

Effect of Different Bio-Enhancers on Antiviral Resistance Inducing Activity of Phytoproteins Obtained from Roots of *Boerhaavia diffusa* and leaves of *Clerodendrum aculeatum*

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ABSTRACT: The virus inhibiting potential of phytoprotein was demonstrated by the ability of phytoproteins to inhibit formation of necrotic lesions in local lesion host (hypersensitive) and delay in development of disease specific symptoms in systemic host, when the respective treated host plants were challenged with viruses. Efficacy of viral inhibitory activity and resistance inducing ability of both phytoproteins could further be enhanced by additions of certain substances of biological origin. Such substances have been referred to as bio-enhancers in the dissertation.

I. INTRODUCTION

The viral diseases are of immense importance as they cause extensive crop damage and severe losses in terms of yield and quality of produce. Hence, their management has been of great interest to the researchers in the field of plant virology. Further, because of the peculiar nature and characteristics of plant viruses in the host system, any therapeutic method to control them has not been found very successful. Where resistant varieties are available, long term and effective protection has been achieved, but for a number of crops no resistant variety is available. However, it has been observed that preventive measures, if adopted, can be of great help in controlling/avoiding the viral diseases. Several reports are available on the effectiveness of a biotic and biotic agents as elicitors of resistance in susceptible plant hosts. These agents appear to act by sensitizing the plant for activation of resistance mechanism. Natural plant substances have also been shown to protect plants against infections from viruses and also to prevent the establishment of viruses in the host (Verma *et al.* 1982, 1985).

II. MATERIALS AND METHODS

The aim of the experiment was to select the best bio-enhancer by screening a range of agents of biological origin, and to optimize concentration for maximum resistance. The different bio-enhancers viz., milk protein, black pepper powder, khand (a semi refined sugar), turmeric powder, ginger powder, papain and jaggery were used in combination with phytoproteins (2.5% w/v) of *B. diffusa* and *C. aculeatum* at strength ranging between 0.5 to 4%. Induction of resistance was evaluated both through local lesion assay using hosts viz., *N. tabacum* cv. Samsun 'NN' and *C. tetragonoloba* which give a hypersensitive reaction to TMV and SRV infections, respectively. Symptom severity of viral infections was assessed in hosts, *N. tabacum* cv. NP-31, *C. juncea* and *L. siceraria*, which show a systemic response to TMV, SRV and CMV, respectively. These plants were grown in soil contained in pots and were maintained under glasshouse conditions. Each pot contained one plant. Treatment involved application/spray of solution [BD/CA phytoprotein + bio-enhancer] on two lower leaves (site) of test plants. The plants of the control set were sprayed with distilled water. After 24 hr, all leaves of all plants were challenged with virus. The local lesions appeared after 3-4 days on hypersensitive host and were counted separately for treated (site) and non-treated (remote) leaves of the same plant and also on the leaves of control plants. Systemic host plants were observed and rated for the development and intensity of symptoms every alternate day after they were challenged.

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Protection of crops from viral diseases by inducing immunity in plants Various plants, like vegetable yielding, pulse yielding (legumes), cash crops and fruit yielding plants, were selected for conducting different experiments and induction of resistance was evaluated on hosts both reacting hyper sensitively (local lesion) or systematically to viruses.

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 at 2-3 leaf stage were used for experimental purposes. Field experiments were done in small plots at university campus and also in agricultural field.

Maintenance of virus culture The cultures of three viruses viz., Tobacco mosaic virus (TMV) (Rothamsted strain), Cucumber mosaic virus (CMV) (a local isolate) and Sun hemp rosette virus (SRV), (a local isolate), (Verma and Baranwal 1983), were maintained on their respective systemic hosts.

Preparation of virus inoculums Virus inoculums was prepared by crushing virus infected leaves, showing distinct mosaic symptoms in distilled water (2 ml/gm) in a mortar. The homogenized tissue was squeezed through two folds of muslin cloth and the filtrate was centrifuged at 8000 rpm for 10 min. The clear supernatant was used as virus inoculum after suitably diluting it 1:2, 1:5, 1:10, 1:20, 1:50 and 1:100 (v/v) with distilled water.

Inoculation The leaves of test plants to be inoculated were sprinkled with 600 mesh carborundum powder. Inoculum was applied gently and uniformly using fore finger on the upper surface of leaves. After inoculation, leaves were washed with distilled water.

Treatment of plants with phytoproteins The dialyzed total protein fraction was suitably diluted with distilled water (1:5, *B. diffusa*; 1:2, *C. aculeatum*) and this fraction was rubbed on the upper surface of the lower (site) leaves of the test plants. The upper (remote site) leaves were left untreated. In field trials, aqueous extract of phytoproteins was sprayed on test plants with the help of a pneumatic hand sprayer.

