

Short Communication

Ecofriendly management of papaya ringspot disease

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Abstract

Papaya plants are prone to several viral diseases. During the last decade, the incidence of papaya ringspot disease caused by *Papaya ringspot virus* (PRSV) has become a major threat to profitable cultivation of papaya across Kerala. A pot culture experiment was carried out to develop a recommendation for the management of papaya ringspot disease using different defense inducers, micronutrients, plant products and microbial formulations. Among the various treatments evaluated, foliar spraying and drenching of leaf extract of *Bougainvillea spectabilis* (10%) recorded lowest disease severity of 6.67 per cent followed by *Pseudomonas fluorescens* (2%) with a disease severity of 11.11 per cent. The leaf samples collected after the final application of these two treatments recorded a significant reduction in the concentration of virus also. Both the treatments were found to be superior in terms of plant height and girth. In addition, assessment of chlorophyll content of leaves revealed that *B. spectabilis* treatment was superior with the highest chlorophyll content (44.67 SPAD units). The present study revealed that foliar application as well as drenching of leaf extract of *Bougainvillea spectabilis* (10%) and *Pseudomonas fluorescens* (2%) reduced the severity of papaya ringspot disease.

Keywords: *Bougainvillea spectabilis*, *Pseudomonas fluorescens*, Papaya Ringspot, Severity.

Papaya (*Carica papaya* L.) is an important fruit crop which is widely cultivated across the globe both in commercial orchards as well as in homesteads. Over the last few decades, reports regarding the susceptibility of papaya to a wide range of viruses belonging to different genera have been of major concern to papaya cultivation and production (Tennant et al., 2007). Among the viral diseases, papaya ringspot disease caused by *Papaya ringspot virus* (PRSV), a *Potyvirus*, is reported to have a devastating effect on the economic yield of papaya fruits causing a yield loss up to 70 per cent (Reddy et al., 2011). Of the different varieties cultivated, the commercially cultivated variety, Red Lady, was observed to be the most susceptible cultivar to Papaya ringspot disease (Chavan et al., 2010). Gonsalves et al. (2010) reported that plants infected at young stage remained stunted and did not produce

any economic yield, indicating the seriousness of the disease. Hence, management of the disease at the right time is very much imperative. Defense inducers like salicylic acid (Zhang et al., 2007; Lewsey et al., 2009; Madhusudhan et al., 2011) and acetyl salicylic acid (Reddy et al., 2006) were proved to be effective against several viral pathogens infecting plants. Barakat et al. (2012) and Ruwanthi et al. (2014) reported the effectiveness of microbial inoculants like plant growth promoting rhizobacteria in reducing the severity of infection caused by different potyviruses. Awasthi and Singh (2009) reported the effect of several plant products, mainly root extracts of *Boerhaavia diffusa* and leaf extract of *Clerodendrum aculeatum*, for the management of papaya ringspot disease. Manjunatha (2012) and Lokesh (2014) reported the potential of various micronutrients in reducing the

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impact of papaya ringspot disease. The influence of silicon on reduction of disease severity of cucurbits infected with PRSV was reported by Elsharkawy and Mousa (2015). Therefore, the present study was taken up to evaluate the efficacy of defense inducers, chemicals, plant products and microbial inoculants in reducing the symptoms of the disease. Some of the common foliar symptoms of papaya ringspot disease are chlorotic mottling, leaf distortion and leaf malformation and extreme reduction of lamina (Dahal et al., 1997; Gonsalves, 1998; Kunkaliker, 2003). On fruits, development of oily ringspots and distortion were predominant symptoms which reduced the economic value of the crop (Singh et al., 2017).

