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Prevention and control of viral diseases in watermelon through botanical biopesticides

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Abstract

In the experiments, conducted for the prevention and control/eco-friendly management of the viral diseases of watermelon, through botanical biopesticides, seed treatment followed by 6 foliar sprays with *B. diffusa* root extract was found most effective against natural infection of viral disease(s) as compared to seed treatment followed by six foliar sprays with *A. indica*, seed treatment followed by six foliar sprays with *C. aculeatum* and seed treatment followed by six foliar sprays with *T. arjuna*. The appearance of disease symptoms was significantly delayed with six foliar sprays along with seed treatment with *B. diffusa* root extract followed by six foliar sprays along with seed treatment with *A. indica*, six foliar sprays of *B. diffusa*, six foliar sprays along with seed treatment with *C. aculeatum*, six foliar sprays of *A. indica*, six foliar sprays of *C. aculeatum*, six foliar sprays along with seed treatment with *T. arjuna* bark extract. A gradual decrease in disease incidence was recorded along with corresponding increase in number of sprays of the antiviral agents. Minimum disease incidence was recorded in plots, which have seed treatment followed by six foliar sprays of *B. diffusa* root extract and was significantly lower over rest of the treatments. As the number of sprays increased along with seed treatment, a gradual increase in vine length, number of fruits plant^{-1}, fruit diameter, fruit weight and fruit yield were recorded in all botanical treatments. Most effective botanical found was *B. diffusa* root extract which exhibited maximum vine length, number of fruits plant^{-1}, fruit diameter, fruit weight and fruit yield followed by *A. indica* leaf extract, *C. aculeatum* leaf extract and *T. arjuna* bark extract.

Introduction

Watermelon [*Citrullas lanatus* (Thumb) Mastum and Nakai, Synonyms: *C. vulgaris*] is one of the most popular desert fruit grown all over world. In India it is grown, mostly as riverbed side crop, in Uttar Pradesh, Rajasthan, Gujarat, Maharastra, Punjab and Haryana. This crop is infested by many biotic and abiotic factors and caused great economic losses. The low productivity of watermelon is mainly due to many diseases incited by viruses, fungi, bacteria, nematodes and phytoplasma. A large number of viruses have been reported from time to time to infect this crop *viz*., watermelon mosaic virus-1 [1], watermelon mosaic virus-2 [2], zuchini yellow mosaic virus [3], cucumber mosaic virus [4-9], papaya ring spot virus [10], watermelon chlorotic stunt virus [11] melon necrotic spot virus [12], watermelon silver mottle virus [13], watermelon bud necrosis virus [16] and watermelon curly mottle virus [14], which induced mild to severe mosaic, yellowing, chlorosis, necrosis, puckering, distortion, mottling and stunting symptoms. Severely infected plants did not bear flowers and could not produce fruits. In a very few cases, if flowers appeared, very small sized distorted fruits were produced.

Plant viruses are persistent threat to production of watermelon in most of the countries including India. Although severe outbreaks of viral diseases tend to occur on a sporadic basis, many plant viruses are ubiquitous in nature and can result in severe outbreaks under favorable conditions. Management of viruses is usually limited to the availability of resistant varieties, besides vector control. However, commercially acceptable varieties that are also resistant to particular virus or several commonly occurring viruses often are not available. Therefore, the solution for the management of viral diseases of watermelon lies in the strategies of the integration of several methods, such as chemical, cultural, varietal, and botanical to control both viruses and their vectors. Synthetic agro chemicals commonly used to kill insect vectors, for the management of viral diseases, cause environmental pollution, health hazards and phytotoxicity besides their very high cast. Use of such agrochemicals can be avoided by some preventive measures/immunization through antiviral substance of plant origin, botanical biopesticides [15-19]. These substances are nonchemical, nonhazardous, easily biodegradable, did not leave any residual effect on soil, water and environment and are eco-friendly, besides their very low cast [20-24].

The objective of the present investigation was to find out ecofriendly management strategies for viruses infecting watermelon through botanical biopesticides in Indian context.