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

Percent decrease in virus infectivity was calculated using the formula:

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

Where: C = Average number of lesions produced on control plants and

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 were made weekly with respect to plant height, flowering and fruiting. Finally, when the crop was mature, fruit length, number of fruits per plant and other yield attributes were also included in assessing efficacy of phytoprotein.

III. RESULTS

Screening of bio-enhancers on virus resistance inducing activity of phytoprotein

Phytoprotein alone or in combination with various bio-enhancers was found to decrease the number of lesions in hypersensitive hosts (Table 1.A.1, 1.A.2, 1.B.1, 1.B.2). Various agents added to the phytoproteins were found to decrease lesion formation in hypersensitive host by varying degrees. Maximum decrease in lesion number 96 % at site and 95 % at remote was reported when milk protein was added to phytoprotein. Milk protein was selected and was further tested for its resistance enhancing potential by using it at different strengths.

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Table: 1.A.1. Effect of different bio-enhancers on antiviral resistance inducing activity of phytoprotein obtained from roots of *Boerhaavia diffusa* in *Nicotiana tabacum* cv. Samsun NN

Treatment	Color of sample	Response*			
		Number of local lesions/leaf		Percent reduction in lesion number	
		Site $\pm$ SEM	Remote $\pm$ SEM	Site	Remote
Control (DW)	—	71 $\pm$ 8.6	76 $\pm$ 9.4	—	—
BD alone	Slightly light brown	16 $\pm$ 3.7	33 $\pm$ 5.2	77.46	56.57
MP alone	Milky white	68 $\pm$ 8.2	74 $\pm$ 7.8	4.23	2.63
BP alone	Light brown	70 $\pm$ 8.0	77 $\pm$ 7.9	1.41	•
K alone	Whitish	73 $\pm$ 8.4	74 $\pm$ 8.1	•	2.63
TP alone	Yellow	70 $\pm$ 6.9	73 $\pm$ 8.3	1.41	3.95
GP alone	Brownish	69 $\pm$ 7.6	77 $\pm$ 8.1	2.82	•
P alone	Light brown	67 $\pm$ 6.9	75 $\pm$ 7.6	5.63	1.32
J alone	Dark brown	74 $\pm$ 8.2	75 $\pm$ 6.9	•	1.32
BD + MP	Slightly milky white	3 $\pm$ 0.5	4 $\pm$ 0.8	95.77	94.73
BD + BP	Dark brown	4 $\pm$ 0.7	7 $\pm$ 1.0	94.37	90.79
BD + K	Whitish	5 $\pm$ 0.6	8 $\pm$ 1.2	92.96	89.47
BD + TP	Slightly yellow	10 $\pm$ 1.3	25 $\pm$ 6.2	85.92	67.11
BD + GP	Brown	6 $\pm$ 0.7	13 $\pm$ 1.8	91.55	82.89
BD + P	Light brown	4 $\pm$ 0.6	6 $\pm$ 0.7	94.37	92.11
BD + J	Dark brown	9 $\pm$ 1.1	16 $\pm$ 1.4	87.32	78.97

DW=distilled water; BD= *B. diffusa* phytoprotein MP=Milk protein; BP=Black pepper powder
K=Khand; TP=Turmeric powder GP=Ginger powder P=Papain
J=Jaggery

* Data represents average number of local lesions formed on 12 leaves each of site and remote site from six plants.

Concentration of bioenhancer used =1.0% (w/v)

Concentration of *B. diffusa* phytoprotein used = 2.5% (w/v)

1.0 g of TMV infected leaf tissue in 100 ml distilled water [virus inoculum (1:100)]

• Increased in insignificant lesion number

Optimum concentration of milk protein used along with phytoproteins

(A) **Hypersensitive host-virus systems** Increasing concentration of milk protein in the phytoprotein decreased the number of lesions. (Plate 1.1, 1.2; Table 1.C.1, 1.C.2, 1.D.1, 1.D.2; Fig. 1.1,1.2). Maximum reduction in lesion number was seen at 4.0% though the difference between strength 4.0% and 2.0% was insignificant.

