Integrated Management Protocol for Bacterial Wilt Disease of Tomato in *Ralstonia solanacearum* Affected Soils in Kerala State

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ABSTRACT

Background: Management of bacterial wilt of tomato in *Ralstonia solanacearum* affected soils is a major issue in tomato cultivation in different agro-ecological regions in Kerala state which affects the production and productivity of the crop. To develop a suitable management protocol for this disease, the field experiments were conducted during 2019 and 2020 in a bacterial wilt-sick field at Kerala Agricultural University, Thrissur, Kerala, India.

Methods: Twelve different treatments *viz.* soil application of bleaching powder, soil test based lime application, streptocycline root dip, drenching of copper hydroxide and copper oxychloride at different doses, seed treatment and seedling dip with *Pseudomonas fluorescens*, drenching of *Pseudomonas fluorescens*, planting grafted seedlings and different integrations and combinations of the above treatments were assessed for the management of bacterial wilt.

Result: The majority of integrations were superior over individual treatments. The higher yield with less disease incidence was obtained from the integration of soil treatment of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@ 2 g/10 L) root dip + drenching of copper oxychloride (@ 0.3%) and therefore, this integration protocol is recommended for management of bacterial wilt of tomato in wilt affected soils in Kerala state.

Key words: Bacterial wilt, Integrated management, Kerala soil, *Ralstonia solanacearum,* Tomato crop.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum*, is a widespread and one of the most destructive soil-borne disease reported in tomato crop (Wicker *et al.,* 2007). This disease is likely to occur under high temperatures and humid conditions and can persist for a longer time (Pradhanang *et al.,* 2003). It causes great concern for tomato production because it can drastically reduce tomato yield up to 90 per cent (Kishun, 1985). Soil acidification can also cause a severe outbreak of bacterial wilt (Li *et al.,* 2017). In Kerala, due to the humid atmospheric condition and acidic soil type, the occurrence of bacterial wilt caused by *Ralstonia solanacearum* is known to cause significant yield losses on different solanaceous crops including tomato. Since various disease management practices developed to control this disease had limited success, the disease continues to be one of the major threats in commercial tomato production.

To manage bacterial wilt disease, practices like intercropping, crop rotation and soil amendment against the pathogen has been reported (Sood *et al.,* 1998; Yadessa *et al.,* 2010; Djeugap *et al.,* 2014). Unfortunately due to the complex nature of *Ralstonia solanacearum,* no method was proven to be successful when applied alone (Nion and Toyota, 2015). Some bacterial wilt resistant cultivars have been developed from the Asian Vegetable Research and Development Centre. Sakthi, Mukthi and Anagha are bacterial wilt resistant varieties from Kerala agricultural university; however, their resistance is restricted to locations, climate and strains of the pathogen and soil characteristics. Even if the pathogen's population is suppressed by crop rotation with non-host plants, it can survive in weed hosts, weakening the effect of crop rotation (Vanitha *et al.,* 2009). The pathogen can survive for longer periods in other wide range of host crops and in the infected tomato debris. Hence, the management of the bacterial wilt of tomato is cumbersome (Tahat *et al.,* 2010).

This is a soil-borne pathogen favoured by acidic soil conditions. Hence, a soil test is mandatory before planting tomato seedlings. Nowadays, tomato is not widely cultivated in Kerala due to the severe incidence of bacterial wilt disease which leads to the import of this vegetable from other parts of the country for meeting the daily requirement for various culinary purposes. Therefore, the management of bacterial wilt disease is one of the most important challenges for the cultivation of tomato in Kerala to make the state self-reliant in tomato production. Keeping this in mind, an experiment was conducted on an integrated management protocol for

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the management of this disease in bacterial wilt sick plot at Kerala Agricultural University, Thrissur.

MATERIALS AND METHODS

Land preparation and raising of tomato seedlings under nursery conditions

The experiment was conducted during the year 2018-19 and 2019-20 at the Kerala Agricultural University, India. The experimental land of the wilt-sick plot was prepared to a fine tilth by thorough ploughing. Good quality farmyard manure of recommended dose was incorporated in the experimental soil before transplanting the seedlings. To raise the tomato seedlings, the seeds of the Akshaya variety of tomato were sown in the nursery under sterile nursery substrate and were grown for one month.

