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Induction of Systemic Resistance Through Antiviral Agents of Plant Origin Against Papaya Ring Spot Virus Disease in Papaya (*Carica papaya* L.)

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Abstract: Both the antiviral agents namely *Boerhaavia diffusa* root extract and *Clerodendron aculeatum* leaf extract were found significantly effective in inducing systematic resistance against *papaya ring spot virus* in papaya. However, *B. diffusa* root extract showed more efficient properties as compared to *C. aculeatum* leaf extract. Maximum reduction in disease incidence (72.25 and 74.00 %) and maximum plant height (119.05 and 126.61 cm), number of fruit (20.04 and 24.05 plant^{-1}) and fruit yield (22.86 and 25.93 Kg plant^{-1}) were observed with the application of *B. diffusa* root extract as seed treatment + nursery treatment + field treatment during 2005-06 and 2006-07 respectively. However, maximum disease incidence (78.79 and 70.56 %) and minimum plant height (87.46 and 92.40 cm), number of fruit (11.36 and 13.50 plant^{-1}) and fruit yield (9.85 and 11.03 kg plant^{-1}).were recorded with the application of *B. diffusa* root extract as seed treatment + nursery treatment + field treatment during both the years.

Key words: Papaya · *Carica papaya* · Papaya ringspot virus · *Boerhaavia diffusa* · *Clerodendron aculeatum* · Antiviral agents · Botanicals

INTRODUCTION

Papaya (*Carica papaya* L.), is an herbaceous tree belonging to family Caricaceae, is a native of Tropical Central America, is being cultivated throughout the tropical and subtropical countries of the World. India is the second largest producer of papaya fruits in the World after Brazil. It is known as wholesome fruit which rank first in respect to nutritional, medicinal and nourishment properties. The unripe fruits are used for extraction of papain, which has proteolytic enzymatic activity [1].

The total area under cultivation of papaya has recorded a regular increase in the recent past but its production has not shown corresponding increase. This might be due to the losses caused by various diseases incited by fungi, bacteria, phytoplasma and viruses. Among these, viral diseases are the limiting factors of papaya cultivation in Northern India. Large numbers of viruses belonging to cucumo-, Gemini-, ilar-, poty-, rhabdo-, tobra- and tospo- virus group have been recorded time to time on papaya. Among them, *papaya leaf curl virus* (*Begomovirus*) and *papaya ring spot virus* (Potyvirus) are more prevalent in India. Papaya ring spot cause heavy loss of 40-90% depending upon the time of infection and age of the plant.

The viral diseases of plants cannot be controlled but can be avoided by some preventive measures like induced systemic resistance through antiviral agents of plant origin. *Boerhaavia diffusa* grows abundantly as a weed and possess a big problem for farmers, if this plant is used as an antiviral agent, the problem of virus management as well as weed control may be solved simultaneously with least financial input. Antiviral properties are present in *B. diffusa* and *C. aculeatum*. They may be easily absorbed into the leaves on which it is sprayed and translocated in whole of the plant to induce the production or synthesis of some proteins which are actually antiviral and defend the plan against infection. Because of its phytochemical nature, it has many other advantages over chemical as it is easily biodegradable does not leave any residue, eco-friendly, non phyto-toxic, easily absorbed by the plant and cheap. Therefore, keeping in view the above experiment entitled "induction of systemic resistance through antiviral agents of plant origin against papaya ring spot virus disease in papaya (*Carica papaya* L.) were carried out".

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MATERIALS AND METHODS

The experiments were conducted with fifteen treatments and three replications under Randomized Block Design (R.B.D.). The plot size was 8 x 6 m (48 m^2) accommodating 12 plants in each plot with spacing of 2x2 m.