Materials and methods

The experiments were conducted at Horticulture Research Farm, N.D. University of Agriculture and Technology, Faizabad, India. Experiment layout was Randomized block design (RBD) with fourteen treatments and three replications. Experimental lay out was made as per treatments and replications. Forty-two plots were laid out for this experiment. The plot size was 6 × 4.5 m (27 m^2) accommodating 9 plants in each plot. The main irrigation channel was provided in eastern side of the experimental field and the sub irrigation channels were prevailed between two replications of the treatment. Seeds of variety Sugar Baby were sown separately in 6m × 4.5m plots with row to row distance of 2 m and plant to plant distance of 1.5 meter. Treatments details are given below:

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Treatments details

1. The experiments were conducted with 14 treatments and 3 replications as below.

T_1=Seed treatment with *Boerhaavia diffusa* root extract (BD) @ 10%

T_2=Seed treatment with *Clerodendrum aculeatum* leaf extract (CA) @ 10%

T_3=Seed treatment with *Azadirachta indica* leaf extract (AI) @ 10%

T_4=Seed treatment with *Terminalia arjuna* bark extract (TA) @ 10%

T_5=Foliar sprays of *Boerhaavia diffusa* root extract (BD) @ 10%

T_6=Foliar sprays of *Clerodendrum aculeatum* leaf extract (CA) @ 10%

T_7=Foliar sprays of *Azadirachta indica* leaf extract (AI) @ 10%

T_8=Foliar sprays of *Terminalia arjuna* bark extract (TA) @ 10%

T_9=T1+T5

T_{10}=T2+T6

T_{11}=T3+T7

T_{12}=T4+T8

T_{13}=Control (water alone)

T_{14}=foliar sprays of insecticides

2. Variety– Sugar baby

3. Design– Randomized block design (RBD)

4. Replications–Three

Preparation of botanical bio pesticides

Roots of *Boerhaavia diffusa* L., leaves of *Clerodendrum aculeatum* L. and *Azadirachta indica* L. and bark of *Terminalia arjuna* L. were collected separately and allowed to dry under shade at room temperature. Dried roots, leaves or bark were ground separately to powder and stored at low temperature. The crude extracts, in each case, were prepared by making the suspension of root, leaf or bark powder separately in the tap water (1g/10 ml). The pulp was stained through two folds of cheese cloth and the homogenate was clarified by centrifugation at 8,000g for 15 minutes. The supernatants obtained following the procedure as described earlier [20,21,23,24], were used for experimental work [16,17].

Procedure of application/treatments

i. Seed treatment: One hundred seeds of watermelon variety, sugar baby were soaked either in the root extract of *B. diffusa*, leaf extract of *C. aculeatum* or *A. indica* or the bark extract of *T. arjuna*, for 30 minutes to one hour. Seeds were then taken out from treatment solutions and spread over a gunny bag, under shade for about two hours. Sowing was done in the afternoon. Seeds soaked exactly in the same way in water for the same time served as control. The treated and untreated seeds were sown in 6 x 4.5 meter plots with row to row distance of 2 m and plant to plant distance of 1.5 meter.

ii. Field sprayings: The first spray of plant extract, in each case separately, was done 4-6 days after germination at cotyledons stage (2 leaf stage) followed by second, third, fourth, fifth and sixth sprayings at fortnightly intervals. In control plots, water alone was sprayed instead of plant extracts. Insecticide Endosulphan @ 1ml/L water was sprayed to eliminate insect vectors, if any.

Observations recorded

Observations were recorded on first appearance of disease, disease incidence, and plant height, number of fruits, fruit yield and yield losses.

First appearance of disease: Observations were recorded at regular intervals for the first appearance of disease symptoms in treated and un-treated plots separately.

Disease Symptoms: Symptoms were recorded at regular time interval in all the treatments on the basis of visual observations.

- = No symptoms

+ = Very mild symptoms

++ = Mild symptoms

+++ = Moderate symptoms

++++ = Severe symptoms

++++++ = Very severe symptoms

Disease incidence: The number of plants infected, with naturally occurring watermelon viruses, out of total number of plants in a plot, was recorded. Per cent disease incidence and per cent disease control were calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants per plots}}{\text{Number of plants (disease + healthy) per plants}} \times 100$$

$$\text{Per cent disease control (PDC)} = \frac{\text{C - T}}{\text{C}} \times 100$$

Vine length: Length of vine in meter was recorded from the ground level to tip of the vine at the last picking (maturing stage).

