Journal of Biological Control, 29(3): 148-156, 2015

Research Article

Evaluation of endophytic microbial consortium for the management of bacterial wilt of tomato caused by *Ralstonia solanacearum*

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ABSTRACT: Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., is a major production constraint in tomato cultivation in Kerala. Five different endophytic microbial consortia were evaluated against *R. solanacearum* under in *planta* condition. Among five, the consortium consisting of three fungi *viz. Trichoderma harzianum*-1 (VSF-3), *T. viride*-1 (CSF-1) and *T. viride*-2 (MyRF-1), one bacteria *viz. Bacillus subtilis* (VSB-1) and one actinomycete, *Streptomyces thermodiastaticus* (ORA-1) was found effective against bacterial wilt under pot culture condition when it was applied in combination of seed treatment (1g/2 ml), seedling root dip for 30 min and soil drenching at 45 DAP (30 ml/ plant). The antagonistic endophytic bacterial consortium was also evaluated under field condition by seed treatment, seedling root dip and soil drenching. Field evaluation of endophytic consortium against bacterial wilt resulted in 40.85 per cent reduction in wilt incidence in highly susceptible variety, PKM-1, 46.94 per cent reduction in susceptible F1 hybrid, COTH-3, and 52.81 per cent reduction in moderately resistant variety, Mukthi. The plots treated with endophytic consortium recorded maximum yield of 2.67 kg, 8.62 kg and 6.38 kg against 0.25 kg, 3.25 kg and 2.08 kg in control in varieties, PKM-1, COTH-3 and Mukthi respectively.

KEY WORDS: Bacterial wilt, *Ralstonia solanacearum*, tomato, endophytes, consortium, resistance

(Article chronicle: Received: 25-06-2015; Revised: 13-07-2015; Accepted: 04-08-2015)

INTRODUCTION

Tomato (*Solanum lycopersicum* Mill) is one of the most important vegetable crops grown throughout the world. Bacterial wilt incited by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., is one of the major production constraints of tomato and it causes extensive losses in Asia and South Pacific regions. The yield loss due to bacterial wilt ranges from 11-93 per cent in India (Kishun, 1987). The warm humid tropical climate and soil conditions prevailing in Kerala are conducive for the occurrence of bacterial wilt and a yield loss up to cent per cent has been reported in susceptible varieties (Sadhankumar, 1995). Management of bacterial wilt disease will be difficult once it is established in the field. Indiscriminate use of chemicals results in environmental pollution, development of resistant strains and detrimental effects on non target organisms including human beings.

The focus on the management of plant diseases has been shifted from chemical pesticides to more ecofriendly methods to reduce environmental hazards and minimize the risk of development of pesticide resistant strains of plant pathogens. Biological control utilizing antagonistic endophytic microorganisms is considered as one of the novel approaches for efficient disease management due to their intimate systemic association with the plants. Endophytes are microorganisms that inhabit for at least one period of their lifecycle inside plant tissues without causing any apparent harm to the hosts (Petrini, 1991) and they benefit the host by promoting plant growth and prevent pathogenic organisms from colonization. Induction of host resistance by endophytic microorganisms has received much attention in recent years as a potential practical method of disease control. Recently, a greater thrust is being given for the development of microbial consortium, since it consists of microbes with different biochemical and physiological capabilities, which permit interactions among themselves and provide better management of diseases by way of synergistic effect and multiple mode of action. Pierson and Weller (1994) tested fluorescent pseudomonads alone and in combinations for the ability to suppress take-all disease in wheat and it was observed that, certain combinations not only suppressed the disease but also enhanced yield in wheat. Raupach and Kloepper (1998) reported that, combined application of antagonistic strains *viz. Bacillus pumilus, B. subtilis*, and *Curtobacterium flaccumfaciens* enhanced growth pro-

motion and disease reduction in cucumber, when compared with the strains tested singly. Sarma and Anandaraj (1998) suggested the consortial approach for disease management in plantation and spice crops. Hence, this present study was undertaken to explore the endophytic microbial consortium for the management of bacterial wilt of tomato.