(B)

Table: 1.A.2. Effect of different bio-enhancers on antiviral resistance inducing activity of phytoprotein obtained from leaves of *Clerodendrum aculeatum* in *Nicotiana tabacum* cv. Samsun NN

Treatment	Colour of sample	Response*			
		Number of local lesions/leaf		Percent reduction in lesion number	
		Site $\pm$ SEM	Remote $\pm$ SEM	Site	Remote
Control (DW)	—	74 $\pm$ 8.2	81 $\pm$ 9.1	—	—
CA alone	Brownish	27 $\pm$ 2.8	51 $\pm$ 6.3	61.51	37.04
MP alone	Milky white	73 $\pm$ 8.4	79 $\pm$ 7.6	1.35	2.47
BP alone	Light brown	70 $\pm$ 7.6	82 $\pm$ 9.3	5.41	•
K alone	Whitish	75 $\pm$ 8.2	80 $\pm$ 8.1	•	1.23

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TP alone	Yellow	71±7.8	78±7.9	4.05	3.70
GP alone	Brownish	74±6.8	79±9.1	0.0	2.47
P alone	Light brown	69±8.4	82±8.6	7.04	•
J alone	Dark brown	72±7.7	78±6.9	2.70	3.70
CA + MP	Milky white	7 ± 0.5	16 ± 0.8	90.54	80.25
CA + BP	Dark brown	13 ± 1.2	29 ± 2.3	82.43	64.20
CA + K	Whitish yellow	17 ± 2.1	39 ± 3.8	72.97	51.85
CA + TP	Yellow	21 ± 2.2	36 ± 3.3	68.91	55.56
CA + GP	Light brown	15 ± 1.3	28 ± 2.4	79.72	65.43
CA + P	Brown	10 ± 1.1	19 ± 2.1	86.49	76.54
CA + J	Dark brown	22 ± 2.0	35 ± 3.2	70.27	56.79

DW=distilled water;
pepper powder
K=Khand;
J=Jaggery

CA= *C. aculeatum* phytoprotein

MP=Milk protein;

BP=Black

TP=Turmeric powder

GP=Ginger powder

P=Papain

* Data represents average number of local lesions formed on 12 leaves each of site and remote site from six plants.

Concentration of all bioenhancer used = 1.0% (w/v)

Concentration of CA phytoproteins used = 2.5% (w/v)

1.0 g of TMV infected leaf tissue in 100 ml distilled water [virus inoculum (1:100)]

• Increased in insignificant lesion number

(B) Systemic host-virus systems Application of phytoproteins containing 2.0% of milk protein was found to delay or suppress characteristic symptom in systemic hosts of TMV, CMV and SRV (Plate 1.3, 1.4, 1.5; Table 1.E.1, 1.E.2). In control plants severe mosaic symptoms were evident by 10 days of virus challenge. These symptoms gradually intensified and by 20 days of virus challenge, stunting in control plants was observed. In plants treated with phytoproteins the symptoms were markedly delayed. Addition of milk protein to phytoproteins appeared to give better protection as compared to phytoproteins alone as was indicated by absence of symptoms or very feebly perceptible mosaic even after 20 days of virus challenge.

IV. DISCUSSION

The antiviral potential of root extract of *Boerhaavia diffusa* and leaf extract of *Clerodendrum aculeatum* has been established through the extensive work conducted by various workers (Verma *et al.* 1973, 1974, 1975, 1978, 1979, 1980, 1982, 1983, 1984; Awasthi *et al.* 1984, 1985; Verma *et al.* 1985, 1986; Awasthi *et al.* 1989; Khan and Verma 1990; Verma *et al.* 1992; Baranwal 1992; Verma *et al.* 1995a,b; Prasad *et al.* 1995; Srivastava 1995; Verma *et al.* 1996, 1998, 1998a, 1999; Gupta *et al.* 2004; Srivastava *et al.* 2004; Singh *et al.* 2004; Awasthi *et al.* 2006). Among these, Prof Verma's work deserves special credit for recognizing antiviral potential and popularizing it among the virologists of various institutes. The virus inhibitory potential of phytoprotein was demonstrated by the ability of phytoprotein to prevent the formation of necrotic lesions in local lesion hosts and in delaying the development of disease specific symptom in systemic hosts when the treated host plants were challenged with virus. This type of induction of resistance in plants has been referred to as induced systemic resistance (ISR). Many agents such as natural plant products, bacteria like *Pseudomonas* spp. etc., and chemicals like benzothiadiazole, are known to elicit this resistance phenomenon. The protection is shown to be induced systemically in various plants viz., tobacco, sun hemp, bottle gourd, tomato, okra, chilli and mungbean etc. The antiviral potential of extracts of these plants has been proven against many viruses like TMV (Verma *et al.* 1973, 1975, 1979, 1980, 1983, 1984, 1985), CMV (Verma and Kumar 1979, 1980), CGMMV (Verma and Kumar 1979, 1980; Verma and Dwivedi 1983; Verma and Khan 1984; Verma *et al.* 1984), GMV (Verma and Mukerji 1975; Verma and Awasthi 1979; Verma *et al.* 1979), SRV (Verma and Mukerji 1975; Verma and Awasthi 1979; Verma *et al.* 1979; Verma and Khan 1984; Verma *et al.* 1984; Verma and Khan 1985) TRSV (Verma and Awasthi 1979; Verma *et al.* 1979), TmYMV (Verma and Kumar 1979; 1980; Verma and Dwivedi 1983; Verma *et al.* 1984), PLRV (Verma and Kumar 1979, 1980) and others.

