

Original Research Article

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Studies on Induced Resistance by Chemicals against Papaya Ring Spot Virus (PRSV) in Papaya

Hemlata Kshirsagar^{1*} and Pankaj Deore²

¹Department of Plant Pathology, College of Agriculture Dhule, Maharashtra, India

²Mahatma Phule Agriculture University, Rahuri, Maharashtra, Maharashtra, India

*Corresponding author

ABSTRACT

Papaya ring spot disease caused by papaya ringspot virus (PRSV) is the major limiting factor in papaya growing regions in Maharashtra. In these study chemicals inducing resistance was assessed in in vitro condition against PRSV. Eight chemicals which didn't show any phytotoxicity (Chitosan, 6-Benzyladenine, Salicylic acid, IAA, IBA, NAA, Humic acid, Trisodium phosphate) inducing resistance were tested at different concentrations. The eight chemicals inducing resistance exhibited variable response in three modes of applications viz., seed soaking, pre inoculation spraying and post inoculation spraying. Among the chemicals tested, none was found efficient in inducing complete resistance against PRSV, but they increased the incubation period thereby delaying the symptom expression, as compared to control. The maximum incubation period was observed in spraying of Chitosan, at 72 hours prior to virus inoculation, which was most effective in reducing PRSV disease incidence in papaya. In seed soaking application, lowest PRSV incidence was recorded in Trisodium phosphate treatment while, by spraying of chemicals at 72 hrs. pre- and post-inoculation, the lowest PRSV incidence was recorded in humic acid treatment. All the chemical treatments delayed the symptoms expression as compared to control treatment. Pre-inoculation chemicals were the best gave the most effective control and Chitosan, 6-Benzyladenine, Salicylic acid, were the best treatments. Though all the chemical treatments enhanced the plant height of papaya, but with the chemicals viz., Salicylic acid, 6-Benzyladenine and Trisodium phosphate applied as seed treatment, there was no significant increase in plant height and same is the case with Naphthalene acetic acid and indole-3-acetic acid applied 72 hrs. prior and after inoculation.

Keywords

Papaya ring spot virus, Induced resistance, Different chemicals, Incubation period, Disease incidence

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Introduction

Papaya, encompasses most of the desirable qualities of a whole as well as processed fruit (Amar Singh, 1996). Besides medicinal values, the seeds and unripe fruits of papaya are rich in sulphur containing chemicals like

Benzyl-isothionate which has been reported as a germicide and insecticide (Olaya, 1985).

Papaya ringspot virus (PRSV) is the most economically important virus in papaya (Barbosa and Pagui, 1982) and has become a major constraint in papaya cultivation, in

India including Maharashtra (Kale, 1999). Symptoms consist of intense yellow mosaic on leaves, small shoestring-like new leaves along with dark green and slightly sunken rings on the fruit. Trees infected at a very young age remain stunted and never produce any fruit (Reddy *et al.*, 2007). As, application of insecticides to control the insect vector is the only way to reduce the disease and biotechnological interventions are yet to be commercialized, alternate management controls are investigated. Plants possess a range of defenses that can be actively expressed in response to various pathogens and parasites, ranging from microscopic viruses to insect herbivores. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance which is characterized by broad-spectrum disease resistance (Kessmann *et al.*, 1994). The activated disease resistance is effective against both the inducing pathogen and other unrelated pathogens, which may be bacteria, viruses or fungi.

Several investigators studied the effectiveness of chemical resistance inducers against root rot disease (Segarra *et al.*, 2006) and hence this study was aimed to study the effect of chemically induced resistance in the management of PRSV in papaya.

Materials and Methods

Collection, isolation and maintenance of PRSV samples

The papaya ring spot disease samples representing ideal symptoms were collected from the farmers' fields of Dhule district (20.9042° N, 74.7749° E), Maharashtra. PRSV samples were collected separately in polyethylene bags and labeled.

The collected samples of PRSV were established and maintained on highly

susceptible papaya variety *i.e.*, Red Lady by mechanical sap inoculation in an insect free shade net house, which served as a source of virus for further studies. The aforementioned variety was used for subsequent studies.