Number of fruits per plant: The total number of fruits were counted at each picking separately and summed up for all pickings for a plot. Number of fruits per plant was calculated by dividing total number of fruits in a plot with number of plants in a plot.

Fruit diameter: Diameter of fruits selected randomly in plots of treated and control replications were measured in centimeter.

Fruit weight: Five fruits randomly selected in each treatment were weighted. The fruit weight was calculated with total weight of five fruits divided by total number of fruits.

Fruit yield/plot: Total fruits yield of all the pickings for each plot was recorded in kg.

Avoidable yield losses: Yield loss was calculated by the following formula.

$$\text{Yield loss (\%)} = \frac{\text{Yield of protected plant - yield of unprotected plant}}{\text{Yield of protected plants}} \times 100$$

Statistical analysis: Data obtained for each treatment were statistically analyzed following the procedure of Randomized Block Design. Calculation was made after applying the test of significance of means. The percentage data for disease incidence was transformed to Archsine [25].

$$\text{Sin}^{-1} = \sqrt{\text{Percent disease incidence before statistical analysis}}$$

Results

Disease initiation

Experimental data presented in Table 1 have clearly indicated that all the extracts significantly delayed the appearance of disease symptoms

Table 1. Effect of botanicals on disease initiation and disease incidence in watermelon plants.

Treatments	Disease initiation (DAS)	Disease incidence (%)	Reduction in disease incidence (%)
T_1 = Seed treatment with *Boerhaavia diffusa* root extract (BD)	20.00	75.28	8.58
T_2 = Seed treatment with *Clerodendrum aculeatum* leaf extract (CA)	19.50	77.85	5.46
T_3 = Seed treatment with *Azadirachta indica* leaf extract (AI)	20.00	76.76	6.78
T_4 = Seed treatment with *Terminalia arjuna* bark extract (TA)	19.00	78.58	4.57
T_5 = Foliar sprays of BD	48.75	50.06	39.23
T_6 =Foliar sprays of CA	36.00	59.88	27.28
T_7 =Foliar sprays of AI	43.50	53.73	34.75
T_8 =Foliar sprays of TA	28.50	62.68	23.88
$T_9 = T_1+T_5$	66.50	37.68	54.24
$T_{10} = T_2+T_6$	47.75	44.77	45.63
$T_{11} = T_3+T_7$	58.00	39.46	52.08
$T_{12} = T_4+T_8$	35.25	53.70	34.79
T_{13} = Control	18.50	82.35	0.00
T_{14} = Foliar spray of insecticide	70.50	35.35	57.07
CD at 5%	1.988	3.045	-
SEm ±	0.683	1.047	-

at all the doses. However, 6 foliar sprays along with seed treatment with *B. diffusa* root extract was found most effective treatment which delayed appearance of symptom till 66.50 days after sowing (DAS) followed by 6 foliar sprays along with seed treatment with *A. indica* (58.00 DAS), 6 foliar sprays of *B. diffusa* (48.75 DAS), 6 foliar sprays along with seed treatment with *C. aculeatum* (47.75 DAS), 6 foliar sprays of *A. indica* (43.50), 6 foliar sprays of *C. aculeatum* (36.00 DAS), 6 foliar sprays along with seed treatment with *T. arjuna* bark extract (35.25 DAS), 6 foliar sprays of *T. arjuna* (28.50 DAS) seed treatment with *B. diffusa* and *A. indica* (20.00 DAS), seed treatment with *C. aculeatum* (19.50 DAS) and seed treatment with *T. arjuna* (20.00 DAS) as compared to untreated plants (18.50 DAS), respectively. However, in the plants having insecticides treatment the disease(s) initiation recorded was 70.50 DAS.

Disease incidence

Results presented in Table 1 revealed a gradual decrease in disease incidence with corresponding increase in number of sprays with *B. diffusa* root extract, *C. aculeatum* leaf extract *A. indica* leaf extract and *T. arjuna* bark extract (TA). Minimum reduction in disease incidence (54.24) percentage was recorded in seed treatment followed by 6 foliar sprays with *B. diffusa*, followed by seed treatment and 6 foliar sprays of *A. indica* leaf extract (52.08%) T_{10} (45.63%), T_5 (39.23%), T_{12} (34.79%), T_7 (24.75%), T_6 (34.75%), T_8 (23.88%), T_1 (8.58%), T_3 (6.78%), T_4 (4.57%) and T_2 (5.46%) as compared to control. However, 57.07% reduction in disease was recorded plants treated with insecticide (Figure 1, 2 and 3).