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The antiviral properties of the plant extracts have been shown due to certain novel proteins present in the extracts. These proteins can be applied in the form of aqueous extracts of the plant part as purified proteins, the latter being more effective. The antiviral potential has been shown to exist in extracts of plants like, *Euphorbia hirta* (Verma *et al.* 1979), *Mirabilis jalapa* (Verma and Kumar 1980); *Cuscuta reflexa* (Awasthi 1981); *Clerodendrum aculeatum* (Verma *et al.* 1984), *Bougainvillea spectabilis* (Verma and Dwivedi 1984), *Pseuderanthemum atropurpureum* (Verma *et al.* 1985), *Boerhaavia diffusa* (Verma and Baranwal 1988), *Celosia cristata* (Baranwal and Verma 1992) and *Clerodendrum inerme* (Prasad *et al.* 1995).

Table: 1.B.1. Effect of different bio-enhancers on antiviral resistance inducing activity of phytoprotein obtained from roots of *Boerhaavia diffusa* in *Cyamopsis tetragonoloba*

Treatment	Colour of sample	Response*			
		Number of local lesions/leaf		Percent reduction in lesion number	
		Site	Remote	Site	Remote
Control (DW)	—	205±16.42	221±18.92	—	—
BD alone	Slightly light brown	55±6.21	118±12.67	73.17	46.61
MP alone	Milky white	201±15.62	223±18.34	1.95	•
BP alone	Light brown	209±16.52	218±17.63	•	1.36
K alone	Whitish	213±18.39	220±15.92	•	0.45
TP alone	Yellow	198±18.64	225±18.69	2.93	•
GP alone	Brownish	211±16.94	217±19.32	•	1.81
P alone	Light brown	196±15.36	216±18.64	4.39	2.26
J alone	Dark brown	199±16.13	224±15.38	2.39	•
BD + MP	Slightly milky white	21±3.45	41±3.49	89.76	81.45
BD + BP	Dark brown	35±4.65	56±5.62	82.93	74.66
BD + K	Whitish	40±4.26	77±6.94	80.49	65.16
BD + TP	Slightly yellow	42±5.65	85±8.26	79.51	61.54
BD + GP	Brown	32±3.48	54±6.23	84.39	75.56
BD + P	Light brown	25±3.16	45±4.28	87.80	79.65
BD + J	Dark brown	33±4.2	65±5.82	83.90	70.59

DW : distilled water;
MP : Milk protein;
K : Khand;
GP : Ginger powder
J : Jaggery

BD : *B. diffusa* phytoproteins
BP : Black pepper powder
TP : Turmeric powder
P : Papain

* Data represents average number of local lesions formed on 6 leaves each of site and remote site from six plants.

Concentration of bio-enhancer used = 1.0% (w/v)

Concentration of *B. diffusa* phytoprotein used = 2.5% (w/v)

1.0 g of SRV infected leaf tissue in 10 ml distilled water [virus inoculum (1:10)]

• Increased in insignificant lesion number

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Table: 1.B.2. Effect of different bio-enhancers on antiviral resistance inducing activity of phytoprotein obtained from leaves of *Clerodendrum aculeatum* in *Cyamopsis tetragonoloba*

Treatment	Color of sample	Response*			
		Number of local lesions/leaf		Percent reduction in lesion number	
		Site	Remote	Site	Remote
Control (DW)	—	209±19.65	226±20.12	—	—
CA alone	Brownish	86±10.82	148±13.62	58.85	34.51
MP alone	Milky white	204±18.64	220±19.68	2.39	2.65
BP alone	Light brown	211±19.08	219±18.62	•	3.10
K alone	Whitish	198±17.68	225±19.34	5.26	•
TP alone	Yellow	209±16.28	221±17.96	•	2.21
GP alone	Brownish	206±16.39	217±16.94	1.46	3.98
P alone	Light brown	203±15.82	227±18.78	2.87	•
J alone	Dark brown	210±16.54	218±17.85	•	3.54
CA + MP	Milky white	43±4.86	76±8.67	79.43	66.37
CA + BP	Dark brown	60±5.98	89±9.35	71.29	60.62
CA + K	Whitish yellow	68±7.24	96±10.21	67.46	57.52
CA + TP	Yellow	65±7.24	92±9.46	68.90	59.29
CA + GP	Light brown	58±6.92	85±7.85	72.25	62.39
CA + P	Brown	47±5.23	78±8.22	77.51	65.49
CA + J	Dark brown	56±5.86	81±7.96	73.20	64.16

DW=distilled water;

MP=Milk protein;

K=Khand;

GP=Ginger powder

J=Jaggery

CA= *C. aculeatum* phytoprotein

BP=Black pepper powder

TP=Turmeric powder

P=Papain

* Data represents average number of local lesions formed on 6 leaves each of site and remote site from six plants.

