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Effect of immune elicitors on the incubation period and disease incidence of PRSV infected plants of papaya (*Carica papaya* L.) cv. red lady at the seedling stage

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Abstract
The papaya ringspot virus (PRSV) is a deadly virus that causes ringspot disease of papaya in papaya growing regions of Andhra Pradesh. In this experiment, eight chemicals (Chitosan @ 200 ppm, Hydroquinone @ 200 ppm, Humic acid @ 20 ppm, Salicylic acid @ 150 ppm, Iso-nicotinic acid @ 150 ppm, 2- thiouracil @ 2500 ppm, Benzyl adenine @ 20 ppm, Cinnamic acid @ 150 ppm) with the potential to induce systemic acquired resistance (SAR) against PRSV were used. Chemicals were applied through four spraying methods: 5 days before, 5 days after, 5 & 10 days before, and 5 & 10 days after inoculation. Pre- inoculation application has shown superior results than post-inoculation. Single and double spraying of chemicals are statistically on par with each other. None of the chemicals evaluated exhibited total resistance to PRSV; however, they did extend the incubation period, resulting in a delay in symptom expression; Cinnamic acid and Benzyl adenine effectively delayed the incubation period and recorded the lowest disease incidence compared to other treatments as well as untreated plants. The results showed no significant difference between single and double sprays due to no significant interaction effects.

Keywords: Papaya ringspot virus, Incubation time, Systemic acquired resistance, Cinnamic acid, Benzyl adenine

Introduction
Papaya (*Carica papaya*) is an important tropical fruit known for its rich nutritional profile and numerous health benefits. Papaya is a significant source of antioxidants, vitamins (such as Vitamin C and A), and minerals like potassium and magnesium. Its consumption is linked to improved digestion, reduced inflammation, and potential anticancer properties.

Papaya is an important fruit crop in Andhra Pradesh, but it is susceptible to the deadly papaya ringspot disease, caused by the papaya ringspot virus (PRSV). This virus, easily transmitted through aphid species such as *Myzus persicae, Aphis gossypii* and *A. craccivora* (Kalleshwaraswamy and Kumar, 2008) [10], causes severe economic losses and challenges for both Indian and global growers. Symptoms include yellow mosaic leaves, leaf distortion, stem stunting, sunken fruit rings, and small, tasteless fruits (Premchand *et al.,* 2023) [14]. Young infected trees may never bear fruit resulting in severe economic loss to the growers (Tripathi *et al.,* 2008) [18]. In our experiment, PRSV-inoculated red lady papaya seedlings were used to study the effect of various immune elicitor chemicals on the incubation period and incidence of the disease on different days.

Materials and Methods
In this experiment, the Red Lady (Taiwan-786) papaya variety, known for its high yield and sweet flavour, was utilized. The F1 hybrid seeds were sourced from Known-You Seed (India) Pvt. Ltd. in Pune, ensuring high-quality planting material. Papaya ringspot virus (PRSV) samples showing appropriate symptoms were collected from a nearby farmer's field in Anantharajupeta, Annamayya district, Andhra Pradesh (13.9916° N, 79.3344° E). The samples were collected prior to inoculation, placed in an ice box, and immediately used for

mechanical sap inoculation to avoid denaturation of the viral RNA. The study inoculated PRSV in plants 50 days after sowing using a standard inoculum from phosphate buffer containing mercapto-ethanol. Disease samples were mixed with celite, and inoculation was done using sterilized cotton wool. Leaves were rinsed with sterile water, and plants were labelled and observed for up to 40 days for symptom manifestation. Using the standard procedure of Panse and Sukhatme (1985) [13], statistical analysis was carried out with the experimental design Factorial-Completely Randomized Design (FCRD).

Chemicals

Eight chemicals *viz.*, Chitosan @ 200 ppm (C_1), Hydroquinone @ 200 ppm (C_2), Humic acid @ 20 ppm (C_3), Salicylic acid @ 150 ppm (C_4), Iso- nicotinic acid @ 150 ppm (C_5), 2-thiouracil @ 2500 ppm (C_6), Benzyl adenine @ 20 ppm (C_7), Cinnamic acid @ 150 ppm (C_8) and water spray (C_9) were chosen as the different levels in the second factor. These chemicals were imposed on the seedlings at different times of spraying as mentioned below.

The chemicals were applied by the following methods

Spraying 5 days before inoculation (T_1): Fifteen healthy seedlings, 50 days old at the 6-7 leaf stage, were sprayed with chemicals on the 45th day after sowing. Mechanical sap inoculation was done on the 50th day.

Spraying 5 days after inoculation (T_2): Fifty-day-old seedlings at the 6-7 leaf stage were inoculated with PRSV extract. Chemical solutions were applied 5 days after inoculation, on the 55th day after sowing.