Disease symptoms

Symptoms severity was reduced in watermelon plants, treated with extracts from these plants.

Vine length (m)

Significant increase in number of vine length was recorded with all the botanicals. Maximum increase in vine length (3.93 m) was recorded in T_9 (seed treatment followed by 6 foliar sprays of *B. diffusa* root extract) which was at par with the treatment T_{11} (3.70m) and significantly superior over rest of the treatments. On the other hand, maximum increase in vine length (106.84%) was observed in the treatment T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) followed by T_{11} (97.43%), T_5 (85.26%), T_{10} (67.89%), T_7 (653.26%), T_{12} (29.47%), T_{13} (13.15%), T_8 (12.105) and T_4 (2.565). However, in insecticide treated plants 114.73 per cent increase in vine length was recorded (Table 2).

Number of fruits plant^{-1}

It is evidenced from the results presented in Table 2 that the significant increase in number of fruits per plant was recorded with all the botanicals. Maximum number of fruits per plant (2.60) was recorded in T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) which was at par with T_{11} (2.36) and significantly superior over rest of the treatments. On the other hand, maximum increase in number of fruits per plant (120.33%) was observed in treatment T_9 (seed treatment followed by 6 foliar sprays with *B. diffusa* root extract) followed by T_{11} (100.00%), T_{10} (78.81%), T_5 (77.96%), T_{12} (61.01%), T_7 (56.77%), T_6 (48.30%), T_8 (23.72%), T_1 (19.49%), T_3 (16.94%), T_2 (11.86%), T_4 (5.93%) as compared untreated plant. However, an increase in fruit per plant was also recorded in insecticide treated plant (130.05%).

Fruit diameter (cm)

A gradual increase in fruit diameter was recorded in all the botanicals with increase in number of sprays. Maximum fruit diameter (21.16 cm) was recorded in the treatment T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) which was at par with T_{11} (seed treatment and six foliar spray with *A. indica*) and significantly superior rest of the treatments. On the other hand, maximum increase in fruit diameter (94.01%) was recorded in treatment T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) as compared to other treatments (Table 2).

Fruit weight (kg)

A gradual increase in fruit weight was recorded in all the botanicals with increase in number of sprays. Maximum fruit weight (3.62 kg/plant) was recorded in treatment T_9 (seed treatment and six foliar sprays with *B. diffusa* root extract) which was at par with T_{11} (seed treatment and 6 foliar sprays of *A. indica*), T_5 (foliar sprays of *B. diffusa*) and significantly superior over rest of the treatments. On the other hand, maximum increase in fruit weight (214.78%) was recorded in treatment T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) as compared to the other treatments (Table 3).

Fruit yield plot^{-1} (kg)

Result present in the Table 3 indicated that significant increase in fruit yield per plant was recorded in all the botanicals with increasing in number of sprays. Maximum fruit yield per plot (78.39 kg/plot) was recorded in treatment T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) which was significantly superior over rest of the treatments. On the other hand, maximum increase in fruit yield (160.43%) was recorded in treatment T_9 (seed treatment followed by six foliar sprays with *B. diffusa* root extract) as compared to the other treatments. However, in insecticide treated plant fruit yield per plant 65.25 kg/plot was recorded.

Table 2. Effect of botanicals on vine length, No of fruits/plant and fruit diameter of watermelon.