Concentration of bio-enhancer used =1.0% (w/v)

Concentration of *C. aculeatum* phytoprotein used = 2.5% (w/v)

1.0 g of Sun hamp Rosette Virus infected leaf tissue in 10 ml distilled water [virus inoculum (1:10)]

• Increased in insignificant lesion number

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Table: 1.C.1. Effect of different concentrations of milk protein (MP) on antiviral resistance inducing activity of phytoprotein obtained from roots of *Boerhaavia diffusa* (BD) in *Nicotiana tabacum* cv. samsun NN

Treatment	Response*			
	Number of local lesions/leaf			
	†Dilution of virus (TMV)			
	1:50		1:100	
	Site	Remote	Site	Remote
Control (DW)	118.8±15.2	127±14.6	78±8.9	86±9.2
A	36±4.6	61±7.5	21±1.9	39±4.7
B	24±3.4	40±5.1	15±1.6	26±3.1
C	14±1.3	25±3.6	9±1.0	16±1.4
D	9±0.8	13±1.1	5±0.6	8±0.9
E	8±0.6	14±1.2	5±0.5	7±0.6
F	121±16.1	124±13.8	73±8.3	89±8.7

DW=Distilled water

A=BD alone;

B=BD+MP (0.5%);

C=BD+MP (1.0%);

D=BD +MP (2.0%);

E=BD+MP (4.0%);

F=MP (4.0%) only;

* Data represents average number of local lesions formed on 12 leaves each of site and remote site from six plants

Concentration of *B. diffusa* phytoprotein used = 2.5% (w/v)

†1.0 g of TMV infected leaf tissue in 50/100 ml distilled water [virus inoculum (1:50, 1:100)]

Table: 1.C.2. Effect of different concentrations of milk protein (MP) on antiviral resistance inducing activity of phytoprotein obtained from leaves of *Clerodendrum aculeatum* (CA) in *Nicotiana tabacum* cv. samsun NN

Treatment	Response*			
	Number of local lesions/leaf			
	† Dilution of virus (TMV)			
	1:50		1:100	
	Site	Remote	Site	Remote
DW Control)	116±17.6	132±16.9	77±8.2	89±9.3
A	49±5.4	83±9.8	32±4.1	55±6.9
B	36±4.1	64±7.6	23±2.6	41±5.7
C	27±3.1	52±6.2	16±1.3	25±2.9
D	17±1.8	23±2.8	10±1.2	16±1.8
E	15±1.4	24±2.7	9±1.0	16±1.4
F	113±15.8	122±7.3	79±8.1	86±7.8

DW=distilled water

A=CA alone;

B=CA+MP (0.5%);

C=CA+MP (1.0%);

D=CA +MP (2.0%);

E=CA+MP (4.0%);

F=MP (4.0%) only;

* Data represents average number of local lesions formed on 12 leaves each of site and remote site from six plants

Concentration of *C. aculeatum* phytoprotein used = 2.5% (w/v)

†1.0 g of TMV infected leaf tissue in 50/100 ml distilled water [virus inoculum (1:50, 1:100)]

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Table: 1.D.1. Effect of different concentrations of milk protein (MP) on antiviral resistance inducing activity of phytoprotein obtained from roots of *Boerhaavia diffusa* (BD) in *Cyamopsis tetragonoloba*

Treatment	Response*			
	Number of local lesions/leaf			
	† Dilution of virus (SRV)			
	1:10		1:20	
	Site ± SEM	Remote ± SEM	Site ± SEM	Remote ± SEM
Control (DW)	193 ± 19.46	229 ± 17.61	117 ± 13.7	136 ± 15.6
A	46 ± 4.17	115 ± 14.65	31 ± 6.4	59 ± 8.9
B	34 ± 6.1	85 ± 9.4	23 ± 4.2	38 ± 5.2
C	20 ± 2.8	31 ± 3.1	13 ± 2.8	21 ± 3.9
D	14 ± 1.8	26 ± 2.6	7 ± 1.6	12 ± 2.8
E	13 ± 1.7	24 ± 1.9	6 ± 1.2	14 ± 2.1
F	179 ± 16.4	225 ± 30.39	106 ± 14.0	133 ± 16.6

DW= Distilled water

A=BD alone;

C=BD+MP (1.0%)

E=BD+MP (4.0%);

B=BD+MP (0.5%);

D=BD +MP (2.0%);

F=MP (4.0%) only;