Spraying 5 and 10 days before inoculation (T_3): Fifteen healthy seedlings, 50 days old at the 6-7 leaf stage, were sprayed with chemicals on the 40th and 45th days after sowing. Mechanical sap inoculation was done on the 50th day.

Spraying 5 and 10 days after inoculation (T_4): Fifty-day-old seedlings at the 6-7 leaf stage were inoculated with PRSV extract. Chemical solutions were applied 5 and 10 days after inoculation, similar to other treatments, and maintained for further observation.

Observed parameters

i) Incubation period (days)

The number of days taken to produce the symptom expression from the day of inoculation.

ii) Disease incidence (%)

The incidence of the disease was calculated at 30 and 40 DAI by using the below formula given by Chiang *et al.*, (2017) [3]

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Results

Incubation period (days after inoculation)

The investigation revealed that both the timing of chemical application and the chemical agents significantly influenced the incubation period of PRSV, though no interaction effect was noted between these factors. The longest incubation period was observed with pre-inoculation sprays at 5 and 10 days before inoculation (T_3) (24.06 days), statistically on par with spraying 5 days before inoculation (T_1) (23.83 days). The shortest incubation period was recorded with post-inoculation sprays, particularly 5 days after inoculation (T_2) (22.72 days), statistically similar to spraying 5 and 10 days after inoculation (T_4) (23.00 days). Among the chemical agents, Cinnamic acid at 150 ppm (C_8) resulted in the longest incubation period (25.88 days) at par with Benzyl adenine at 20 ppm (C_7) (25.25 days), while the shortest incubation period was with water spray (C_9) (18.00 days) (Table 1).

Disease incidence (%)

The results showed that the time of spraying and the chemicals had a significant influence on the disease incidence of PRSV-inoculated papaya seedlings on the 30th and 40th day after inoculation as displayed in Table 2 and Table 3.

At 30 days after inoculation (%)

The chemical treatments significantly influenced PRSV disease incidence at 30 DAI. The lowest disease incidence was observed with Cinnamic acid at 150 ppm (C_8) and Benzyl adenine at 20 ppm (C_7), both at 16.97%, while the highest was with water spray (C_9) at 41.07%. Pre-inoculation spraying at 5 and 10 days before inoculation (T_3) showed the lowest incidence (24.60%), comparable to spraying 5 days before inoculation (T_1) (25.00%). The highest incidence was recorded with post-inoculation spraying 5 days after inoculation (T_2) (30.16%), similar to spraying 5 and 10 days after inoculation (T_4) (28.97%). No significant interaction was found between the chemicals and the timing of application (Table 2).

At 40 days after inoculation (%)

The study showed significant differences in PRSV disease incidence at 40 DAI based on the timing of chemical application. The lowest incidence was observed with pre-inoculation sprays at 5 and 10 days before inoculation (T_3) (34.13%), statistically in the same line with spraying 5 days before inoculation (T_1) (34.52%). The highest incidence was recorded with post-inoculation sprays, particularly 5 and 10 days after inoculation (T_4) (40.08%), on par with spraying 5 days after inoculation (T_2) (39.68%). Among the chemical agents, Cinnamic acid at 150 ppm (C_8) resulted in the lowest disease incidence (25.89%), comparable to Benzyl adenine at 20 ppm (C_7) (26.79%), while the highest incidence was observed with water spray (C_9) (52.68%). No significant interaction was found between the chemicals and the timing of application (Table 3).

Discussion

Incubation period (days after inoculation)

The chemical treatments on papaya seedlings extended the incubation period of PRSV, with pre-inoculation applications being more effective than post-inoculation. Pre-inoculation spraying activates plant defense mechanisms earlier, aligning with Kshirsagar *et al.* (2020) [11]. The number of sprays either single or double showed no significant difference which might be attributed to the short interval between the sprayings. Cinnamic acid delayed the

pathogen symptoms by enhancing plant defense pathways, producing phenolic compounds and lignin (Ruwizhi *et al.* 2020) [15], and modulating oxidative stress responses (Halasa et al. 2023) [7]. Found cinnamic acid derivatives, especially compounds A5 and 8d, effective against TMV and TSWV. Benzyl adenine delayed pathogen symptoms by promoting cell division and growth (Hurny and Benková, 2017) [8], aiding tissue repair, and enhancing defense mechanisms through increased secondary metabolites and proteins. Kshirsagar *et al.* (2020) [11] observed similar effects in PRSV-inoculated papaya seedlings.