Phytotoxicity test of chemicals used in the study

Eight treatments viz. Chitosan, 6-Benzylaminopurine (6-BAP), Humic acid (HA), Salicylic acid(SA), Indole Acetic Acid (IAA), Indole Butyric Acid(IBA), Naphthalene acetic acid (NAA) and Trisodium phosphate(TSP) were used for the study. For the phytotoxicity test, papaya seedlings (var: Red lady) were used. Different doses of the treatments viz. Chitosan(10ppm, 50ppm, 100ppm and 250 ppm), 6-BAP (1ppm, 2.5 ppm, 5ppm and 10ppm), Humic and Salicylic acid(0.5ppm, 1ppm, 5ppm and 10ppm each) and IAA, IBA, NAA and Trisodium phosphate (50ppm, 100ppm, 250ppm and 500ppm each) were dissolved in their respective solvents and was used immediately for spraying. Phytotoxicity symptoms were recorded periodically and the highest dose/concentration of chemical not showing any phytotoxicity was selected for the further studies

Induction of resistance through chemicals

The pot culture experiment was conducted to study the effect on induced resistance by chemicals against papaya ring spot virus (PRSV) in Papaya. For each of the test chemical, the highest dose/concentration of chemical not showing any phytotoxicity symptoms in phytotoxicity test was used. Nine treatments included Chitosan @250ppm, 6-BAP @ 10ppm, HA @ 10ppm, SA @ 10ppm, IAA @500ppm, IBA @ 500ppm, NAA @ 500ppm, TSP @ 500ppm along with an untreated virus inoculated control. The experiments were laid in completely

randomized design with three replications per treatment. Ten plants per treatment were considered for the study.

The test chemicals were applied by the following methods

Soaking of seeds

The method of Lange (1961) with minor modifications was used. Fifteen healthy seeds per treatment were soaked in chemical solution for 60 minutes in sterilized beaker.

The treated seeds were then raised in plastic polythene bags containing in insect proof shed net house. The fifty days old were sap inoculated at 6 to 8 leaf stage and the observations were recorded at 15 days interval.

Pre inoculation spraying of chemicals

Fifty days old seedlings at 6 to 8 leaf stage were sprayed with chemical solutions in their respective concentration as per the method of Hofgaard *et al.*, (2010) with minor modifications, the sprayed seedlings were sap inoculated with the PRSV extract after 72 hrs. of application of chemicals and the observations were recorded at 15 days interval.

Post inoculation spraying of chemicals

The fifty days old seedlings at 6 to 8 leaf stage were sap inoculated with PRSV extract. The chemical solutions were prepared in requisite concentration and applied on the inoculated seedlings, 72 hrs. after inoculation. The observations were recorded at 15 days interval after inoculation.

Observed parameters

a) Phytotoxicity of the test chemicals, if any

b) Percent disease incidence (PDI) at 60 days after inoculation and calculated by the following formula as given by Chiang *et al.*, (2017)

$$\text{PDI} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

c) Incubation period i.e. Number of days required to produce the symptoms after inoculation.

d) Symptoms of PRSV inoculated papaya plants viz. Vein clearing (vc), Chlorosis (c), Mosaic (m), Blistering (b), Leaf distortion (ld), Shoe stringing (ss) and Necrosis (n)

e) Changes in plant height (cm) was observed 30 and 60 days after inoculation of PRSV and finally calculated the per cent increase or decrease in the plant height, by applying the formula

$$\text{Increase/ decrease in plant height (\%)} = \frac{T - C}{T} \times 100$$

Where, T = Plant height in treated plants

C = Plant height in inoculated untreated plants

Statistical analysis

Statistical analysis was carried out as per the procedure given by Panse and Sukhatme (1995). To compare different numerical observations, the data was statistically analyzed by using CRD.

Results and Discussion

Collection, Isolation and maintenance of PRSV samples

The PRSV infected papaya samples collected exhibited the symptoms viz., severe mosaic,

leaf distortion, shoe stringing and fruits with ringspot. All the inoculated papaya cv. Red lady seedlings showed the PRSV symptoms, which were used as a source of virus inoculum for further studies.

Phytotoxicity test of chemicals

The results obtained in phytotoxicity test are presented. It was observed that all the eight chemicals tested at four different concentrations did not show any phytotoxicity symptoms like chlorosis, epinasty, russeting, tip burning and necrosis on leaves up to ten days after spray.

Hence, the final concentration of the chemicals *viz.*, Chitosan (250ppm), 6-BAP (10ppm), Humic acid (10ppm), Salicylic acid (10ppm), IAA (500ppm), IBA (500ppm), NAA (500ppm) and Trisodium phosphate (500ppm) were used for further studies.