The experiment was conducted under insect-proof net house conditions. Fourteen treatments including chemicals, plant products, micronutrients and microbial formulations were evaluated for their efficiency in reducing the severity of papaya ringspot disease. The pot culture experiment was laid out in Completely Randomized Design with three replications having nine plants per treatment. The variety used for the experiment was the most susceptible one *viz.*, Red Lady. The treatments consisted of foliar spraying as well as soil drenching of salicylic acid (0.15 g L⁻¹), acetyl salicylic acid (0.15 g L⁻¹), *Pseudomonas fluorescens* (KAU formulation) (2%), PGPR mix II (KAU formulation) (2%), *Lecanicillium lecanii* (KAU formulation) (2%), leaf extract of *Mirabilis jalapa* (10%), leaf extract of *Bougainvillea spectabilis* (10%), Perfekt (commercial viricide) (0.1%), Sampoorna (micronutrient formulation) (1%), Solubor (1%), humic acid (0.2%), potassium silicate (0.3%) and Solubor (0.1%) along with untreated control. Three week old seedlings were planted in grow bags filled with potting mixture of soil, cowdung and corphith in the ratio of 1:1: 0.5. The first application of the treatments was done one month after planting (MAP) prior to challenge inoculation of the test plants with the virus inoculum. One week after the first application of treatments, the test plants were subjected to

mechanical inoculation with virus inoculum. One week after challenge inoculation of plants, four more applications were given at fortnightly intervals. Observations on per cent disease severity were recorded after each treatment application from the day of first appearance of the symptoms. The per cent disease severity was measured as Vulnerability index (V) in accordance to the 0-5 scale developed by Bos (1982) as mentioned below:

- 0 = no symptom
- 1 = slight vein clearing, very little mottling of light and dark green colour in younger leaves
- 2 = mottling of leaves with light and dark green
- 3 = blisters and raised surfaces on the leaves
- 4 = distortion of leaves
- 5 = stunting of plant with negligible or no flowering and fruiting

Based on the scoring, V was calculated using the following equation,

$$V = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5) \times 100}{n_t (n_c - 1)}$$

where, n_0, n_1, \dots, n_5 = number of plants in disease category 0 to 5 respectively
 n_t = total number of plants
 n_c = total number of categories

This index obtained was expressed as percent disease severity (PDS). The general view of experiment is given in Figure 1.

The plant height and the mean girth of the plants were also recorded at weekly intervals from two weeks after the first spray. The chlorophyll content



Figure 1: General view of insect - proof net house

in the test plants was recorded using a SPAD meter two weeks after final application of treatments and the apical leaves were subjected to DAC- ELISA to assess the influence of each treatment on the virus titre.

Analysis of variance was performed on the data collected in the experiment using online statistical software, Web Agri Statistical Package (WASP 2.0.). Multiple comparisons among treatment means were done using DMRT. In order to determine the best treatment, the treatments were ranked with respect to the per cent disease severity following the method proposed by Arunachalam and Bandyopadhyay (1984). The scores allotted for observations after each application were summed up and the treatment with the maximum value was considered as the best treatment.

The disease appeared in all the test plants in 14 - 15 days after inoculation (DAI). A downward trend was observed in the PDS in most of the treatments mainly, leaf extract of *B. spectabilis* (10%), and *P. fluorescens* (2%), salicylic acid (0.15g/L), leaf extract of *M. jalapa* (10%) and Solubor (0.1%). Significant difference was noticed among the treatments at all intervals of observations. The PDS

recorded from the first appearance of the disease two weeks after every treatment application is given in Table 1. The results indicated that after the second application, plants treated with the leaf extract of *B. spectabilis* (10%) (T7) and Solubor (0.1%) (T13) exhibited lower disease severity viz., 20 and 24.44 per cent respectively. Statistical analysis revealed that both the treatments were on par. The severity recorded in plants treated with *L. lecanii* (T5) was on par with untreated control plants (T 14). The PDS recorded in case of Sampoorna (T9) was also considerably high viz., 53.33 per cent after the second application. All the other treatments gave a PDS ranging from 28.89 to 37.77 per cent. The severity was found to decrease following the third application of treatments and the PDS decreased from 20 per cent to 11.11 per cent in case of plants treated with 10 per cent leaf extract of *B. spectabilis* (T7). Two per cent *P. fluorescens* (T3) and 0.1 per cent Solubor (T 13) were also observed to be superior, both of which resulted in a disease severity of 15.55 per cent. These three treatments were found to be on par after third application. However, *L. lecanii* (T5) treated plants did not show any reduction following third application and the severity recorded was 77.77 per cent which was found to be on par with the untreated control plants.