Based on the results of the soil analysis, an appropriate quantity of good quality lime was applied (as acidic conditions favour the bacterial wilt) and mixed well in respective treatments (T2, T9, T10 and T11) to adjust the soil pH to neutral conditions. A fertilizer dose of 75:40:25 kg N: P_2O_5: K_2O per hectare was applied. Half the dose of nitrogen, full phosphorous and half of potash were applied as basal dose before transplanting.

Seed treatment of tomato seeds with *Pseudomonas fluorescens* (10 g/kg seed) was done for appropriate treatments (T6 and T11). These protrays were irrigated as per requirement. One month old tomato seedlings, as well as grafted tomato seedlings were transplanted into the experimental wilt-sick plot (3.0 × 2.4 m^2) of individual treatments during the month of June. Each experimental treatment plot had four rows and each row had five tomato seedlings transplanted at 60 × 60 cm distance. For this 660 tomato seedlings and 120 grafted tomato seedlings were used.

The experiment was carried out in RBD design with three replications during the years 2019 and 2020.

Evaluation of integrated disease management (IDM) protocol for the management of bacterial wilt

The details of experimental treatments/integration of treatments are mentioned in Table 1, which was assessed for the management of bacterial wilt. In the first treatment (T1), soil drenching of bleaching powder @ 15 kg/ha was carried out. After testing the soil pH, the lime requirement of soil was calculated for each plot of 7.2 m^2 size based on standard lime requirement given in the KAU package of practices (Table 2). Then the required quantity of quality lime was mixed thoroughly with the soil before transplanting of seedlings in T2 treatment. The roots of tomato seedlings were dipped in streptocycline (2 g/10 L) for 30 minutes before transplanting in case of T3 treatment. In T4, the drenching 250 ml of 0.2% copper hydroxide per plant was done 20 days after transplanting and it was repeated thrice at 10 days intervals. Drenching 250 ml of 0.3% copper oxychloride per plant was done 20 days after transplanting of seedlings and it was repeated thrice at 10 days intervals in T5 treatment. In T6 treatment the thick slurry of *Pseudomonas fluorescens* (10 g/kg seed) was used for tomato seed treatment prior to sowing of seeds and along with that dipping of this germinated seedlings in 2% *Pseudomonas fluorescens* solution was also done before transplanting it in the bacterial wilt sick plot. In T 7 treatment, drenching of 250 ml of *Pseudomonas flourescens* @ 2% per plant was done 20 days after transplanting and it was repeated thrice at 10 days intervals. Planting grafted plants of tomato in bacterial wilt sick plot was the T8 treatment. Integration of T1, T2, T3, T4 and integration of T1, T2, T3, T5 was T9 and T10 treatments respectively. T11 treatment was the integration of T2, T6 and T7 treatments. T12 was kept as the control plot without applying any management strategies.

The incidence and severity of bacterial wilt were observed daily after transplanting of the tomato seedlings in respective treatment in wilt sick plot. For confirmation of bacterial wilt, the string test (Borkar, 2018) was carried out of the wilted plants. The disease incidence and its progression were recorded at 30, 60, 90 and 120 days of transplanting and was calculated as the percentage of diseased plants as compared to the total number of plants growing in each plot as described by Bainsla *et al.,* (2016).

$$\text{Bacterial wilt PDI (\%)} = \frac{\text{Number of dead plants due to bacterial wilt disease}}{\text{Total number of plants established}} \times 100$$

Statistical analysis

Statistical significance was done by using the data of the percentage of affected plants and transformed to arcsine square root equivalents before a one-way analysis of variance (ANOVA). All statistical analyses were performed with the Wasp 2.0 software program. Differences among the treatments were assessed with a one-way analysis of variance (ANOVA) at the end of each treatment. A combined analysis was carried out to pool the data. The yield recorded year-wise was pooled, statistically analysed and presented.

RESULTS AND DISCUSSION

Bacterial wilt disease management efficacy of various protocols during the year 2019

During the year 2019, the performance of treatments such as planting grafted plants of tomato (T8) and integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10 L) root dip + drenching of copper oxychloride @ 0.3% (T10) were on par and superior as compared to all other treatments as they have the lowest per cent disease incidence.