Effect of the test chemicals on PRSV expression

Effect on Incubation period

There was a significant variation in incubation period (Table 1) of PRSV, in the three methods employed for the application of the chemicals. Minimum incubation period was recorded in untreated control (18 days) in all the three methods. In seed soaked in chemicals, maximum incubation was observed with TSP treatment (26 days), which was at par with SA (25 days). In pre-inoculation treatments, maximum incubation period was recorded with Chitosan (28 days) but it was at par with HA (27 days) and 6-BPA (27 days).

In post-inoculation category, highest incubation period was recorded in HA and Chitosan treatments (each 26 days), and both were at par with SA treatment (25 days).

Effect on PRSV incidence

In seeds soaked in chemicals the results (Table 2) revealed that there was minimum PRSV incidence with TSP (66.66%), as compared to untreated control followed by HA (73.33%) and SA treatment (76.66%) but they were all at par with each other. IAA, IBA and NAA (each 100%), did not have any effect on disease control. In pre-inoculation treatments, the most effective treatments were HA (70.00%), Chitosan (73.33%), SA (76.67%) and 6-BPA (83.33%) and they were all at par with each other. In this case also IAA, IBA and NAA recorded very high disease incidence.

Finally, in post-inoculation also minimum per cent disease incidence was recorded in treatments with HA (73.33%), followed by SA (76.67%), Chitosan (80.00%) and 6-BPA (83.33%) treatments and they were statistically at par with each other. Like other two categories IAA, IBA and NAA recorded a disease incidence which was at par with untreated control.

Symptomatology on papaya plants

When seeds were soaked in chemicals, 30 days after inoculation (DAI) only vein clearing was recorded in all the treatments; whereas, in untreated control there were severe symptoms of vein clearing, chlorosis, mosaic and leaf distortion (Table 3). At 60 days after inoculation, in inoculated untreated control more severe and pronounced symptoms were expressed along with shoe-string symptoms.

In Chitosan, HA, SA, and IAA treatments, chlorosis further progressed to mosaic and leaf distortion; while, in 6-BAP chlorosis progressed to mosaic only. In IBA and NAA treatments mosaic, leaf distortion, blistering and necrosis were observed.

In pre-inoculation studies, at 30 DAI no symptoms were recorded in Chitosan, 6 BAP and SA treatments. Only vein clearing was recorded in rest of the treatments except in untreated control, which recorded the severe symptoms *viz.*, vein clearing, chlorosis, mosaic and leaf distortion (Table 3)

At 60 days after inoculation, in inoculated untreated control the symptoms further progressed to shoe stringing. In chitosan, 6-BPA, HA, and SA treatments the symptoms progressed to Chlorosis, vein clearing and leaf distortion, IBA and NAA had similar symptoms as untreated control.

In post-inoculation, at 30 DAI, only vein clearing was recorded in all the treatments except untreated control, which expressed the severe symptoms *viz.*, vein clearing, chlorosis, mosaic and leaf distortion. All other

treatments had a mixed expression of different types of treatments (Table 3).

Effect of chemicals on height of papaya plants

The results (Table 4) indicated a significant difference in plant height at 60 DAI

When seeds were soaked in chemicals, at 60 DAI, the maximum increase in plant height was recorded with IAA treatment (2.25cm) followed by SA (2.20cm), IBA (2.15cm), Chitosan (1.85cm) and NAA (1.20cm) treatments, all of which were at par. However, minimum increase in height was recorded with TSP treatment (0.20cm) followed by 6-BPA (0.60cm), control (0.67cm), HA (0.90cm) and NAA (1.20cm) treatments, all of which were at par.

Table.1 Effect of various chemicals on incubation period of PRSV

Tr. No.	Treatments	Conc. ppm	Incubation period (days) *		
			Seed soaking in chemicals	Pre inoculation spraying of chemicals	Post inoculation spraying of chemicals
1	Chitosan	250	22	28	26
2	6-BAP	10	21	27	24
3	HA	10	23	27	26
4	SA	10	25	26	25
5	IAA	500	24	22	21
6	IBA	500	22	21	22
7	NAA	500	20	20	20
8	TSP	500	26	25	24
9	Control	-	18	18	18
SE±			0.55	0.61	0.53
CD @ 5 %			1.65	1.83	1.58

*: Mean of three replications

Table.2 Effect of various chemicals on incidence of PRSV at 60 Days after inoculation

