suitably diluting it to 1:2, 1:5, 1:10, 1:20, 1:50 and 1:100 (v/v) with distilled water.

Inoculation

The host plants were trimmed to 4 fully expanded leaves in each, a day before the inoculation. The leaves of test plants to be inoculated were dusted with carborundum powder (400-600 mesh). Inoculum was applied gently and uniformly using fore finger on the upper surface of leaves. Immediately after inoculation, leaves were rinsed with distilled water. Number of local lesions, appeared 4-6 days after inoculation, were counted and used for calculation.

Treatment of host plants with phytoproteins

The dialyzed total protein fraction was suitably diluted with distilled water (1:2, *Clerodendrum aculeatum*; 1:5, *Boerhaavia diffusa*). This fraction was applied separately, by rubbing with forefinger dipped in the protein solution, on to the upper surface of the basal/lower (site) leaves on the test plants. The upper (remote site) leaves on the same plant were left untreated. In field trials, aqueous extract of phytoproteins was sprayed on to test plants with the help of a pneumatic hand sprayer.

Infectivity bioassay

An aqueous solution of each inhibitor/phytoprotein was applied separately by rubbing onto the leaves of their respective test hosts. The virus, in each case was challenge inoculated 24 h later. Leaves of control plants were rubbed exactly in the same way with distilled water. The experiments were repeated thrice before confirming the results.

Disease incidence and disease severity was assessed respectively with respect to number of diseased plants and symptom severity in systemic host. The active virus titre in the infected plants was measured by infectivity assay on local lesion hosts. The induced resistance was expressed as reduction in lesion number in host reacting hyper sensitively to virus infection.

Percent decrease in virus infectivity was calculated using the formula

$$= \frac{C - T}{C} \times 100$$

Where: C=Average number of local lesions produced on control plants, T=Average number of local lesions produced on treated plants.

To evaluate the efficacy of phytoprotein mediated resistance under field conditions, regular sprays with these phytoproteins were administered till the plants were in the susceptible phase.

Observations on disease incidence, disease control, plant height, flowering and fruiting were recorded at weekly intervals, till the maturity of the crop. Finally, when the crop was mature, fruit length, number of fruits per plant and other yield attributes were also included in assessing the efficacy of phytoproteins.

Application of insecticide

Insecticide "Rogor" was used in the concentration of 1 ml. per litre of water to make the glass house free of insect vector (aphids and white flies), if any.

The experiments were carried out under the temperature and humidity controlled glass house conditions. The seeds of vegetable crops (Tomato and Bottle gourd) were sown in earthen pots. Upon germination, the seedlings, depending upon plant species were thinned to approximately 6-10 plants/pot. Two bio enhancers viz., M9 and M3 were added separately to each of the phytoproteins at strength of 1:5. In addition to bio enhancer, a spreader was also added so that phytoproteins evenly spread all over the surface of leaves. For the confirmation of validity and significance of results, of the experiments conducted in different environmental conditions, data obtained were analyzed statistically [28].

Results

Data presented in Tables 1-4 have revealed that minimum disease incidence with maximum protection against virus (es) infection was recorded in treatments with phytoproteins, isolated from *Clerodendrum aculeatum* and *Boerhaavia diffusa*, along with bio enhancer. The plants treated with phytoproteins mixed with M9 and M3 exhibited minimum symptoms severity and incidence of cucumber mosaic virus, tomato mosaic virus and tomato leaf curl virus in their respective hosts. The beneficial effects were variable in different treatments.

CA mixed with M9 and M3

Addition of M9 and M3 to phytoprotein solution was found to increase the potential of CA phytoprotein mediated induction of resistance in plants of Lycopersicon lycopersicum, and *Lagenaria siceraria* against viral infections. The height of treated plants was increased. In most of the cases, increase in plant height was about 130% as compared to control plants. Height of plants treated with modified phytoprotein (bio enhancer+phytoprotein) was more than plants treated with CA phytoprotein alone (Figures 1 and 2).

Figure 1: Effect of *Clerodendrum aculeatum* (CA)/*Boerhavvia diffusa* (BD) phytoprotein with bio enhancer M9/M3 (L-Lysine monohydro Chloride / L-Asparagine) on growth and disease incidence in *Lycopersicon esculentum.* (a) Untreated control plants showing viral disease symptoms (left) and healthy plants with CA+M9 treated (right) at early stages, (b) Untreated control plants showing viral disease symptoms (left) and healthy plants with BD+M3 (right) at early stages, (c) Untreated control plants showing high viral symptom at later stages, (d) CA +M9 treated plants showing better growth and high fruiting at later stage, (e) BD+M9 treated plants showing better growth and high fruiting at later stage.