However, drenching of copper oxychloride @ 0.3%, 20 DAT and thrice at 10 days intervals (T5), seed treatment (10 g/kg seed) and seedling dip in @ 2% with *Pseudomonas fluorescens* (T6), integration of soil test based lime application + seed treatment (10 g/kg seed) and seedling dip @ 2% with *Pseudomonas fluorescens* + drenching of

Pseudomonas fluorescens @ 2% (T11) were the least effective treatments.

However, the highest yield was given by drenching of *Pseudomonas fluorescens* @ 2%, 20 DAT and thrice at 10 days intervals (T7) due to the larger fruit size of these plants. This was followed by seedling root dip in streptocycline @ 2 g/10L (T3) and integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10L) root dip + drenching of copper hydroxide @ 0.2% (T9). The lowest yield was obtained from T5, T6 and T8.

Bacterial wilt disease management efficacy of various protocols during the year 2020

During the year 2020, the performance of treatments such as planting grafted plants of tomato (T8) and integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10 L) root dip + drenching of copper oxychloride @ 0.3% (T10) were on par and superior as compared to all other treatments as they have the lowest per cent disease incidence.

However, in this year the least effective treatments were drenching of copper oxychloride @ 0.3%, 20 DAT and thrice at 10 days interval (T5), seed treatment (10 g/kg seed) and seedling dip in *Pseudomonas fluorescens* @ 2% (T6).

Drenching of *Pseudomonas fluorescens* @ 2%, 20 DAT and thrice at 10 days intervals (T7) and integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10 L) root dip + drenching of copper oxychloride @ 0.3% (T10) had given the highest yields and the lowest yield was given by T6 and T8 treatments.

Bacterial wilt disease management efficacy of various protocols (pooled)

As per pooled analysis, the performance of the treatments such as planting grafted plants of tomato (T8) and integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10L) root dip + drenching of copper oxychloride @ 0.3% (T10) were on par and superior as compared to all other treatments as they have the lowest per cent disease incidence (Table 3).

The least effective treatment was seed treatment (10 g/kg seed) and seedling dip in *Pseudomonas fluorescens* @ 2% (T6) which had given the highest per cent disease incidence. The highest per cent disease reduction over control (29.97%) was obtained by planting grafted plants of tomato (T8) which was followed by (29.59%) integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10L) root dip + drenching of copper oxychloride @ 0.3% (T10) (Table 3). The plants in the treated plots were compared with the control plot (Fig 1) for the management of bacterial wilt disease of tomato.

The highest yield was obtained by drenching of *Pseudomonas fluorescens* @ 2%, 20 DAT and thrice at 10 days interval (T7) followed by integration of bleaching powder (15 kg/ha) + soil test based lime application + streptocycline (@2 g/10 L) root dip + drenching of copper oxychloride @ 0.3% (T10). The lowest yield was again given by T6 and T8 (Table 4).

Various workers have demonstrated the significance of various components like bleaching powder, lime, copper

Table 1: The treatment details adopted for the integrated management of bacterial wilt of tomato.

Treatments	Treatment details
T1	Soil application of bleaching powder @15 kg/ha
T2	Soil test based lime application
T3	Seedling root dip in streptocycline @ 2 g/10 L
T4	Drenching of copper hydroxide @ 0.2%, 20 days after transplanting (DAT) and thrice at 10 days intervals
T5	Drenching of copper oxychloride @ 0.3%, 20 DAT and thrice at 10 days intervals
T6	Seed treatment (10 g/kg seed) and seedling dip in @ 2% with *Pseudomonas fluorescens*
T7	Drenching of *Pseudomonas fluorescens* @ 2%, 20 DAT and thrice at 10 days intervals
T8	Planting grafted plants of tomato
T9	Integration of T1, T2, T3 and T4
T10	Integration of T1, T2, T3 and T5
T11	Integration of T2, T6 and T7
T12	Control

*DAT - Days after transplanting.

Table 2: Soil test based lime requirement.