MATERIALS AND METHODS

Healthy tomato plants were collected from 16 different locations representing north, central and south Kerala for the isolation of endophytes. Endophytic bacteria were isolated from root and stem samples as suggested by McInroy and Kloepper (1995). The stem and root bits were disinfected with 20% hydrogen peroxide and 1.05% sodium hypochlorite respectively and rinsed with sterile 0.02 M tris phosphate buffer (pH 7). An aliquot of 1 ml of the final buffer wash was transferred to sterile petri plate to which nutrient agar (NA) was added and it served as sterility check. Each sample was triturated in 9 ml of final buffer wash and dilutions were prepared up to 10^{-6}. One ml from each dilution was pipetted into sterile Petri Plates to which 15 ml each of molten and cooled NA and King's B media were poured separately and the plates were incubated at 28 ± 2^0C for 48 h. The isolation of endophytic fungi from stem and root samples was carried out according to Haiyan *et al*., (2005). The root and stem samples, after surface disinfestation, were triturated in tris phosphate buffer and from serial dilutions prepared up to 10^{-3}, 1 ml was poured into sterile Petri Plates to which Martins Rose Bengal Agar and Trichoderma Selective Medium (TSM) were poured separately and the plates were incubated at room temperature for 72 h. Endophytic actinomycetes were isolated from root and stem samples adopting the protocol of Tan *et al*. (2006). Each sample was triturated in 9 ml of buffer and 1 ml was pipetted into Petri Plates to which 15 ml of Kenknight's Agar medium was poured and the plates were incubated at 28 ± 2^0C for seven days. A total of 154 predominant colonies of various endophytic microorganisms were obtained and the pure cultures were maintained on potato dextrose agar slants for further studies. The endophytes were characterized based on cultural and morphological characteristics. The isolated endophytes were subjected to both *in vitro* and *in planta* evaluation against the bacterial wilt pathogen. The promising endophytes were selected for mutual compatibility studies and the compatible ones were again selected for the development of different consortia. Endophytes selected for the consortia were *Trichoderma viride*-1 (MyRF-1), *T. viride-2* (CSF-1), *T. harzianum*-1 (VSF-3), *T. harzianum*-2 (ASF-3), and *Penicillium melini* (VRF-1) (fungi), *Bacillus subtilis* (VSB-1) (bacteria) and *Streptomyces thermodiastaticus* (ORA-1) and *S. griseous* (VRA-1) (actinomycetes).

Development of consortia

Based on antagonistic potential, mutual compatibility, type and species of endophytic microorganisms, five microbial consortia were formulated consisting of four to six microorganisms (fungi, bacteria, and actinomycetes) in different combinations. Consortial inoculum was prepared by inoculating PDB with 48 h old bacterial culture (@ 1 loopful/100 ml) and five and seven day old fungal and actinomycete culture (@ 1 cm disc/100 ml) separately to get concentrations of 10^6 spores ml^{-1} for fungi, 10^8 cfu ml^{-1} for bacteria and 10^5 cfu ml^{-1} for actinomycetes. The inoculation dates of different endophytes were adjusted accordingly to complete the incubation period of all endophytes on the same day. The cultures of the organisms of the specific consortium were mixed and diluted with sterile water to prepare 30% concentration.

Screening of different consortia under *in planta* condition against bacterial wilt pathogen

Five different consortial formulations of antagonists were tested against *R. solanacearum* under *in planta* condition. Nursery was raised in earthern pots (size 9" x 9") containing sterilized potting mixture using a highly susceptible variety, PKM-1 and 25 day old seedlings were transplanted in the polybags having sterilized potting mixture. The prepared consortial suspensions were applied to the soil at the time of planting as soil drenching.

The bacterial wilt pathogen of tomato, *Ralstonia solanacearum*, was isolated from the infected plants as suggested by Kelman (1954). The wilted plants collected from the field was washed under running tap water and then subjected to ooze test. A loopful of the turbid suspension was streaked on triphenyl tetrazolium chloride (TZC) medium and the plates were incubated at room temperature for 48 h. Typical white colonies with pinkish centre were selected, purified, and maintained on NA slants. Challenge inoculation of the pathogen was done 30 days after planting (DAP) with fresh bacterial ooze suspension by soil drenching with wounding. Each treatment was replicated thrice with 12 plants in each. The most effective consortium was selected based on the wilt incidence recorded at 7, 10 and 14 days after inoculation.