Table 1. Per cent disease severity recorded after each treatment application

Treatment No.	Treatment	Per cent disease severity (PDS)			
		3WAI	5WAI	7WAI	9WAI
T1	Salicylic acid - 0.15 g L ⁻¹	31.11* (5.16) ^{cd}	22.22(4.70) ^c	22.22(4.70) ^d	17.77(4.25) ^{de}
T 2	Acetyl salicylic acid - 0.15 g L ⁻¹	35.55(5.99) ^{bc}	60.00(7.73) ^b	51.11(7.14) ^c	48.89(7.02) ^b
T 3	<i>Pseudomonas fluorescens</i> - 2%	28.89(5.41) ^{de}	15.55(3.92) ^d	13.33(3.65) ^f	11.11(3.37) ^f
T 4	PGPR mix II - 2%	28.89(5.41) ^{de}	24.44(4.93) ^c	22.22(4.70) ^d	20.00(4.52) ^{ede}
T 5	<i>Lecanicillium lecanii</i> - 2%	60.00(7.77) ^a	77.77(8.81) ^a	77.77(8.81) ^b	88.89(9.45) ^a
T 6	<i>Mirabilis jalapa</i> leaf extract - 10%	28.89(5.41) ^{de}	22.22(4.70) ^c	20.00(4.47) ^{de}	17.77(4.25) ^{de}
T 7	<i>Bougainvillea spectabilis</i> leaf extract- 10%	20.00(4.52) ^f	11.11(3.29) ^d	8.89(2.93) ^g	6.67(2.67) ^g
T 8	Perfekt - 0.1%	31.11(5.61) ^{cd}	26.67(5.16) ^c	24.44(4.93) ^d	24.44(4.98) ^c
T 9	Sampoorna - 1%	53.33(7.33) ^a	62.22(7.88) ^b	55.55(7.45) ^c	53.33(7.33) ^b
T 10	Solubor -1%	37.77(6.18) ^b	24.44(4.93) ^c	22.22(4.70) ^d	22.22(4.75) ^{cd}
T 11	Humic acid -0.2%	35.55(5.99) ^{bc}	24.44(4.93) ^c	22.22(4.70) ^d	20.00(4.75) ^{cd}
T 12	Potassium silicate -0.3%	35.55(5.99) ^{bc}	22.22(4.70) ^c	20.00(4.47) ^{de}	17.77(4.25) ^{de}
T 13	Solubor -0.1%	24.44(4.98) ^{ef}	15.55(3.92) ^d	15.55(3.92) ^{ef}	15.55(3.98) ^e
T 14	Untreated control	60.00(7.77) ^a	84.44(9.18) ^a	95.55(9.77) ^a	97.77(9.91) ^a
CD (0.05)		0.51	0.65	0.57	0.60

* Mean of three replications. Figures in parentheses are transformed values; WAI - weeks after inoculation

Table 2. Per cent reduction of disease over control after final treatment application

Treatment	PDS after final treatment application	Per cent reduction over control
Salicylic acid - 0.15 g L ⁻¹ (T1)	17.77(4.25) ^{de}	81.82
Acetyl SA - 0.15 g L ⁻¹ (T2)	48.89(7.02) ^b	50.00
<i>P. fluorescens</i> - 2% (T3)	11.11(3.37) ^f	88.63
PGPR mix II - 2% (T4)	20.00(4.52) ^{ede}	79.54
<i>L. lecanii</i> - 2% (T5)	88.89(9.45) ^a	9.08
<i>M. jalapa</i> leaf extract - 10% (T6)	17.77(4.25) ^{de}	81.82
<i>B. spectabilis</i> leaf extract-10% (T7)	6.67(2.67) ^g	93.17
Perfekt - 0.1% (T8)	24.44(4.98) ^e	75.00
Sampoorna - 1% (T9)	53.33(7.33) ^b	45.45
Solubor -1% (T10)	22.22(4.75) ^{cd}	77.27
Humic acid -0.2% (T11)	20.00(4.75) ^{ed}	79.54
Pot. silicate -0.3%(T12)	17.77(4.25) ^{de}	81.82
Solubor -0.1% (T13)	15.55(3.98) ^e	84.10
Untreated control (T14)	97.77(9.91) ^a	