Treatments	Soil pH		Soil pH class		Lime ($CaCO_3$) requirement (kg/ha)		Lime ($CaCO_3$) requirement (g/plot)	
	2019	2020	2019	2020	2019	2020	2019	2020
T2	4.4	5.31	Extremely acidic	Strongly acidic	850	350	612	252
T9	4.9	4.83	Very strongly acidic	Very strongly acidic	600	600	432	432
T10	4.8	4.98	Very strongly acidic	Very strongly acidic	600	600	432	432
T11	5.0	4.92	Strongly acidic	Very strongly acidic	350	600	252	432

Fig 1: A. Treated plant of tomato in bacterial wilt sick plot and B. Control plant of tomato in bacterial wilt sick plot.

Table 3: Progression of bacterial wilt of tomato after 30, 60, 90 and 120 days after transplanting.

Treatments	PDI (Pooled)				Per cent disease reduction over control
	30 DAT	60 DAT	90 DAT	120 DAT	
T1	0.00 (6.75)^{de}	7.51 (19.56)^{de}	12.03 (21.53)^{cd}	18.67 (25.16)^{de}	23.8
T2	0.00 (5.55)^{ef}	8.98 (16.60)^{ef}	13.24 (19.66)^{de}	21.16 (23.04)^{ef}	21.31
T3	0.00 (4.83)^{fg}	6.51 (14.26)^{fg}	10.85 (17.61)^{def}	16.87 (20.41)^{ef}	25.6
T4	0.00 (4.74)^{fgh}	7.98 (15.75)^{f}	12.72 (19.60)^{de}	19.82 (22.71)^{ef}	22.65
T5	0.69 (8.41)^{cd}	16.61 (24.52)^{bc}	29.16 (29.66)^{b}	32.38 (30.66)^{bc}	10.09
T6	6.00 (13.66)^{b}	18.63 (26.23)^{b}	36.60 (33.51)^{ab}	37.12 (33.87)^{ab}	5.35
T7	0.52 (8.68)^{c}	9.74 (22.63)^{bcd}	14.03 (24.36)^{c}	25.11 (28.29)^{cd}	17.36
T8	0.00 (2.96)^{h}	2.08 (10.64)^{g}	5.63 (14.37)^{f}	12.50 (18.33)^{f}	29.97
T9	0.00 (4.74)^{fgh}	6.69 (14.35)^{f}	11.53 (17.95)^{def}	17.73 (20.76)^{ef}	24.74
T10	0.00 (3.28)^{gh}	4.60 (13.07)^{fg}	9.12 (16.71)^{ef}	12.88 (18.60)^{f}	29.59
T11	0.66 (7.86)^{cd}	10.83 (22.00)^{cd}	18.20 (24.99)^{c}	30.80 (29.39)^{bcd}	11.67
T12-Control	12.26 (17.88)^{a}	24.10 (29.91)^{a}	40.71 (35.73)^{a}	42.47 (36.26)^{a}	0.00
C.D (0.05%)	1.83	3.64	4.22	5.00	
C.V (%)	14.48	11.22	18.85	11.52	

*Values in parenthesis are arc sine transformed.

Table 4: Fruit yield obtained from the cultivation of tomato crop.

Treatments	Yield (tons/ha)		
	2019	2020	Pooled
T1	12.83^{c}	14.79^{ef}	13.81^{d}
T2	11.09^{cd}	16.61^{e}	13.85^{d}
T3	19.22^{b}	23.13^{cd}	21.17^{c}
T4	12.60^{c}	19.06^{de}	15.83^{d}
T5	2.67^{e}	9.48^{gh}	6.07^{f}
T6	0.96^{e}	3.21^{i}	2.09^{g}
T7	33.19^{a}	29.90^{ab}	31.54^{a}
T8	3.10^{e}	5.16^{hi}	4.13^{fg}
T9	19.95^{b}	27.50^{bc}	23.73^{c}
T10	20.99^{b}	34.22^{a}	27.61^{b}
T11	7.63^{d}	11.67^{fg}	9.65^{e}
T12-Control	1.15^{e}	2.45^{i}	1.80^{g}
C.D (0.05%)	4.07	4.46	2.80
C.V (%)	19.85	16.02	11.58