* Data represents average number of local lesions formed on 6 leaves each of site and remote site from six plants.
Concentration of *B. diffusa* phytoprotein used = 2.5% (w/v)

† 1.0 g of SRV infected leaf tissue in 10/20 ml distilled water [virus inoculum (1:10, 1:20)]

Table: 1.D.2. Effect of different concentrations of milk protein (MP) on antiviral resistance inducing activity of phytoprotein obtained from leaves of *Clerodendrum aculeatum* (CA) in *Cyamopsis tetragonoloba*

Treatment	Response*			
	Number of local lesions/leaf			
	† Dilution of virus (SRV)			
	1:10		1:20	
	Site± SEM	Remote ± SEM	Site± SEM	Remote ± SEM
DW Control)	206 ± 23.6	227 ± 21.4	132 ± 14.1	146 ± 15.6
A	82 ± 11.7	149 ± 14.1	51 ± 6.6	95 ± 9.2
B	61 ± 9.1	96 ± 7.9	36±4.2	74±6.2
C	42 ± 3.9	76 ± 6.2	27 ± 1.3	58 ± 3.6
D	27 ± 2.3	56 ± 3.1	16 ± 2.1	35 ± 2.8
E	30 ± 3.7	57 ± 4.1	19 ±2.3	36 ± 3.2
F	189 ± 16.6	231 ± 20.4	128 ± 12.8	143 ± 17.4

DW=Distilled water

A=CA alone;

C=CA+MP (1.0%)

E=CA+MP (4.0%);

B=CA+MP (0.5%);

D=CA +MP (2.0%);

F=PM (4.0%) only

* Data represents average number of local lesions formed on 6 leaves each of site and remote site from six plants.
Concentration of *C. aculeatum* phytoprotein used = 2.5% (w/v)

† 1.0 g of SRV infected leaf tissue in 10/20 ml distilled water [virus inoculum (1:10, 1:20)]

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Table: 1.E.1. Induction of antiviral resistance of phytoprotein from *Boerhaavia diffusa* (BD) in *Nicotiana tabacum* cv.NP-31, *Crotalaria juncea* and *Lagenaria siceraria* against Tobacco Mosaic Virus (TMV), Sun hemp Rosette Virus (SRV) and Cucumber Mosaic Virus (CMV), respectively

Treatment	Response*			
	Time (in days) after treatment	Plant species		
		<i>N. tabacum</i> cv. NP-31	<i>C. juncea</i>	<i>L. siceraria</i>
Control (DW)	10	++	++	+
	15	+++	+++	++
	20	++++	++++	+++
BD (10% w/v)	10	+	+/-	+/-
	15	+	+	+
	20	++	++	+
BD (10% w/v) + MP (2.0%)	10	+/-	—	—
	15	+	+/-	—
	20	+	+/-	+/-
BD (20% w/v)	10	+/-	—	—
	15	+/-	+/-	—
	20	+	+/-	+/-
BD (20% w/v) + MP (2.0%)	10	—	—	—
	15	—	—	—
	20	+/-	+/-	—

* Data represents the average from 6 plants

Number of '+' signs denotes intensity of disease severity; '—' No symptoms (healthy plants); +/- feebly perceptible mosaic; + mild mosaic; ++ severe mosaic; +++ severe mosaic with leaf size reduction; ++++ very severe mosaic and stunted growth.

Virus dilution = 1:2 (1.0 g TMV/SRV/CMV infected leaf tissue in 2 ml distilled water)

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Table: 1.E.2. Induction of antiviral resistance of phytoprotein from *Clerodendrum aculeatum* (CA) in *Nicotiana tabacum* cv.NP-31, *Crotalaria juncea* and *Lagenaria siceraria* against Tobacco Mosaic Virus (TMV), Sun hemp Rosette Virus (SRV) and Cucumber Mosaic Virus (CMV), respectively

Response*				
Treatment	Time (in days) after treatment	Plant species		
		<i>N. tabacum</i> cv. NP-31	<i>C. juncea</i>	<i>L. siceraria</i>
DW Control	10	++	++	+
	15	+++	+++	++
	20	++++	++++	+++
CA (10% w/v)	10	+	+	+/-
	15	+	+	+/-
	20	++	++	+
CA (10% w/v) + MP (2.0%)	10	+/-	+/-	—
	15	+	+/-	+/-
	20	+	+	+
CA (20% w/v)	10	+/-	+/-	—
	15	+	+	+/-
	20	++	++	+
CA (20% w/v) + MP (2.0%)	10	—	—	—
	15	+/-	+/-	—
	20	+/-	+	+/-

* Data represents the average from 6 plants

Number of '+' signs denotes intensity of disease severity; '—' No symptoms (healthy plants);

+/- feebly perceptible mosaic; + mild mosaic; ++ severe mosaic; +++ severe mosaic with leaf size reduction; ++++ very severe mosaic and stunted growth.