Roots of *Boerhaavia diffusa* (BD) and leaf of *Clerodendron aculeatum* (CA) were collected and allowed to dry under shade at room temperature. Dried roots/leaves were ground to powder and stored at low temperature. The crude extracts were prepared by making the suspension of root or leaf powder in distilled water (1g/10 ml). After 24 hrs, pulp was strained through two folds of cheese cloth and the homogenate was clarified by centrifugation at 3000 rpm for 15 minutes. The supernatants were used for experimental works. Milk protein was mixed with antiviral agents before using of solution.

Three hundred seeds of each papaya cultivar viz. Coorg honeydew, Washington, Pusa Giant and Pusa Dwarf were soaked in BD/CA solution for 24 hrs. In controls, three hundred seeds of each variety were soaked in water for the same time. These seeds of each cultivar were sown separately in polythene bags filled with sandy loam soil + FYM (10:1) mixture.

In the nursery, first spray of BD/CA @ 5% was done at two leaf stage of seedlings. The second and third sprays of *B. diffusa* root extract and *C. aculeatum* leaf extract were done at weekly intervals following the first spraying. In control plots, water alone was sprayed instead of antiviral agents. Treated and untreated seedlings were transplanted in three replications in the field as per treatments.

In the field, ten sprays of BD/CA solution were done. The first spray of BD/CA solution@ 5% was done at 15 days after planting and rest of the sprays were done at fortnightly intervals following the first spraying. In control plots, milk protein alone was sprayed instead of antiviral agents. Observations were recorded on disease incidence, plant height, number of fruits and fruit yield at fruit ripening stage.

RESULTS AND DISCUSSION

Disease Incidence: Experimental data clearly indicate that minimum disease incidence (21.86, 18.35%) was observed under treatment T_{14} (seed + nursery + field treatments with *B. diffusa* root extract) which was at par with T_{12} (nursery + field treatments with *B. diffusa* root extract) and significantly lower over rest of the treatments during 2005-06 and 2006-07. On the other hand, maximum reduction in disease incidence of 72.25 and 74.00 per cent were recorded with the application of treatment T_{14} followed by T_{12}, T_{10} (seed + field treatments with *Boerhaavia diffusa* root extract), T_{13} (seed + nursery + field treatments with *C. aculeatum* leaf extract), T_9 (seed + nursery + field treatments with *C. aculeatum* leaf extract), T_8, T_{11}, T_9, T_7, T_6, T_5, T_4, T_3, T_2 and T_1 during both the years.

Table 1: Effect antiviral agents plant origin on incidence of papaya ring spot virus disease under field condition (Average of four varieties)

	2005-06		2006-07		2005-06		2006-07	
Treatments	Disease incidence (%)	Disease reduction (%)	Disease Incidence (%)	Disease reduction (%)	Plant Height (cm)	Per cent Increase	Plant Height (cm)	Per cent Increase
T_1 = Seed treatments with CA	72.03	8.58	64.35	8.80	89.21	2.00	94.36	2.12
T_2 = Seed treatments with BD	69.82	11.38	61.99	12.15	90.39	3.35	95.65	3.52
T_3 = Three foliar sprays of CA in nursery	68.38	13.21	60.41	14.38	92.24	5.46	97.48	5.50
T_4 = Three foliar sprays of BD in nursery	64.13	18.61	57.95	17.87	92.85	6.16	98.26	6.34
$T_5 = T_1 + T_3$	63.70	19.15	56.16	20.41	93.49	6.90	98.84	6.97
$T_6 = T_2 + T_4$	57.01***	27.64	51.85***	26.52	96.07***	9.84	101.64***	10.00
T_7 = Ten foliar sprays of CA in field	44.98	42.91	39.37	44.20	104.02	18.93	109.16	18.14
T_8 = Ten foliar sprays of BD in field	36.37	53.84	32.44	54.03	109.33	25.00	116.64	26.20
$T_9 = T_1 + T_7$	40.97	48.00	35.71	49.39	106.56	21.84	114.81	24.25
$T_{10} = T_2 + T_8$	31.32	60.25	28.75	59.25	112.70	28.86	120.35	30.25
$T_{11} = T_3 + T_7$	37.23	52.75	32.89	53.39	108.10	23.60	116.42	26.00
$T_{12} = T_4 + T_8$	27.42	65.20	25.62	63.69	115.55	32.12	125.57	35.90
$T_{13} = T_5 + T_7$	33.88	57.00	30.81	56.34	111.06	26.98	118.14	27.86
$T_{14} = T_6 + T_8$	21.86***	72.25	18.35***	74.00	119.05***	36.12	126.61***	37.42
T_{15} = Control	78.79	00.00	70.56	00.00	87.46	00.00	92.40	00.00
Sem±	2.01		1.95		2.76		3.00	
CD (P=0.05)	5.87		5.68		8.03		8.73	