After the fourth application, the plants subjected to foliar spraying and soil drenching of 10 per cent leaf extract of *B. spectabilis* (T7) exhibited the least PDS viz., 8.89 per cent and significantly differed from all other treatments. Two per cent *P. fluorescens* (T3) and 0.1 per cent Solubor (T13) were on par after fourth application with a PDS of 13.33 and 15.55 per cent respectively. All the other treatments showed reduction in PDS compared to the previous application, ranging from 20 to 55.55 per cent except for *L. lecanii* (T5) which did not result in any reduction. Finally, after the fifth application, plants treated with 10 per cent *B. spectabilis* leaf extract (T7) gave the least PDS viz., 6.67 per cent as against 97.77 per cent in untreated control plants. The present study revealed that T7, *Bougainvillea* leaf extract (10%), was found to be the best treatment which gave consistently low values of PDS throughout the experiment. Initially, the PDS recorded was 20 per cent which decreased to 6.67 per cent after final application. Hence, *Bougainvillea* treated plants gave a per cent reduction of 93.17 per cent in disease severity over untreated control plants. This was followed by *P. fluorescens* (2%) (T3) and Solubor (0.1%) (T13) with 88.63 and 84.10 per cent reduction over untreated control plants (Table 2).

A similar observation in the delay in appearance of symptoms was reported in case of papaya ringspot disease by spraying *Bougainvillea* leaf extracts 30

minutes before the inoculation of the virus (Shaik, 1996). Other workers have also reported reduction in disease incidence due to potyviruses like *Bean common mosaic virus* (BCMV) and *Bittergourd yellow mosaic virus* (BGYMV) following application of leaf extracts of *Bougainvillea* (Prasad and Kudada, 2005; Rajinimala et al., 2009). Foliar spraying and soil drenching with 2 per cent *P. fluorescens* (T3) and 0.1 per cent Solubor (T13) resulted in disease severity of 11.11 and 15.55 per cent respectively. These findings are in line with the observations recorded by Kumar et al. (2005) who reported that *P. fluorescens* was effective in reducing the disease incidence caused by BCMV, a potyvirus infecting French bean. Similarly, in a study conducted by Ashwini (2015) on the efficacy of *P. fluorescens* on BGYMV in bitter gourd, highest yield was recorded in case of plants sprayed with 2 per cent *P. fluorescens*. There are numerous reports about the use of micronutrients especially boron for management of papaya ringspot disease (Basha, 2002; Kunkaliker, 2003; Lokesh, 2014). However, the observations on PDS in case of Sampoorna micronutrient mix (T9) showed disparity from the previous reports. The PDS recorded was 53.33 per cent in contrast to 15.55 per cent in plants which were treated with 0.1 per cent Solubor (T13).

To conclude, the data was further scrutinized statistically based on the ranking method proposed by Arunachalam and Bandyopadhyay (1984), where

Table 3. Determination of best treatment by ranking method developed by Arunachalam and Bandyopadhyay (1984)

Treatment	Treatment details	3WAI	5WAI	7WAI	9WAI	Total
T1	Salicylic acid - 0.15 g L ⁻¹	0.58	0.75	0.57	0.64	2.54
T2	Acetyl salicylic acid - 0.15 g L ⁻¹	0.41	0.5	0.42	0.29	1.62
T3	<i>P. fluorescens</i> - 2%	0.75	1	0.86	0.86	3.47
T4	PGPR mix II - 2%	0.75	0.75	0.57	0.57	2.64
T5	<i>L.lecanii</i> - 2%	0.17	0.25	0.29	0.14	0.85
T6	<i>M. jalapa</i> leaf extract - 10%	0.75	0.75	0.64	0.64	2.78
T7	<i>B.spectabilis</i> leaf extract- 10%	1	1	1	1	4.0
T8	Perfekt - 0.1%	0.58	0.75	0.57	0.43	4.33
T9	Sampoorna - 1%	0.17	0.5	0.42	0.29	1.38
T10	Solubor -1%	0.33	0.75	0.57	0.5	2.15
T11	Humic acid -0.2%	0.41	0.75	0.57	0.5	2.23
T12	Potassium silicate -0.3%	0.41	0.75	0.64	0.64	2.44
T13	Solubor -0.1%	0.91	1	0.79	0.71	3.41
T14	Untreated control	0.17	0.25	0.14	0.14	0.70