Evaluation of selected endophytic consortium under pot culture condition

A pot culture experiment was carried out to study the efficacy of the selected endophytic consortium adopting different methods of application using the highly susceptible variety, PKM-1. The different treatments adopted were seed treatment (T_1) (1 g/2 ml), seedling root dip (T_2), soil drenching (30 ml/plant) at the time of planting (T_3), seed

treatment + soil drenching at the time of planting (T_4), seed treatment + seedling root dip (T_5), seed treatment + seedling root dip+ soil drenching at 45 DAP (T_6), control (pathogen alone) (T_7) and absolute control (T_8). The experiment was laid out in completely randomized design with three replications having 12 plants in each. Microbial consortium of 30% concentration was prepared as mentioned earlier. Challenge inoculation of the pathogen was done with fresh bacterial ooze suspension having concentration of OD_{600} = 0.3 @ 10 ml/plant by soil drenching with wounding on lateral roots with a knife at 30 days after planting in all treatments except T_8 which served as absolute control without any treatment. Observations on wilt incidence were recorded at 10, 14, and 21 days after inoculation.

Recovery of endophytes

The endophytes were reisolated both from individual application of endophytic cultures and from pot culture experiment. Individual culture suspensions were applied to the plants at the time of planting. The endophytes were reisolated from soil, stem and root at 60 DAP and at the time of harvest on their respective media to confirm their endophytic nature and survivability in soil. The isolated colonies were compared with the original cultures to confirm their identity.

Field evaluation of microbial consortium against bacterial wilt disease

A field experiment was conducted during October 2013 to January 2014 in wilt sick plot of Vellanikkara to evaluate the efficacy of the endophytic microbial consortium against bacterial wilt disease using three different varieties PKM-1 (highly susceptible), COTH-3 (F1 hybrid) and Anagha (highly resistant). The experiment was laid out in 8.64 m^2 sized plot in randomized block design with three replications and spacing of 60 x 60 cm. Five different treatments were applied for the three varieties including endophytic microbial consortium (30%) (T_1), rhizosphere microbial consortium (30 %) (T_2), *Pseudomonas fluorescens* KAU reference culture (2%) (T_3), soil drenching with copper hydroxide (2g/l) (T_4), and control (T_5). Consortial suspension of endophytic isolates *viz. T. harzianum-1* (VSF-3), *T. viride-1* (CSF-1), *T. viride-2* (MyRF-1), *B. subtilis* (VSB-1), and *S. thermodiastaticus* (ORA-1) and consortial suspension of rhizosphere organisms *viz. T. harzianum* (CT-30) and *P. fluorescens* (VB-1) were prepared as mentioned earlier and diluted to 30 per cent concentration. Seed treatment + seedling root dip+ soil drenching at 45 DAP was adopted for the application of all biocontrol treatments. Copper hydroxide @ 2 g/l was applied as soil drenching at the time of planting and 45 DAP. Soil drenching was given @ 6 l/m^2.

Per cent wilt incidence was calculated using the formula

$$\text{Per cent wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants observed}} \times 100$$

Another field experiment was carried out to study the effect of endophytic microbial consortium against bacterial wilt in moderately resistant variety (Mukthi) with two treatments consisted of endophytic consortium alone and a control.

Observations on wilt incidence at 30, 45 and 60 DAP, plant height, days to flowering, days to first harvest, number of fruits per plant, fruit yield per plant, average fruit weight and yield per plot were recorded.

Statistical analysis

Data were analyzed following analysis of variance for randomized block design (Gomez and Gomez, 1984). The post hoc test adopted was Duncan's Multiple Range Test (DMRT) to test the significance of experimental results.