Table 2: Effect of seed treatments and foliar sprays with *B. diffusa* root and *C. aculeatum* leaf extracts on number of fruits and fruit yield per plant infected with papaya ring spot disease in papaya during 2005-06 (Average of four varieties)

	2004-05		2005-06		2004-05		2005-06	
Treatments	Fruits Plant^{-1}	Per cent increase	Fruits Plant^{-1}	Per cent increase	Fruit yield (Kg Plant^{-1})	Avoidable yield losses (Kg Plant^{-1})	Fruit yield (Kg Plant^{-1})	Avoidable yield losses (Kg Plant^{-1})
T_1 = Seed treatments with CA	12.03	5.86	14.31	5.98	10.66	0.81	12.09	0.96
T_2 = Seed treatments with BD	12.25	7.84	14.58	8.00	10.95	1.10	12.45	1.32
T_3 = Three foliar sprays of CA in nursery	12.43	9.45	14.85	10.00	11.23	1.38	12.75	1.62
T_4 = Three foliar sprays of BD in nursery	12.81	12.46	15.22	12.76	11.68	1.83	13.26	2.13
$T_5 = T_1 + T_3$	13	14.42	15.49	14.75	12.02	2.17	13.81	2.68
$T_6 = T_2 + T_4$	13.77***	21.24	16.49***	22.12	12.84***	2.99	14.80***	3.67
T_7 = Ten foliar sprays of CA in field	15.99	40.72	18.90	40.00	16.43	6.58	18.40	7.27
T_8 = Ten foliar sprays of BD in field	17.32	52.50	20.66	53.00	18.57	8.72	21.17	10.04
$T_9 = T_1 + T_7$	16.68	46.83	19.88	47.25	17.30	7.45	19.75	8.62
$T_{10} = T_2 + T_8$	18.51	62.98	22.13	63.90	20.48	10.63	22.87	11.74
$T_{11} = T_3 + T_7$	17.14	50.86	20.56	52.26	18.12	8.27	20.40	9.27
$T_{12} = T_4 + T_8$	19.01	67.35	22.94	69.94	21.72	11.87	24.63	13.50
$T_{13} = T_5 + T_7$	17.93	57.80	21.36	58.20	19.25	10.00	21.84	10.71
$T_{14} = T_6 + T_8$	20.04***	76.43	24.05***	78.15	22.86***	13.01	25.93***	14.80
T_{15} = Control	11.36	00.00	13.50	00.00	9.85	00.00	11.13	00.00
Sem±	0.53	-	0.59	-	0.75	-	0.66	-
CD (P=0.05)	1.55	-	1.72	-	2.08	-	1.94	-

Plant Height: Data pertaining to plant height presented in Table 1 clearly indicate that maximum plant height of 119.05 and 126.61cm was recorded with the application of treatment T_{14} which was at par with treatments T_{13}, T_{12} and T_{10} and significantly superior over rest of the treatments during 2005-06 and 2006-07. On the other hand maximum plant height increases (36.12 and 37.42%) in treatment T_{14} followed by T_{12}, T_{10}, T_{13}, T_8, T_{11}, T_9, T_7, T_6, T_5, T_4, T_3, T_2 and T_1 during both the years.