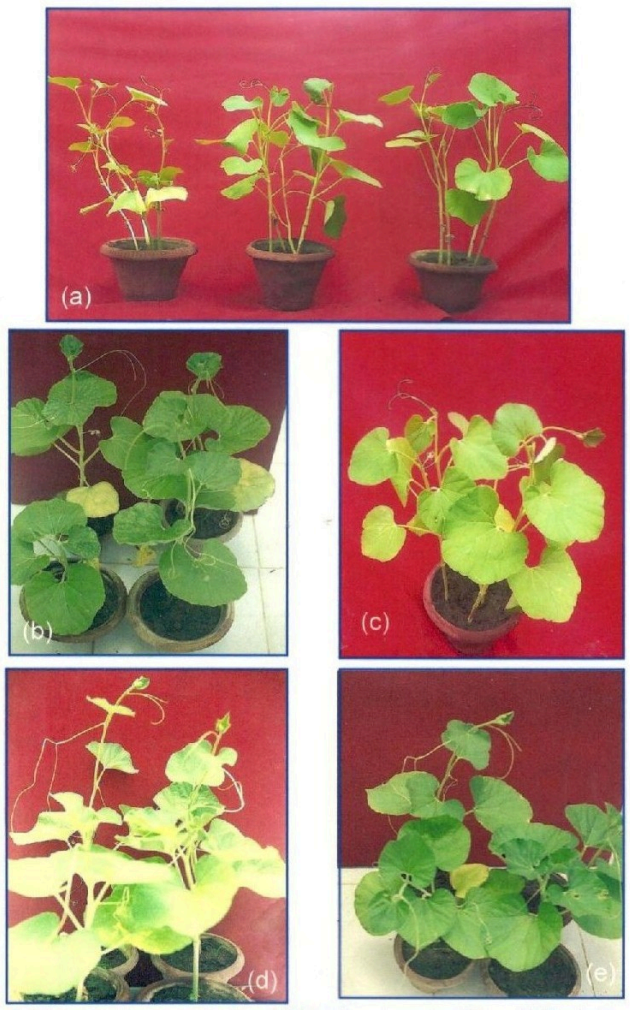


Figure 2: Effect of *Clerodendrum aculeatum* (CA)/*Boerhavvia diffusa* (BD) phytoprotein with bioenhancer M9/M3 (L-Lysine monohydro Chloride/L-Asparagine) on growth and disease incidence in *Lagenaria siceraria*. (a) Untreated control plants showing viral disease symptoms (left) and healthy plants with CA treated (middle) and healthy plant with CA+M9 treated (right) at early stages, (b) Untreated control plants showing viral disease symptoms at later stages, (c) CA+M3 treated plants showing better growth at later stages, (d) CA+M9 treated plants showing better growth at later stages, (e) BD+M9 treated plants showing better growth at later stage.

The phytoprotein from CA mixed together with M9 was more efficacious in protecting crops than CA alone. Enhanced flowering, fruiting, and crop yield was recorded in plants treated with CA phytoprotein only. Further increase in the above parameters was recorded with the use of modified CA phytoprotein. The addition of M9/M3 decreased disease severity and also the number of diseased plants (Figures 1, 2 and Graphs 1, 2).

S No.	Parameters	Response*			
		Control	Treated		
		DW	CA	CA+M9	CA+M3
†1	Diseased plants (%)	41	24	9	12
2	Disease severity	+ + + +	+ +	+	+

3	Flowering plants (%)	96.5	98	99	98
4	Fruiting in plants (%)	95	96	98	98
5	Number of fruits/plant	4.4 ± 0.44	6.8 ± 1.20	9.6 ± 1.24	8.6 ± 1.13
6	Leaf area-'l × b' (cm)	4.2+2.23	7.9+4.50	8.8+5.60	8.0+4.90
7	Plant height (cm)	74.2 ± 8.20	93.3 ± 9.22	96.2 ± 10.8.60	94.7 ± 9.25
8	Total crop yield (kg)	6.4 ± 0.64	10.2 ± 1.02	12.8 ± 0.84	11.3 ± 0.68

*Data based on average of 100 replicate plants ± SEM; Experiment was repeated thrice.

Where number of '+' signs denote intensity of disease severity; †After 60 days of growth

CA concentration was used 1:5; M9 and M3 were used 4 mg/ml; M9=L-Lysine monohydro chloride; M3=L-Asparagine+SEM=Standard Error of Mean.