* Values are scores allotted to the observations of PDS WAI - weeks after inoculation

a score was allotted to every observation taken for each treatment. The sum total of the scores allotted for each observation in every treatment was found to be maximum in case of T7 i.e., 10 per cent leaf extract of *B. spectabilis*. (Table 3). In terms of plant height, two treatments viz., 2 per cent *P. fluorescens* (T3) and 10 per cent leaf extract of *B. spectabilis* (T7) exhibited a height of 71 and 70 cm respectively as against 35.60 cm in untreated control plants and were found to be equally superior after the final application. However, in the case of mean girth of stem, T3 was significantly different and gave better

result than all other treatments (Table 4). In addition to this, the virus titre in the test plants was assessed through DAC - ELISA and the absorbance values indicating the virus titre are presented in Table 5. The results revealed that both *Bougainvillea* leaf extract and *P. fluorescens* were on par. The data presented in Table 6 indicated that *Bougainvillea* treatment on plants (T7) was superior to other treatments and gave the maximum chlorophyll content of 44.67 SPAD units as against 17.66 SPAD units in untreated control plants. Plants treated with *P. fluorescens* (T3) also revealed considerably

Table 4. Effect of treatments on biometric parameters in cm

Treatment	After 1 st spray*		After 2 nd spray*		After 3 rd spray*		After 4 th spray*		After 5 th spray*	
	Plant height	Girth of stem	Plant height	Girth of stem	Plant height	Girth of stem	Plant height	Girth of stem	Plant height	Girth of stem
T1	15.51	1.56 ^{ab}	40.53 ^f	1.90 ^b	47.30 ^e	2.03 ^b	49.20 ^f	2.03 ^b	49.20 ^f	2.06 ^c
T2	15.5	1.53 ^b	31.33 ⁱ	1.70 ^{cd}	40.63 ^h	1.78 ^c	41.33 ⁱ	1.78 ^d	41.33 ⁱ	1.78 ^{de}
T3	15.22	1.70 ^a	57.63 ^a	2.33 ^a	65.43 ^a	2.46 ^a	71.00 ^a	2.48 ^a	71.00 ^a	2.91 ^a
T4	14.91	1.66 ^{ab}	49.10 ^c	1.76 ^{bc}	57.30 ^c	1.86 ^c	59.20 ^d	1.86 ^{cd}	59.2 ^c	1.86 ^d
T5	15.13	1.00 ^{fgh}	32.5 ^h	1.46 ^{ef}	38.83 ⁱ	1.49 ^c	39.70 ^j	1.49 ^f	39.70 ^j	1.50 ^e
T6	16.73	1.06 ^{de}	55.13 ^b	1.58 ^{de}	61.16 ^b	1.65 ^d	61.1 ^c	1.65 ^c	61.10 ^b	1.68 ^f
T7	14.3	1.23 ^{cd}	57.13 ^a	1.81 ^{bc}	66.30 ^a	1.80 ^c	69.03 ^b	1.9 ^c	70.00 ^a	2.50 ^b
T8	13.43	1.10 ^{def}	38.53 ^s	1.46 ^{ef}	42.80 ^g	1.53 ^c	43.80 ^h	1.53 ^f	43.80 ^h	1.53 ^s
T9	14.36	0.93 ^{sh}	42.03 ^c	1.30 ^g	42.27 ^g	1.36 ^f	47.00 ^g	1.36 ^g	47.00 ^g	1.36 ^h
T10	14.26	0.83 ^h	17.26 ^l	0.90 ^h	32.32 ^j	0.86 ^h	36.00 ^k	0.93 ⁱ	56.16 ^d	1.00 ⁱ
T11	13.8	1.13 ^{odef}	38.02 ^s	1.76 ^{bc}	44.97 ^f	1.78 ^c	49.20 ^f	1.83 ^{cd}	49.20 ^f	1.83 ^{de}
T12	14.66	1.20 ^{ode}	47.13 ^d	1.70 ^{cd}	51.26 ^d	1.78 ^c	53.26 ^e	1.78 ^d	53.26 ^e	1.78 ^{def}
T13	15.57	1.26 ^c	30.60 ⁱ	1.35 ^{fg}	41.77 ^{gh}	1.43 ^{ef}	47.50 ^g	1.80 ^{cd}	60.66 ^{bc}	1.73 ^s
T14	10.03	0.93 ^{sh}	20.20 ^k	1.00 ^h	23.33 ^k	1.13 ^s	24.50 ^l	1.13 ^h	35.60 ^k	1.38 ^h
CD (.05)		0.135	0.510	0.152	1.680	0.111	1.499	0.111	1.487	0.102