Tr. No.	Chemicals	Conc. ppm	Mean PRSV incidence* (%)		
			Seed soaking in chemicals (PDI)	Pre inoculation spraying of chemicals(PDI)	Post inoculation spraying of chemicals(PDI)
1	Chitosan	250	80.00 (63.93)	73.33 (59.00)	80.00 (63.93)
2	6-BAP	10	90.00 (68.86)	83.33 (66.15)	83.33 (66.15)
3	HA	10	73.33 (59.00)	70.00 (57.00)	73.33 (59.00)
4	SA	10	76.66 (61.22)	76.67 (61.71)	76.67 (61.22)
5	IAA	500	96.66 (83.86)	96.67 (83.86)	96.67 (83.86)
6	IBA	500	100.00 (90.00)	93.33 (77.71)	96.67 (83.86)
7	NAA	500	100.00 (90.00)	96.67 (83.86)	100 (90.00)
8	TSP	500	66.66 (54.78)	96.67 (83.86)	93.33 (77.71)
9	Control	-	100.00 (90.00)	100 (90.00)	100 (90.00)
		SE±	3.03	3.61	3.82
		CD@5%	9.09	10.83	11.44

*: Mean of three replications, figures in parentheses arc sine values

DI: Disease Incidence

PDI: Per cent disease incidence

Table.3 Reactions of PRSV at various intervals on Papaya cv. Red Lady

Tr. No.	Chemicals	Conc. ppm	Seed soaking in chemicals		Pre inoculation spraying of chemicals		Post inoculation spraying of chemicals	
			30 DAI	60 DAI	30 DAI	60 DAI	30 DAI	60 DAI
			1	Chitosan	250	vc	vc,c, m,lld	-
2	6-BAP	10	vc	vc,c,m	-	vc,c, ,lld	Vc	vc,c,lld
3	HA	10	vc	vc,c, m,lld	vc	vc,c, ,lld	Vc	vc,c, m,lld
4	SA	10	vc	vc,c, ,lld	-	vc,c, ,lld	Vc	vc,c,lld
5	IAA	500	vc	vc,c, ,lld	vc	vc,c, ,lld	Vc	vc,c, ,n
6	IBA	500	vc	vc,c,m, lld,b,n	vc	vc,c,m, lld,b,n	Vc	vc,c, , lld,n
7	NAA	500	vc	vc,c,m, lld,b,n	vc	vc,c,m, lld,b,n	Vc	vc,c, , lld,n
8	TSP	500	vc	vc,c,	vc	vc,c, , lld,b	Vc	vc,c, , lld,n
9	Control	-	vc,c, m,lld	vc,c,m,lld, b,n,ss	vc,c, m,lld	vc,c,m, lld,b,n,ss	vc,c, m,lld	vc,c,m,lld, b,n,ss

DAI: Days After Inoculation

Table.4

Sr. No.	Treatment	Conc. ppm	Height of papaya plants (cm)					
			Seed soaking in chemicals (Mean)		Pre inoculation spraying of chemicals (Mean)		Post inoculation spraying of chemicals (Mean)	
			ATI	60 DAI	ATI	60 DAI	ATI	60 DAI
1	Chitosan	250	23.07	24.92	30.5	32.9	29.1	30.55
2	6-BAP	10	22.3	22.9	27.3	28.35	29.1	30.8
3	HA	10	25.78	26.68	33.95	35.8	32.2	36.1
4	SA	10	24.17	26.37	27.4	28.75	32.8	34.3
5	IAA	500	25.5	27.75	23.75	25.4	22.7	24.2
6	IBA	500	27.4	29.55	24.25	25.65	22.35	24.3
7	NAA	500	30.4	31.6	25.05	25.4	16.8	17.55
8	TSP	500	29.1	29.3	26.95	29.5	27.35	29.75
9	Control	-	23.15	23.82	23.15	23.82	23.15	23.82
	SE±		0.42	0.72	0.57	0.67	0.57	0.67
	CD @5%		1.26	2.14	1.7	2	1.71	2

ATI: Height of papaya plants (cm) at the time of inoculation (50 days old seedlings)

DAI: Days after Inoculation

In pre-inoculation, at 60 DAI, the maximum increase in plant height was recorded with TSP treatment (2.55cm) but it was at par with all the treatments except NAA (0.35cm), control (0.67cm) and 6-BPA (1.05cm) treatments. However, minimum increase in height was recorded with NAA treatment (0.35cm) but it was at par with control (0.67cm), 6-BPA (1.05cm), SA (1.35cm) and IBA (1.40cm) treatments.

In post-inoculation, at 60 DAI, the maximum increase in plant height was recorded with HA treatment (3.90cm) followed by TSP treatment (2.40cm), both the treatments were at par with each other. However, minimum increase in height was recorded with control treatment (0.67cm) but it was at par with all the treatments except TSP (2.40cm) and HA (3.90cm) treatments.