Table 1: Effect of addition of bio enhancers on virus resistance inducing activity of phytoprotein, isolated from leaves of *Clerodendrum aculeatum*, on protection of tomato (*Lycopersicon esculentum*) crop.

S No.	Parameters	Response*			
		Control	Treated		
		DW	CA	CA+M9	CA+M3
†1	Diseased plants (%)	44.5	17.5	4.4	13.5
2	Disease severity	+ + + +	+ +	+	+
3	Flowering plants (%)	91.4	97	98.5	97.5
4	Fruiting in plants (%)	89.4	94.5	98	96.4
5	Number of Fruits/Plant	4.2 ± 0.50	6.2 ± 0.60	8.5 ± 0.80	7.4 ± 0.70
6	Leaf area-'l × b' (cm)	40.3+4.23	69.7+8.45	87.40+13.34	72.52+11.22
7	Plant height (cm)	105.6 ± 16.20	131.4 ± 18.10	138.0 ± 19.90	134.4 ± 19.10
8	Total crop yield (kg)	7.4 ± 0.06	12.9 ± 0.82	19.8 ± 1.62	15.8 ± 1.20

*Data based on average of 50 replicate plants ± SEM; Experiment was repeated thrice. Where number of '+' signs denote intensity of disease severity; †After 60 days of growth CA concentration was used. 1:5; M9 and M3 were used 4 mg/ml; M9=L-Lysine monohydro chloride; M3=L-Asparagine+SEM=Standard error of mean.

Table 2: Effect of addition of bio enhancers on virus resistance inducing activity of phytoprotein, isolated from leaves of *Clerodendrum aculeatum*, on protection of bottle gourd (*Lagenaria siceraria*) crop.

Modifiers in combination with CA phytoprotein induced significant resistance as compared to CA alone. However, CA phytoprotein in combination with M9 was more effective than either CA alone or CA with M3.

BD mixed with M9 and M3

Addition of M9 and M3 to *Boerhavvia diffusa* phytoprotein solution increased the potential of phytoprotein in inducing resistance in, *Lycopersicon esculentum* and *Lagenaria siceraria* plants against common viral infections. The height of plants was increased by nearly 120% as compared to control (untreated) plants. Increase in height of plants treated with modified phytoprotein was more than plants treated with BD phytoprotein alone. The differences were statistically insignificant. As in the previous experiment, in this case too, the treated plants showed increase in flowering, fruiting, and crop yield as compared to controls. Here too the addition of bio enhancer to BD phytoprotein, further improved overall growth of the plants as compared to crops sprayed with BD phytoprotein alone. The addition of M9/M3 decreased disease severity and also the number of diseased plants. Modified induced better resistance as BD phytoprotein compared to BD alone. However, BD phytoprotein in combination with M9 was more effective than BD with M3 and BD alone (Table 2, 4 and Graphs 1, 2).

S No.	Parameters	Response*	
		Control	Treated

		DW	BD	BD+M9	BD+M3
†1	Diseased plants (%)	38.5	28.5	14.5	18.5
2	Disease severity	+ ++ +	+ ++	++	++
3	Flowering plants (%)	95.5	96.5	97.5	97
4	Fruiting in plants (%)	94	94.5	97	96
5	Number of fruits/plant	4.2 ± 0.42	5.2 ± 0.96	7.8 ± 0.95	7.2 ± 0.82
6	Leaf area-'l × b' (cm)	4.0+2.10	6.8+4.20	8.2+5.20	7.6.0+4.22
7	Plant height (cm)	70.2 ± 5.15	81.4 ± 7.44	85.8 ± 8.85	84.6 ± 8.17
8	Total crop yield (kg)	6.0 ± 0.42	9.6 ± 0.82	10.9 ± 0.54	9.9 ± 0.84

*Data based on average of 100 replicate plants ± SEM; Experiment was repeated thrice.

Where number of '+' signs denote intensity of disease severity; †After 60 days of growth

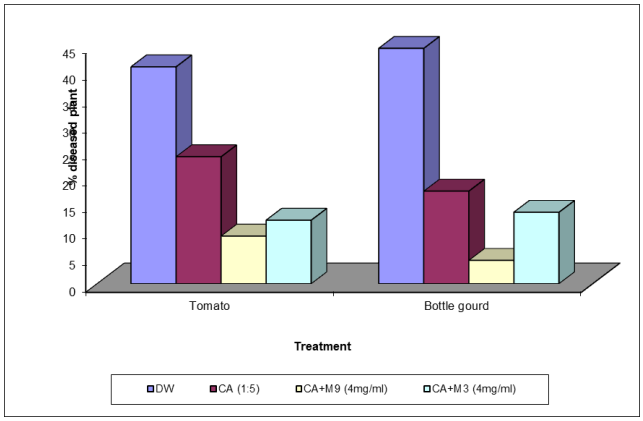
BD concentration was used 1:5; M9 and M3 were used 4 mg/ml; M9=L-Lysine monohydro chloride; M3=L-Asparagine+SEM=Standard error of mean.