Treatments	Vine length (m)	Increase in vine length (%)	No of fruits/plant	Increase in Number of fruits/plant (%)	Fruit diameter (cm)	Increase in fruit diameter (%)
T_1 = Seed treatment with *Boerhaavia diffusa* root extract (BD)	2.22	16.84	1.41	19.49	11.85	16.7
T_2 = Seed treatment with *Clerodendrum aculeatum* leaf extract (CA)	2.05	15.00	1.32	11.86	11.09	9.47
T_3 = Seed treatment with *Azadirachta indica* leaf extract (AI)	2.15	13.15	1.38	16.94	11.58	14.31
T_4 = Seed treatment with *Terminalia arjuna* barkextract (TA)	1.95	2.56	1.25	5.93	10.59	4.54
T_5 = Foliar sprays of BD	3.52	85.26	2.10	77.96	18.10	78.67
T_6 =Foliar sprays of CA	2.40	26.31	1.75	48.30	15.01	48.17
T_7 =Foliar sprays of AI	3.14	65.26	1.85	56.77	14.29	41.06
T_8 =Foliar sprays of TA	2.13	12.10	1.46	23.72	13.25	30.79
T_9 =T_1+T_5	3.93	106.84	2.60	120.33	21.16	99.01
T_{10} =T_2+T_6	3.19	67.89	2.11	78.81	18.78	46.05
T_{11} = T_3+T_7	3.70	94.73	2.36	100.00	20.05	97.92
T_{12} = T_4+T_8	2.46	29.47	1.90	61.01	15.13	49.35
T_{13} = Control	1.90	0.00	1.18	0.00	10.13	0.00
T_{14} = Foliar spray of insecticide	4.08	114.73	2.75	133.05	19.65	93.97
CD at 5%	0.331	-	0.307	-	1.525	-
SEM ±	0.114	-	0.105	-	0.524	-

Table 3. Effect of botanicals on fruit weight, fruit yield of watermelon fruits.

Treatments	Fruit weight (kg/plant)	Increase in fruit weight (%)	Fruit yield (kg/plot)	Increase in fruit yield (%)	Avoidable yield loss (kg/plot)	Avoidable Yield loss (%)
T_1 = Seed treatment with *Boerhaavia diffusa* root extract (BD)	1.75	52.17	39.15	30.06	9.05	23.11
T_2 = Seed treatment with *Clerodendrum aculeatum* leaf extract (CA)	1.45	26.08	34.70	15.28	4.6	13.25
T_3 = Seed treatment with *Azadirachta indica* leaf extract (AI)	1.60	39.13	37.29	23.88	7.19	19.28
T_4 = Seed treatment with *Terminalia arjuna* barkextract (TA)	1.33	15.65	32.65	8.47	2.55	7.81
T_5 = Foliar sprays of BD	3.00	160.86	68.34	127.0	38.24	55.95
T_6 =Foliar sprays of CA	2.65	130.43	54.00	79.40	23.90	49.25
T_7 =Foliar sprays of AI	2.81	144.34	62.27	106.87	32.17	51.66
T_8 =Foliar sprays of TA	2.00	73.91	45.85	52.32	15.75	34.35
T_9 =T_1+T_5	3.62	214.78	78.39	160.43	48.29	61.60
T_{10} =T_2+T_6	2.89	151.30	62.82	108.70	32.72	52.08
T_{11} = T_3+T_7	3.40	195.65	71.46	137.40	41.36	57.87
T_{12} = T_4+T_8	2.39	107.82	50.45	67.60	20.35	40.33
T_{13} = Control	1.15	0.00	30.10	0.00	10.00	0.00
T_{14} = Foliar spray of insecticide	3.20	178.26	65.25	116.77	35.15	53.86
CD at 5%	0.629	-	2.787	-	-	-
SEM ±	0.216	-	0.958	-	-	-

Avoidable yield loss (%)

Maximum avoidable yield loss (61.60%) was recorded in T_9 (seed treatment + 6 foliar sprays with *B. diffusa* root extract) which was significantly higher over rest of the treatments. Minimum per cent avoidable yield losses was recorded in the treatment T_{13} (control) which was significantly lower over rest of the treatments. However, in insecticide treated plants, avoidable yield loss recorded was 53.86% (Table 3). Attempts were made earlier for the management of viral diseases in watermelon crop through insecticides to prevent the movement of insect vectors. But all insecticides caused environmental pollution, health hazards and phytotoxicity. Use of these insecticides cannot be eliminated but can be avoided by some preventive measures including antiviral agents of plant origin. These antiviral agents of plant origin are non-chemicals, non-hazardous, easily bio-degradable, eco-friendly and did not have a residual effect.