Disease incidence (%)

The chemicals chosen have all effectively decreased the incidence of PRSV than the untreated plants at both 30 and 40 DAI. Pre-inoculation applications were more effective than post-inoculation, as they allowed plants to prime their defenses. The frequency of sprays (single vs double) showed no significant difference due to the short interval between treatments. Found cinnamic acid compounds A5 and 8d significantly reduced TMV and TSWV incidence, outperforming ningnanmycin. Gulcu *et al.* (2022) [6] demonstrated trans-cinnamic acid's effectiveness in reducing powdery mildew in hazelnuts by over 90%. Benzyl adenine (BA) helps plants tolerate stress by modulating stress-related hormones and signalling pathways, reducing viral infectivity and disease incidence (Aldwinckle and Selman, 1967). BA also inhibits pathogen growth, such as *Phytophthora colocasiae*, and increases phenolic compounds, enhancing plant defense (Mishra *et al.*, 2008; El-Fawy and Ahmed, 2017) [12, 4].

Table 1: Incubation period of PRSV in papaya seedlings as influenced by different times of spraying and chemicals

	Incubation Period (Days after inoculation)				
	Time of Spraying				
Chemicals	**T_1**	**T_1**	**T_1**	**T_1**	**Mean**
C_1	25.00	24.00	25.50	24.00	24.63bc
C_2	20.50	20.00	21.00	20.50	20.50d
C_3	24.50	24.00	25.50	24.00	24.50bc
C_4	25.00	24.00	24.50	24.00	24.38bc
C_5	23.50	23.00	24.00	24.00	23.63c
C_6	24.50	23.00	24.50	23.50	23.88c
C_7	26.00	24.50	26.50	24.00	25.25ab
C_8	27.50	24.50	27.00	24.50	25.88a
C_9	18.00	17.50	18.00	18.50	18.00e
Mean	23.83a	22.72b	24.06a	23.00b	
	T		T		T
SEm±	0.25		0.36		0.75
CD @ 5%	0.71		1.08		NS

Table 2: Disease incidence of PRSV at 30 DAI in papaya seedlings as influenced by different times of spraying and chemicals

	Disease incidence at 30 DAI (%)				
	Time of Spraying				
Chemicals	**T_1**	**T_2**	**T_3**	**T_4**	**Mean**
C_1	25.00	28.57	25.00	28.57	26.79cd
C_2	35.71	35.71	32.14	35.71	34.82e
C_3	25.00	28.57	21.43	28.57	25.89c
C_4	17.86	25.00	21.43	21.43	21.43b
C_5	28.57	32.14	28.57	32.14	30.36d
C_6	28.57	32.14	28.57	32.14	30.36d
C_7	14.29	21.43	10.72	21.43	16.97a
C_8	10.72	25.00	10.72	21.43	16.97a
C_9	39.29	42.86	42.86	39.29	41.07f
Mean	25.00a	30.16b	24.60a	28.97b	
	T		C		T x C
SEm±	0.89		1.33		2.66
CD @ 5%	2.56		3.82		NS

Table 3: Disease incidence of PRSV at 40 DAI in papaya seedlings as influenced by different times of spraying and chemicals

	Disease incidence at 40 DAI (%)				
	Time of Spraying				
Chemicals	**T_1**	**T_2**	**T_3**	**T_4**	**Mean**
C_1	35.71	39.29	35.71	42.86	38.39c
C_2	42.86	42.86	42.86	46.43	43.75d
C_3	32.14	39.29	32.14	35.71	34.82b
C_4	28.57	35.71	28.57	35.71	32.14b
C_5	39.29	46.43	35.71	42.86	41.07cd
C_6	35.71	42.86	35.71	39.29	38.39c
C_7	25.00	28.57	21.43	32.14	26.79a
C_8	21.43	32.14	21.43	28.57	25.89a
C_9	50.00	50.00	53.57	57.14	52.68e
Mean	34.52a	39.68b	34.13a	40.08b	
	T		C	T x C	
SEm±	0.79		1.19	2.38	
CD @ 5%	2.28		3.41	NS	

Conclusions

The study found that pre-inoculation chemical treatments on papaya seedlings significantly extended the incubation period of Papaya Ringspot Virus (PRSV) and reduced disease incidence more effectively than post-inoculation treatments. This might be due to the activation of plant defense mechanisms before the pathogen attack. The frequency of chemical applications (single vs. double sprays) showed no significant differences due to the short interval of 5 days between the sprays. Cinnamic acid and Benzyl adenine were particularly effective, delaying the pathogen symptoms and reducing the incidence of the disease at an early stage by activating plant defense pathways and modulating oxidative stress responses. Overall, pre-inoculation chemical treatments in particular single spray were more effective in priming plant defenses and reducing PRSV incidence, emphasizing the importance of timing in disease management. The results obtained are in accordance with the findings of Kshirsagar *et al.* (2020) [11], Ruwizhi *et al.* (2020) [15] and Halasa *et al.* (2023) [7].

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