Virus dilution = 1:2 (1.0 g TMV/SRV/CMV infected leaf tissue in 2 ml distilled water)

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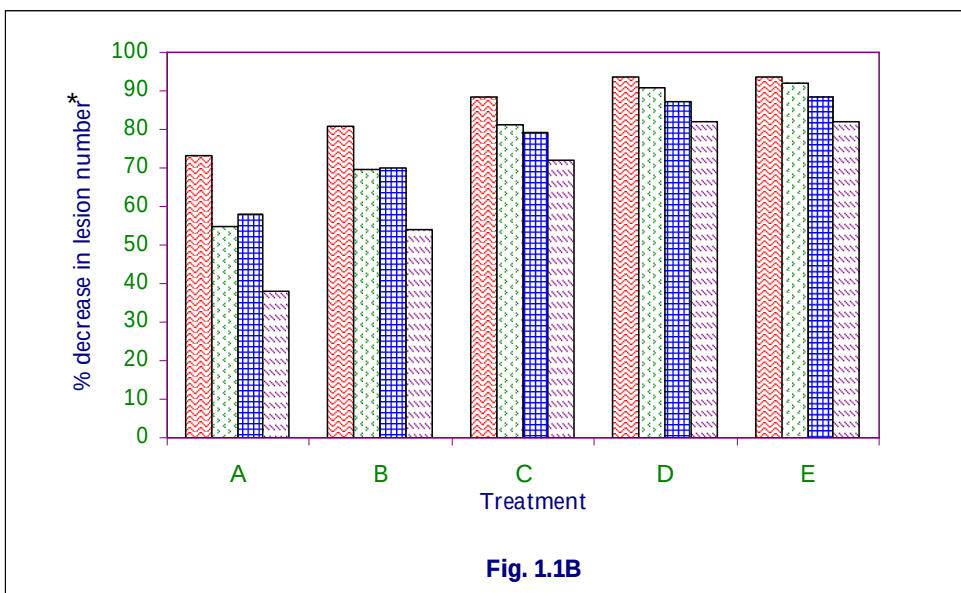
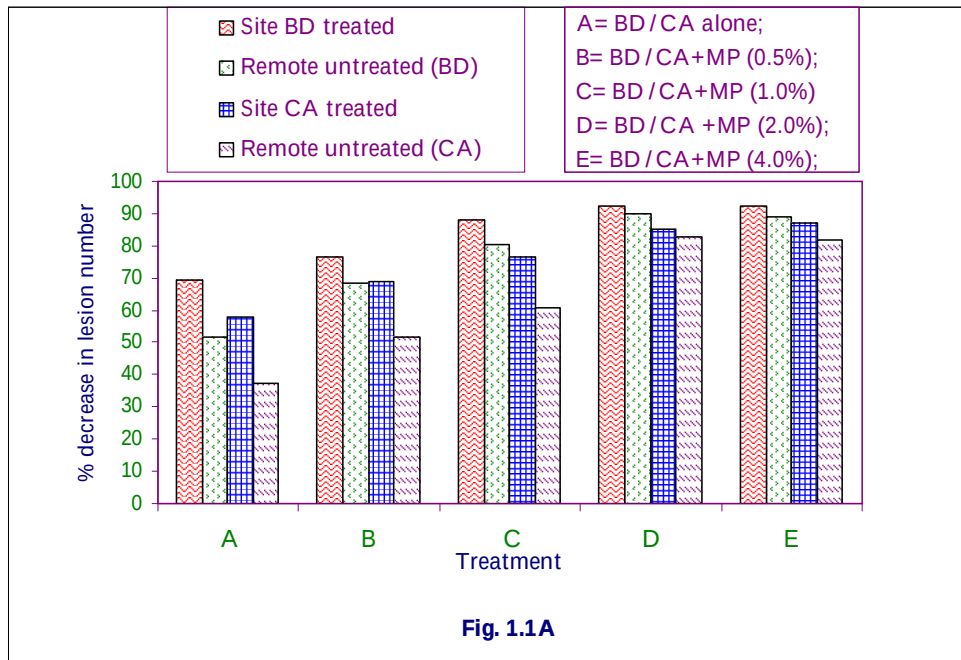


Fig. 1.1 Effect of using different concentration of milk protein on antiviral resistance induction by phytoproteins from *Boerhaavia diffusa* and *Clerodendrum aculeatum* in *Nicotiana tabacum* cv. Samsun NN

* Challenge inoculation were made using TMV at 1:50 (v/v) dilution (Fig.1.1A) and 1:100 (v/v) dilution (Fig.1.1B)