Number of Fruits: Data regarding number of fruits per plant presented in Table 2 revealed that both the antiviral agents increased fruiting significantly except both antiviral agents applied as seed treatment and foliar sprays in nursery. However, maximum number of fruit (20.04 and 24.05 plant^{-1}) was obtained by the application of *B. diffusa* root extract as seed treatment and foliar sprays in nursery and fields (T_{14}). It was at par with T_{12}, T_{10} and significantly superior over rest of the treatments during 2005-06 and 2006-07. Maximum increase in number of fruits (76.43 and 78.15 %) was recorded under treatment T_{14} followed by T_{12}, T_{10}, T_{13}, T_8, T_{11}, T_9, T_7, T_6, T_5, T_4, T_3, T_2 and T_1 treatments during both the years.

Fruit Yield: Experimental data regarding to fruit yield indicate that *B. diffusa* root extract and *C. aculeatum* leaf extract significantly increased fruit yield except both the antiviral agents applied as seed treatment and foliar sprays in nursery. Most effective treatment was found T_{14} which exhibited maximum fruit yield (22.86 and 25.93 kg plant^{-1}). It was at par with T_{12} and significantly superior over rest of the treatments during 2005-06 and 2006-07. However, minimum fruit yield (9.85 and 11.03 kg plant^{-1}) was obtained under untreated (control) plants. Maximum yield loss (13.01 and 14.80 Kg plant^{-1}) were avoided by the application of treatment T_{14} followed by T_{12} (11.87 and 13.50 kg plant^{-1}), T_{10} (10.63 and 11.74 kg plant^{-1}), T_{13} (10.00 and 10.71 kg plant^{-1}), T_8 (8.72 and 10.04 kg plant^{-1}), T_{11} (8.27 and 9.27 kg plant^{-1}), T_9 (7.45 and 8.62 kg plant^{-1}), T_7 (6.58 and 7.27 kg plant^{-1}), T_6 (2.99 and 3.67 kg plant^{-1}), T_5 (2.17 and 2.68 kg plant^{-1}), T_4 (1.83 and 2.13 kg plant^{-1}), T_3 (1.38 and 1.62 kg plant^{-1}), T_2 (1.10 and 1.32 kg plant^{-1}) and T_1 (0.81 and 0.96) during 2005-06 and 2006-07.

Plant virus inhibitory properties were reported in *B. diffusa* root extract and *C. aculeatum* leaf extract by Verma *et al.* [2]; Verma and Awasthi [3]; Verma and Singh [4]. The identified natural antiviral compounds have been used successfully under field conditions [5]. Awasthi and Mukerjee [6] reported that the glycoprotein from *B. diffusa* can prevent 60 to 90 per cent of the virus infection of tomatoes, potatoes, pea and French bean.

Both these antiviral agents may be acts as insect vectors repellent. They forced xylem feeding resulted in much less virus transmission under laboratory as well as fields [7]. The inhibitory effect of *B. diffusa* and *C. aculeatum* may be due to resistance inducer present that induces strong systemic resistance against several viruses in hypersensitive as well as systemic hosts [4].

B. diffusa root extract may be attributed to blocking of host cell receptors or to interference of virus synthesis in the host cells.

It is presumed that the inhibitor in *B. diffusa* root and *C. aculeatum* leaf extract which applied before virus inoculation may induce synthesis of translocable virus inhibitory or protective substances the host plant. They believe that the physiology of host cell is affected in such way that the cells no larger support virus multiplication. No other properties are required than that inhibitors differs from normal components of cells and because of this stimulate and cells to unusual activity [8]. Shaikh [9] observed delay in symptoms expression by spraying of extract of neem, *Bougainvillea, Clerodendron* and *Ocimum* when applied 30 minutes before PRSV inoculation. Awasthi and Singh [10] reported that most effective treatment was seed treatment with *B. diffusa* root extract + three foliar sprays followed by seed treatment with *C. aculeatum* leaf extract + three foliar sprays against viruses in tomato. Singh *et al.* [11] found the successful management of papaya ring spot virus disease by the application of some plant products under field conditions.

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