*Mean of 3 replications

Table 5. Effect of treatments on virus titre

Treatment	Treatment	Virus titre*
T1	Salicylic acid - 0.15 g L ⁻¹	0.689 ^{fg}
T2	Acetyl salicylic acid - 0.15 g L ⁻¹	0.967 ^{cd}
T3	<i>Pseudomonas fluorescens</i> - 2%	0.509 ⁱ
T4	PGPR mix II - 2%	0.714 ^{efg}
T5	<i>Lecanicillium lecanii</i> - 2%	1.253 ^b
T6	<i>Mirabilis jalapa</i> leaf extract - 10%	0.627 ^{gh}
T7	<i>Bougainvillea spectabilis</i> leaf extract- 10%	0.373 ^{hi}
T8	Perfekt - 0.1%	0.834 ^{def}
T9	Sampoorna - 1%	1.023 ^c
T10	Solubor -1%	0.69 ^{fg}
T11	Humic acid -0.2%	0.772 ^{efg}
T12	Potassium silicate -0.3%	0.853 ^{dc}
T13	Solubor -0.1%	0.619 ^{gh}
T14	Untreated control	1.474 ^a

* Absorbance value at 405 nm - Mean of four replications

higher chlorophyll content of 42.67 SPAD units. The results obtained in case of treatments using *Bougainvillea* and *Pseudomonas* are portrayed in Figure 2. The effect of *Bougainvillea* leaf extract may be ascribed to the explanation given by Verma and Dwivedi (1984), who isolated a virus inhibiting agent (VIA) developed in plants following application of the leaf extract. The active molecule was identified as a proteinaceous compound which reached a maximum level within 24 hours after

Table 6. Effect of treatments on chlorophyll content of leaves

Treatment	Treatment	Chlorophyll content (SPAD units)*
T1	Salicylic acid - 0.15 g L ⁻¹	39.30
T2	Acetyl salicylic acid - 0.15 g L ⁻¹	38.72
T3	<i>Pseudomonas fluorescens</i> - 2%	42.67
T4	PGPR mix II - 2%	39.20
T5	<i>Lecanicillium lecanii</i> - 2%	18.83
T6	<i>Mirabilis jalapa</i> leaf extract - 10%	39.10
T7	<i>Bougainvillea spectabilis</i> leaf extract- 10%	44.67
T8	Perfekt - 0.1%	33.075
T9	Sampoorna - 1%	39.10
T10	Solubor -1%	32.36
T11	Humic acid -0.2%	39.10
T12	Potassium silicate -0.3%	37.36
T13	Solubor -0.1%	40.55
T14	Untreated control	17.66

* Mean of three replications

treatment with the leaf extract. The reduction in virus concentration in plants treated with *P. fluorescens* may be attributed to its capacity to induce systemic resistance in the applied plants as has been discussed by Loon et al. (1998) regarding different strains of *P. fluorescens* against viruses infecting plants. Abdalla et al. (2017) reported that plant growth promoting rhizobacteria (PGPR)



Figure 2: Papaya plants after final application a. T7= Test plant treated with 10 per cent leaf extract of *Bougainvillea spectabilis* T14= Untreated control b. T3 = Test plant treated with 2 per cent *Pseudomonas fluorescens* T14 = Untreated control

strains were superior to conventional chemicals in reducing papaya ringspot disease in squash. The values recorded in the remaining treatments ranged from 18.33 to 39.30 SPAD units.

Thus, the present study highlighted the effectiveness of foliar spraying and soil drenching of 10 per cent leaf extract of *Bougainvillea spectabilis* and 2 per cent *P. fluorescens* (KAU formulation) for reducing the severity of papaya ring spot disease along with plant growth promotion.

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