Table 3: Effect of addition of bio enhancers on virus resistance inducing activity of phytoprotein, isolated from roots of Boerhaavia diffusa, on protection of tomato (Lycopersicon esculentum) crop.

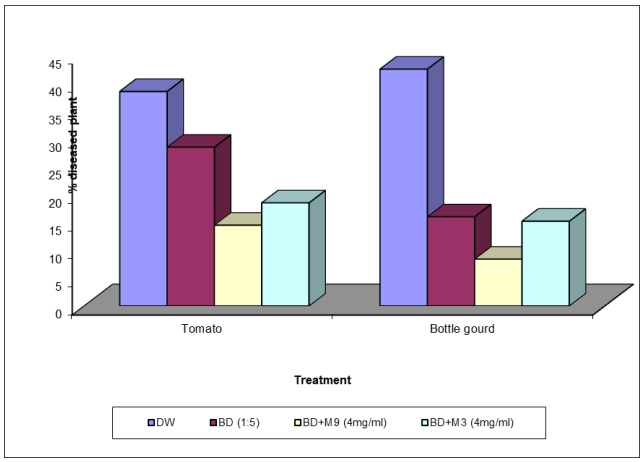
S No.	Parameters	Response*			
		Control	Treated		
		DW	BD	BD+M9	BD+M3
†1	Diseased plants (%)	42.5	16	8.4	15.2
2	Disease severity	+ + + +	+ +	+	+
3	Flowering plants (%)	90.3	95.5	97.5	96.4
4	Fruiting in plants (%)	88.5	93.4	97.7	95.3
5	Number of fruits/plant	4.0 ± 0.62	5.9 ± 0.50	7.4 ± 0.70	6.8 ± 0.60
6	Leaf area-'l × b' (cm)	39.4 ± 4.12	61.7 ± 6.49	80.20 ± 11.34	69.52 ± 10.20
7	Plant height (cm)	102.2 ± 14.4	117 ± 7.20	124 ± 19.8	119 ± 19.10
8	Total crop yield (kg)	7.2 ± 0.06	10.5 ± 0.50	17.6 ± 0.62	15.4 ± 1.00

*Data based on average of 50 replicate plants ± SEM; Experiment was repeated thrice. Where number of '+' signs denote intensity of disease severity; †After 60 days of growth BD concentration was used 1:5; M9 and M3 were used 4 mg/ml; M9=L-Lysine monohydro chloride; M3=L-Asparagine+SEM=Standard error of mean.

Table 4: Effect of addition of bio enhancers on virus resistance inducing activity of phytoprotein, isolated from roots of Boerhaavia diffusa, on protection of bottle gourd (Lagenaria siceraria) crop.



Graph 1: Effect of addition of bio enhancers on virus resistance inducing activity of phytoprotein, isolated from leaves of *Clerodendrum aculeatum* (CA), on protection of tomato (*Lycopersicon esculentum*) and bottle gourd (*Lagenaria siceraria*) crop.



Graph 2: Effect of addition of bio enhancers on virus resistance inducing activity of phytoprotein, isolated from roots of Boerhaavia diffusa (BD), on protection of tomato (Lycopersicon esculentum) and bottle gourd (Lagenaria siceraria) crop.