RESULTS AND DISCUSSION

Evaluation of different microbial consortia against bacterial wilt disease under *in planta* condition

Five different microbial consortia developed (Table 1), were screened against the bacterial wilt disease under *in planta* condition. The observations on wilt incidence are furnished in Table 2. It was noticed that, all treatments were superior to control. Among the five consortia, consortium No. 1 showed lowest wilt incidence of 10.32, 17.26 and 26.19 per cent at 7, 10 and 14 DAI respectively followed by consortium No. 5 which showed 26.19, 31.75, and 47.62 percent at 7,10,14 DAI as compared to control. Based on these results, consortium No.1 consisted of three fungi *viz. Trichoderma harzianum*-1 (VSF-3), *T. viride*-1 (CSF-1) and *T. viride*- 2 (MyRF-1), one bacteria *viz. B. subtilis* (VSB-1) and one actinomycete, *Streptomyces thermodiastaticus* (ORA-1) was selected for further studies. According to Guetsky *et al*., (2002), a combination of bio-control agents with different mechanisms of disease control will have an additive effect in enhancing disease control and biometric characters compared to their individual application. Jetiyanon and Kloepper (2002) also proposed consortial application of different biocontrol agents for improved and stable control against a complex of diseases. Anandaraj and Sarma (2003) evaluated the consortial effect of different combinations of five bacterial strains which had been proved efficient in suppressing *P. capsici* and growth promotion in black pepper and was found highly effective when used in combination.

Table 1. Details of different microbial consortia

Consortia No.	Endophytes
1	*T. harzianum*-1 (VSF-3) + *T. viride*-1 (CSF-1) + *T. viride*-2 (MyRF-1) + *B. subtilis* (VSB-1) + *S. thermodiastaticus* (ORA-1)
2	*T. harzianum*-1 (VSF-3) + *T. viride*-1 (CSF-1) + *T. viride-2* (MyRF-1) + *B. subtilis* (VSB-1)
3	*T. harzianum*-1 (VSF-3) + *T. viride*-1 (CSF-1) + *T. viride*-2 (MyRF-1) + *Penicillium melinii* (VRF-1) + *B. subtilis* (VSB-1) + *S. thermodiastaticus* (ORA1)
4	*T. harzianum*-1 (VSF-3) + *T. viride*-1 (CSF-1) + *T. viride*-2 (MyRF-1) + *T. harzianum* -2 (ASF-3) + *B. subtilis* (VSB-1)
5	*T. harzianum*-1 (VSF-3) + *T. viride*-1 (CSF-1) + *T. viride*-2 (MyRF-1) + *B. subtilis* (VSB-1) + *S. griseous* (VRA-1)

Evaluation of promising endophytic consortium under pot culture condition

Observations on wilt incidence recorded at 10, 14 and 21 days after inoculation is summarized in Table 3. The data indicated that, all treatments were superior to control at all the three intervals. At 10th day after inoculation, incidence was very less, ranged from 0 to 8.33 per cent in different treatments against 27.78 in control (T_7). The treatment, seed treatment + seeding root dip + soil drenching 45 DAP recorded lowest wilt incidence at 14 (5.55 %) and 21 (11.11 %) days after inoculation against 61.11 and 80.55 per cent in control. All other treatments belonged to a homogenous group. The delivery method combining seed treatment, seeding root dip and soil drenching 45 DAP was selected as best for field experiments. The present result is in agreement with Manimala (2003) who observed maximum suppression of bacterial wilt in solanaceous vegetables when the bioagents applied in combination of seed treatment, root dipping and soil application with seed treatment + root dipping + soil application. Similarly, Chakravarty and Kalita (2012) also noticed lowest wilt incidence in brinjal with the same method of application of *P. fluorescens.* In addition, Sivakumar *et al*., (2011) reported the effective suppression of bacterial wilt of brinjal with seed treatment + seedling root dip + soil application + foliar spray of *B. megaterium*. Akbar (2002) also noticed that, the seed treatment and soil drenching of *P. aeruginosa* was more effective than single application in reducing the wilt incidence in tomato.