The papaya specimens showing typical virus infected symptoms were collected and identified on the basis of symptoms as papaya

ring spot virus. The symptoms observed were compared with the symptoms described by Sta. Cruz *et al.*, (2009), Tripathi *et al.*, (2008) and Verma *et al.*, (2007), and were found similar.

The phytotoxicity test attempted with the test chemicals at three concentrations revealed that all the test chemicals were non-phytotoxic to papaya, and on this basis their highest concentration were selected for further studies. This was in accordance with the findings of Spletzer and Enyedi (1999) who reported that 200 mM SA when added in MS medium to the hydroponically grown tomato plants caused no change in leaf turgor or signs of phytotoxicity on the foliage. Le (2006) tested chemical inducers like the salicylic acid for controlling anthracnose in chilli and no phytotoxicity was reported at higher concentrations.

In all the treatments significant variations were observed in respect of incubation period

of PRSV. However, minimum incubation period was observed in control treatment (18 days), followed by NAA (20 days); whereas, it was maximum with Chitosan (28 days), followed by 6-BPA (27 days) and HA (27 days). It was also observed that due to the chemical treatments there was increase in incubation period as compared to control among all three applications. However, there was a significant variation in incubation period when applied in different manner or applied at different times of inoculation. Similar findings were reported by Gandhi *et al.*, (2009) and Reddy *et al.*, (2006) who opined salicylic acid was responsible for delaying the virus infection on blackgram and urdbean respectively. As chemicals can prolong the incubation period it can play a significant role in the management of PRSV.

Among the three application methods of the test chemicals, spraying of chemicals at 72 hrs. prior to PRSV inoculation was found most effective in reducing PRSV disease incidence in papaya.

In seed soaking application, lowest PRSV incidence was recorded in TSP treatment (6.67%) but the same treatment in other applications recorded higher disease incidence; In chemical application by spraying at 72 hrs. prior and 72 hrs. after PRSV inoculation, the lowest PRSV incidence was recorded in HA treatment. In similar studies, Yang *et al.*, (2011) reported that the resistance inducing agents polypeptide and 3-acetyl-3-hydroxyindole (AHO) delayed the TMV incident in tobacco.

Madhusudhan (2008) also observed that Acibenzolar-S-methyl (ASM; S-methylbenzo (1,2,3) thiadiazole-7-carbothiate) pre-treatment to tomato and tobacco plants reduced the concentration of Tomato mosaic tobamovirus (ToMV) and Tobacco mosaic

tobamovirus (TMV) in tomato and bell pepper seedlings, respectively. Elsharkawy and Mousa (2015) reported Silicon nanopowder treated cucumber plants had reduced severity of PRSV as compared with the control due to strong activations of peroxidases (POX) and phenylalanine ammonialyase (PAL) genes.

All three methods of the chemicals applications delayed the symptoms expression, as compared to control treatment, which indicated that time and type of application of chemicals inducing resistance play important role in PRSV symptom expression. However, pre-application of chemicals was found to be the best for Chitosan, 6-BPA and SA.

Deya *et al.*, (2008) reported that SA applied to the leaves of *Vicia faba* prior to Bean Yellow Mosaic Virus (BYMV) inoculation had enhanced the plant resistance against BYMV which was manifested by reduction in percentage of infected plants and decrease in disease severity

The results obtained indicated that the entire test chemicals applied by either of the method significantly enhanced the height of papaya plants but same chemical showed variation in increase height of papaya plant when applied in different manner or different time of inoculation. It was also observed chemicals *viz.*, SA, 6-BPA and TSP when applied as seed treatment reported increase in plant height less than control same is the case with NAA applied 72 hrs. prior to inoculation and for IAA and NAA applied 72 hrs. after inoculation. From this it is pointed out that some chemicals have deleterious effect on papaya plant height when plants were inoculated with PRSV and so time of chemical application and way of chemical application is important factor while using chemicals for inducing resistance.

Le *et al.*, (2012) reported that the SAR chemicals (oxalic acid) when applied as seed soaking to protect rice crop against grassy stunt virus disease, had enhanced the plant height, reduced the disease incidence and also increased the grain yield in rice and this finding is in tandem with the present finding.

Thus the above findings revealed that none of the test chemical was effective in inducing complete resistance in papaya against PRSV, but they delayed the incubation period and symptoms production, as compared to control. The mode of action of inducing resistance against PRSV by the chemicals needs to be investigated further as several morphological and biochemical changes within the host plants are probably the reason for such defense responses.

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