References

1. Verma HN, Awasthi LP, Mukerjee K (1979) Induction of Systemic resistance by antiviral plant extracts in non-hypersensitive hosts. J Pl Dis Prot 87: 735-740.
2. Awasthi LP (1981) The purification and nature of an antiviral protein from Cuscuta reflexa plants. See comment in PubMed Commons below. Arch Virol 70: 215-223.
3. Awasthi LP, Verma HN, Kluge S (2016) A possible mechanism of action for the inhibition of plant viruses by an antiviral glycoprotein isolated from Boerhaavia diffusa roots. J Virology and Antiviral Research 5: 1-8.
4. Verma HN, Sivastava A, Gupta RK, (1998) Seasonal variation in systemic resistance inducing basic protein isolated from leaves of Clerodendrum aculeatum. Ind Plant Pathol 16: 9-13.
5. Verma HN, Baranwal VK, Srivastava S (1998b) Antiviral substances of plant origin. In Plant virus disease control, edited by A Hadidi, R.K. Khetarpal, A. Kozenzava (American Phytopathol. Society (APS) Press, St. Paul, Minnesota) 154-162.
6. Raychaudhari M, Varma A, (1978) Mosaic disease of muskmelon caused by minor variant of C.G.M.M.V. Phytopath Z.93: 120-125.
7. Vani S (1987) Studies on viral disease of muskmelon and watermelon. Ph. D. Thesis, P.G.School, IARI, New Delhi.
8. Raychaudhari M, Varma A, (1975) Virus disease of cucurbits in Delhi, Proc. 62nd Indian Sci Cong. Part III P. 74.
9. Jain RK, Pappu HR, Pappu SS, Krishna Reddy M, Vani A (1998) Water melon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. Arch Viral 143: 1637-1644.
10. Mandal B, Chaudhari V, Jain RK, Varma A (2003) First report of Natural infection of Luffa acutangula by watermelon bud necrosis virus in India. Plant Dis 87: 598.
11. Varma A, malathi VG (2003) Emerging geminivirus problem: A serious threat to sustainable crop production. Ann App Biol 142: 145-164.
12. Desai SV (1933) Studies on the nature of causative agent of the mosaic disease of tomato. Ind J Agri Sci 3: 626-639.
13. Vasudeva RS, Samraj J, (1948). A leaf curl disease of tomato. Phytopathology 38: 364- 359.
14. Marrou J, Quiot JB, Duteil M, Labonne G, Leclant F, et al. (1973) Ecology and epidemiology of cucumber mosaic virus III Interest of the exposure of bait plants in the study of cucumber mosaic virus dissemination. Ann Phytopathol 11: 291-306.
15. Todd JM, Ponniah S, Subramanyam CP (1975) Study on tomato spotted wilt virus disease of tomato. Mad Agri J 63: 162.
16. Chenulu VV, Giri VK (1985) Viral and mycoplasmal diseases of tomato and their management. Pers Plant Virol, pp: 45-74.
17. Chaubey AN, Awasthi LP, Singh SP (2014) Eco-friendly management of viral diseases of potato. Int Res J Life Sci 2: 8-12.
18. Pandey AK, Awasthi LP, Pandey VP, Sharma NK, Kumar A, et al. (2014) Bio-efficacy of root extract of Boerhaavia diffusa on yellow disease of ginger. Am Inte J Research in Formal, Applied and Natural Sciences 5: 79-80.
19. Awasthi LP, Singh Shyam, Sharma NK, Singh SK (2011) Induction of systemic resistance through antiviral agents of plant origin against papaya ring spot virus disease in papaya (Carica papaya L.). Intern J Sustainable Agriculture 3: 54-57.
20. Awasthi LP, Singh SP, Verma HN, Kluge S (2013) Further studies on the antiviral agent(s) isolated from host plants, pre-treated with Boerhaavia diffusa glycoprotein. Virol Mycol 3: 124.
21. Awasthi LP, Singh, Samir Pratap, Tripathi D (2014) Eco-friendly Management of the Viral Diseases of Chilli (Capsicum annum L.). Research & Review: J Agriculture Sci Tech 3: 11-16.
22. Singh AK, Najam A, Verma HN, Awasthi LP (2010) Control of Natural viral infection on Okra (Ablemoschus esculentus) by root extract of Boerhaavi diffusa. Inte J Plant Protection 2: 195-198.
23. Singh SK, Awasthi LP, Singh S, Sharma NK (2011) Protection of mungbean and urdbean crops against vector borne mungbean yellow mosaic virus through botanicals. Curr Bot 2: 08-11.
24. Verma HN, Awasthi LP (1979a) Antiviral activity of Boerhavvia diffusa root extract and the physical properties of the virus inhibitor. Cand J Bot 57: 926-932.
25. Verma HN, Awasthi LP (1979b) Prevention of virus infection and multiplication by leaf extract of Euphorbia hirta and the properties of the virus inhibitor. New Botanist 6: 49-59.
26. Verma HN, Awasthi LP (1980) Occurrence of a highly antiviral agent in plants treated with Boerhaavia diffusa inhibitor. Cand J Bot 58: 2141-2144.
27. Verma HN, Kumar V (1980) Prevention of plant virus diseases by Mirabilis jalapa leaf extract. New Botanist 7: 87-91.
28. Snedecor GW (1961) The statistical methods, Allied Pacific Private Limited, Bombay.