Table 2. In *planta* evaluation of microbial consortia against bacterial wilt of tomato

Consortia No.	Endophytes	Per cent wilt incidence		
		7 DAI	10 DAI	14 DAI
1	*T. harzianum*- 1 + *T. viride*- 1 + *T. viride*- 2 + *B. subtilis* + *S. thermodiastaticus*	10.32^{c} (0.34)	17.26^{c} (0.41)	26.19^{c} (0.53)
2	*T. harzianum*- 1 + *T. viride*- 1 + *T. viride*- 2 + *B. subtilis*	42.06^{b} (0.71)	47.62^{b} (0.76)	53.18^{b} (0.82)
3	*T. harzianum*- 1 + *T. viride* -1 + *T. viride*- 2 + *Penicillium melinii* + *B. subtilis* + *S. thermodiastaticus*	31.75^{bc} (0.60)	42.06^{b} (0.71)	57.94^{b} (0.87)
4	*T. harzianum*- 1 + *T. viride* -1 + *T. viride*- 2 + *T. harzianum* -2 + *B. subtilis*	42.86^{b} (0.71)	42.86^{b} (0.71)	53.97^{b} (0.83)
5	*T. harzianum*- 1 + *T. viride*- 1 + *T. viride*- 2 + *B. subtilis* + *S. griseous*	26.19^{bc} (0.53)	31.75^{bc} (0.60)	47.62^{b} (0.76)
	Control (without consortia)	83.33^{a} (1.16)	83.33^{a} (1.16)	100.00^{a} (1.37)

DAI- Days after inoculation
Treatment means with same alphabets in superscript, do not differ significantly
Figures in parenthesis are arc-sine transformed values

Table 3. Evaluation of selected microbial consortium against bacterial wilt of tomato in pot culture

Sl No.	Treatments	Per cent wilt incidence		
		10 DAI	14 DAI	21 DAI
1	T_1 - Seed treatment	5.56	16.67^{b} (4.05)	22.22^{bc} (4.61)
2	T_2 - Seedling dip	0.00	08.33^{ab} (2.61)	16.66^{ab} (3.92)
3	T_3 - Soil application at the time of planting	8.33	16.67^{b} (4.05)	22.22^{bc} (4.61)
4	T_4 - Seed treatment + Soil application at the time of planting	0.00	13.89^{ab} (3.17)	19.44^{b} (4.31)
5	T_5 - Seed treatment + Seedling dip	0.00	08.33^{ab} (2.61)	13.89^{ab} (3.17)
6	T_6 - Seed treatment + Seedling dip + Soil application at 45 DAP	0.00	05.55^{ab} (2.22)	11.11^{ab} (2.99)
7	T_7 - Control (with pathogen)	27.78	61.11^{c} (7.84)	80.55^{c} (9.00)
8	T_8 - Absolute control	0.00	0^{a} (0.71)	0^{a} (0.71)

Treatment means with same alphabets in superscript do not differ significantly

Table 4. Recovery of endophytes from individual application

Endophytes	60 Days after planting			At the time of harvest		
	Soil (cfu g^{-1})	Root (cfu g^{-1})	Stem (cfu g^{-1})	Soil (cfu g^{-1})	Root (cfu g^{-1})	Stem(cfu g^{-1})
T. harzianum-1	14 x 10^4	6 x 10^1	3 x 10^1	11 x 10^4	5 x 10^1	1 x 10^1
T. viride-1	6 x 10^4	4 x 10^1	4 x 10^1	3 x 10^4	2 x10^1	3 x 10^1
T. viride-2	12 x 10^4	3 x 10^1	1 x 10^1	5 x 10^4	3 x 10^1	1 x 10^1
B. subtilis	76 x 10^8	39 x 10^4	22 x 10^3	48 x 10^8	30 x 10^4	20 x 10^3
S. thermodiastaticus	15 x 10^5	8 x 10^1	5 x 10^1	10 x 10^5	6 x 10^1	3 x 10^1

DAI – Days after inoculation
Treatment means with same alphabets in superscript, do not differ significantly
Figures in parenthesis are square root transformed values