hydroxide, copper oxychloride, *Pseudomonas fluorescens* in the management of bacterial wilt disease and disease-causing bacterium *Ralstonia solanacearum*. Bleaching powder acts as a bactericide, which reduced *R. solanacearum* population in the soil, resulting in the good health of the plant (Sharma and Kumar, 2009). Soil acidification is a major problem in modern agricultural systems and is an important factor affecting the soil microbial community and soil health. Similar to our results, the application of lime as soil pH amendments improved soil pH and reduced the occurrence of bacterial wilt in China (Li *et al.,* 2017). Similarly, seedling root dip in streptocycline @ 0.1% for 2 h before transplanting was the most effective against the disease as it was recorded 50.63 percent disease reduction as compared to control (Salvi *et al.,* 2020). Copper hydroxide WP showed a control value of 62.5% as a bactericide and showed a strong inhibitory effect on tomato bacterial wilt and therefore recommended to control the disease (Han *et al.,* 2011). Copper oxychloride is also found to be effective in the management of bacterial wilt disease (Bannihatti and Suryawanshi, 2019). The lowest bacterial wilt incidence (35.18%) was recorded in soil drenching of *P. fluorescens* (Jinnah *et al*., 2002). Grafting also helps to manage bacterial wilt disease and the use of resistant rootstocks is an important component of an integrated pest and disease management program for tomato. Grafting is also effective at reducing damage and crop loss caused by other soil-borne plant pathogens such as *Fusarium oxysporum* f. sp. *lycopersici, Sclerotium rolfsii* and root-knot nematodes *Meloidogyne* spp (Rivard and Louws, 2008; Rivard *et al.,* 2010). According to Revathi *et al.,* (2018) neem cake + *Trichoderma harzianum* + *P. fluorescens* + streptocycline + copper oxychloride had given highest (29.24%) per cent disease reduction over control. Copper oxychloride seed treatment @ 3 g/kg seed showed 36.38% per cent disease reduction over control (Bannihatti and Suryawanshi, 2019).

Pseudomonas fluorescens is a known antagonist of plant pathogenic bacteria and has been found to be a very potential bio-control agent against soil-borne plant pathogenic bacteria under both greenhouse and field conditions (Anuratha and Gnanamanikam, 1990). Many strains of *P. fluorescens* are known to enhance plant growth promotion and reduce the severity of various diseases (Mulya *et al*., 1996). Seed treatment with antagonistic *P. fluorescens* strain significantly improved the quality of seed germination and seedling vigour. The disease incidence was significantly reduced in plants raised from *P. fluorescens* treated seeds followed by challenge inoculation with *R. solanacearum* (Vanitha *et al.,* 2009). The lowest bacterial wilt incidence (35.18%) was recorded in soil drenching of *P. fluorescens* and plant height, number of branches/plant, number of fruits/plant, total fruit weight/plant and fruit yield (t/ha) was significantly highest in this treatment. Soil drenching by *P. fluorescens* suspension (10^9 cfu/ml) was useful for controlling wilt and increasing yield of tomato (Jinnah *et al*., 2002).

The development of the resistant variety requires significant amount of time that may lead to excessive yield loss in the farmer's field until that period. So, the development of a reliable grafting technique prevents yield loss during the developmental phase of a resistant variety. These limitations lead to acknowledge the importance of grafting techniques that can be readily used by the farmers. The use of resistant rootstocks also enabled economically viable tomato production in soils naturally infested with *R. solanacearum* (Rivard *et al.,* 2012).

All these findings were similar to our results. However, our results on the integrated management approach for the management of bacterial wilt of tomato gave a better result than the individual component used by these authors in the management of the diseases.

CONCLUSION

To manage the bacterial wilt of tomato in *Ralstonia solanacearum* affected soils with optimization of yield, in Kerala state, an integration protocol comprising of bleaching powder (15 kg/ha) + soil test-based lime application + streptocycline (@ 2 g/10 L) root dip + drenching of copper oxychloride @ 0.3% is recommended. This protocol not only manage the bacterial wilt disease but also increases the yield of tomato in the wilt-affected soils.

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