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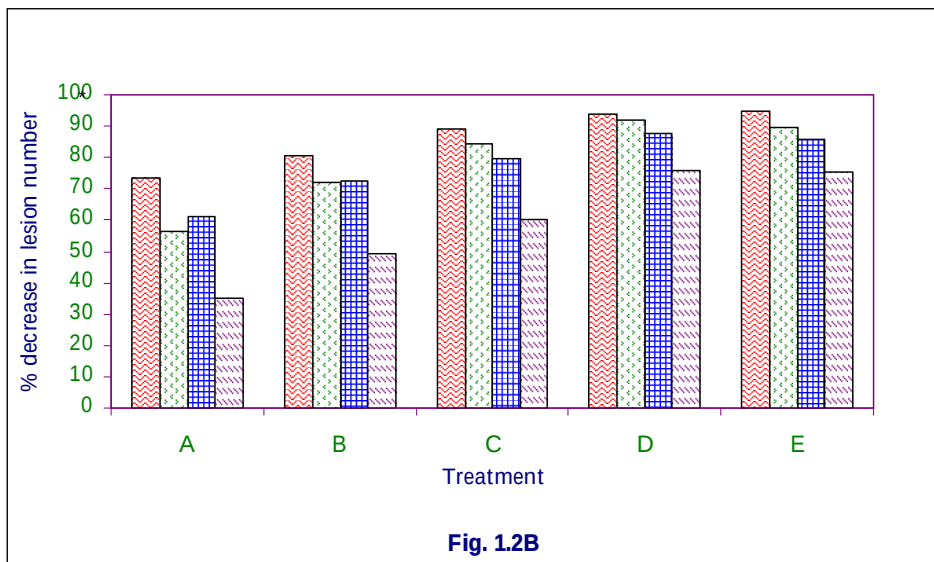
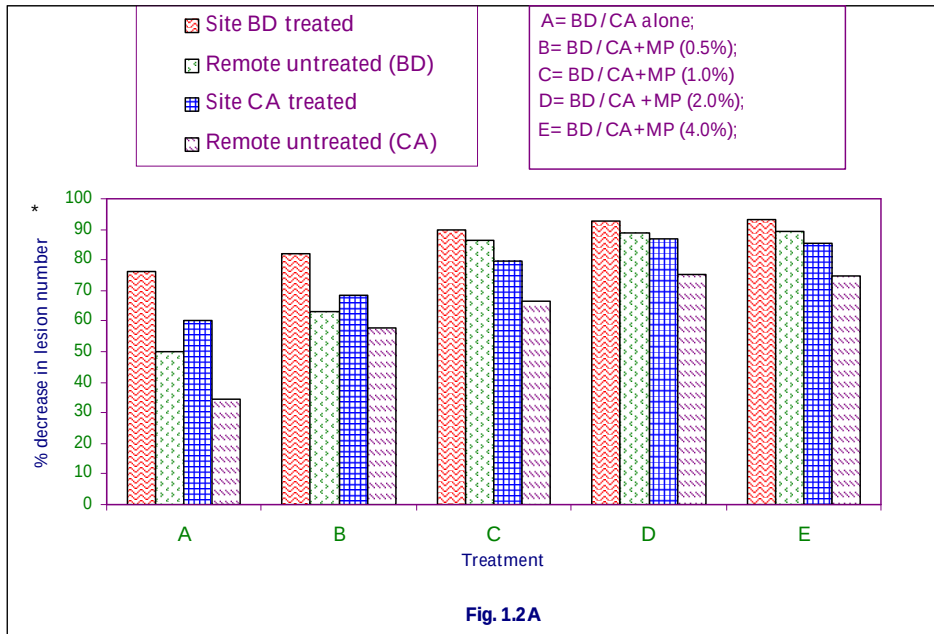


Fig. 1.2 Effect of using different concentration of milk protein on antiviral resistance induction by phytoproteins from *Boerhaavia diffusa* and *Clerodendrum aculeatum* *Cyamopsis tetragonoloba*
* Challenge inoculation were made using SRV at 1:10 (v/v) dilution (Fig.1.2A) and 1:20 (v/v) dilution (Fig.1.2B)

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V. CONCLUSION

1. Adding bio-enhancers to phytoproteins and then applying it to plants provided plants with better resistance towards the subsequent viral infection. The effectiveness of various bio-enhancers was variable and amongst all those used, milk protein gave best response when mixed with phytoproteins.
2. Mixing bio-enhancer at a strength of 2.0% was found to be optimum and further increase in strength did not have any significant effect on the resistance inducing ability of these phytoproteins.
3. The development of symptoms was markedly delayed in systemic hosts treated with milk protein added to phytoproteins.
4. Addition of bio-enhancer to BD phytoprotein provided plants with better resistance towards viral infection than when it was added to CA phytoprotein.

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