Discussion

The antiviral potential of the botanical biopesticides isolated from a few higher plants has been established through the extensive work conducted by various workers [16-19,24,26-37]. The virus inhibitory potential of phytoprotein was demonstrated by the ability of phytoprotein to prevent the formation of necrotic lesions in hypersensitive hosts and in delaying the development of disease specific symptom in systemic hosts. This type of induction of resistance in plants has been referred to as induced systemic resistance (ISR).

The antiviral properties of the plant extracts have been shown due to certain novel proteins present in the extracts [29,38]. These proteins can be applied in the form of aqueous extracts of the plant part as purified proteins, the latter being more effective [18,19,30,32,39]. The precise mechanism of protection offered by these phytoproteins

Figure 1. Effect of botanical pesticides on mosaic disease of watermelon.

Figure 2. Effect of botanical pesticides on mosaic disease of watermelon.

Figure 3. Effect of botanical pesticides on mosaic disease of watermelon.

has been worked out [26] and it has been shown that application of phytoproteins, induced formation of some virus inhibiting agent (VIA) in the treated plants. The VIA formation is sensitive to Actinomycin D, implying that VIA is a protein [29,40].

An interesting virus disease preventing system that is based on a natural insect repellent rather than on a virus inhibitor has been developed by Awasthi and Rizvi [41]. Plant products were found to activate the defense system in susceptible plants. Spraying of extracts of non-host plants like *B. diffusa, Clerodendrum aculeatum* and a few others on susceptible plants like tobacco, tomato, and potato etc. induced systemic resistance in these plants towards subsequent virus infection. The development of macro molecules like polypeptides, glycoprotein or protein in the sprayed host has been responsible for acquiring the resistance [29,30].

It is clear from the results that all the botanicals were effective in reducing disease incidence, symptom severity and their delayed appearance. The protective effect of these botanicals was more pronounced, if the number of sprays was increased from two to six. It was evident that plants which received six sprays were found to have highest reduction in disease incidence and gave highest biomass and yield. Similar results were obtained by Verma *et al.* [26] and Verma and Singh [42] on mungbean and urdbean through leaf extract of *B. diffusa* and *C. aculeatum*. Verma and Verma [43] also reported the management of some disease of mungbean and urdbean through leaf extract of *C. aculeatum.* The inhibitory effect of *B. diffusa* and *C. aculeatum* may be due to resistance inducers present in the plants induced systemic resistance against several viruses in hypersensitive as well as systemic hosts [22,44,45]. The inhibitory effect of *B. diffusa* root extract may be attributed in blocking of host cell receptors or to interference of virus synthesis in the host cell.

It has been demonstrated, that the glycoproteinaceous inhibitor present in *B.diffusa* when applied before virus inoculation induced synthesis of some translocable virus inhibitory or protective substances in the host plants [21]. The mechanism of virus inhibition by *B. diffusa* was studied and it was speculated that it alters the physiology of host cell in such a way that host cells no longer can support virus multiplication and ultimately the virus multiplication is affected to a great extent [23]. No other properties are required than that inhibitors differ from normal component of cells and because of this stimulate the cells to unusual activity. In present case also multiple sprays of *A. indica* on cucumber plants induced host cells to resist infection and reduced virus multiplication. Only very mild and delayed symptoms appeared on plants which received six sprays.

We have also observed in our findings that fortnightly sprayings of the extract from *A. indica ,B.diffusa , C. aculeatum* and *T .arjuna* reduced disease incidence and increased the yield. Thus, weekly / fortnightly sprays with partially purified concentrated preparation of antiviral agents, the disease incidence can be considerably lowered and yield of plants markedly improved [9,30].These statements support our experimental findings. Furthermore, repeated and continuous use of these plant products is not associated with any health hazards and environmental pollution.

It is clear from the forgoing discussion that watermelon crop may be protected against infection and spread of a complex infection of gemini, cucumo and poty viruses by the multiple sprays of *B.diffusa*, *A. indica, C. aculeatum* and *T. arjuna* since cotyledon stage. Antiviral substance isolated from these plants, not only prevented viral infection but also enhanced the growth of plants, as we have observed better plant growth, significant increase in number of branches, flowering, fruiting and higher yields.

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