Recovery of endophytes

Reisolation of individual endophytes yielded high population of each organism from soil, root, and stem at 60 DAP. Soil samples showed *Trichoderma* spp, *Bacillus* and *Streptomyces* population of 6-14 x 10^4, 76 x 10^8 and 15 x 10^5 cfu g^{-1} whereas reisolation from root and stem yielded 3-6 x 10^1, 39 x 10^4 and 8 x 10^1 cfu g^{-1} and 1-4 x 10^1, 22 x 10^3 and 5 x 10^1 cfu g^{-1} respectively. At the time of harvest, the population of *Trichoderma* spp, *Bacillus* and *Streptomyces* were found to be 3-11 x 10^4, 48 x 10^8 and 10 x 10^5 cfu g^{-1} of soil, 2-5 x 10^1, 30 x 10^4 and 6 x 10^1 cfu g^{-1} of root and 1-3 x 10^1, 20 x 10^3 and 3 x 10^1 cfu g^{-1} of stem respectively and showed much reduction in bacterial population (Table 4).

In pot culture experiment, the endophytes were reisolated from soil, root, and stem at 60 DAP and harvest on their respective selective media and the result is shown in Table 5. Reisolation from soil samples showed higher population of endophytic *Trichoderma* spp, *Bacillus* and *Streptomyces* recording 22 x 10^4, 68 x 10^6 and 13 x 10^5 cfu g^{-1} respectively and the endophytic population reisolated from root and stem were comparatively less showing 14 x 10^1, 44 x 10^4, and 6 x 10^1 cfu g^{-1} and 6 x 10^1, 28 x 10^3 and 3 x 10^1 cfu g^{-1} respectively. At the time of harvest, the population of *Trichoderma* spp, bacteria and actinomycetes were found to be 19 x 10^4, 48 x 10^8 and 10 x 10^5 cfu g^{-1} of soil, 10 x 10^1, 30 x 10^4 and 5 x 10^1 cfu g^{-1} of root and 5 x 10^1, 20 x 10^3 and 3 x 10^1 cfu g^{-1} of stem respectively. Comparison of the reisolated organisms with their original cultures and microscopic observation showed similarity and thus confirmed their endophytic nature and survivability in the soil.

Field evaluation of endophytic microbial consortium against bacterial wilt disease

It is observed from the data presented in Table 6 that, all treatments were superior to control for all the three varieties in all the three intervals of observations. At 30 DAP, wilt incidence was comparatively less in all treatments of three varieties which ranged from 0 to 22.22 per cent except in the control plot of highly susceptible variety (PKM-1) which recorded 47.22 per cent incidence.

In PKM-1, the disease incidence ranged from 8.33 to 20.84 per cent in different treatments against 47.22 per cent in control at 30 DAP. Lowest incidence (8.33%) was observed in plants treated with copper hydroxide @ 2g/l. A drastic increase in wilt incidence was noticed at 45 DAP which varied from 52.78 to 84.72 per cent with minimum (52.78%) in copper hydroxide against 97.22 per cent in control. It is well known about the efficacy of copper hydroxide in reducing the bacterial wilt incidence in solanaceous vegetables (Akbar, 2002 & Mathew, 2002). Chemical control is always better than any other management practices like cultural and biological methods. However, at 60 DAP, no significant increase in wilt incidence was noticed in various treatments and the minimum incidence (58.33%) was observed in treatment with endophytic consortium recording 40.85 per cent reduction over control.

In susceptible hybrid variety (COTH-3), at 30 DAP, both endophytic consortium and copper hydroxide treatments recorded lowest incidence of 6.94 per cent against

Table 5. Recovery of endophytes from pot culture experiment

Samples	60 Days after planting			At the time of harvest		
	Trichoderma (cfu g^{-1})	*Bacillus* (cfu g^{-1})	*Streptomyces* (cfu g^{-1})	*Trichoderma* (cfu g^{-1})	*Bacillus* (cfu g^{-1})	*Streptomyces* (cfu g^{-1})
Soil	22 x 10^4	68 x 10^8	13 x 6 x	19 x 10^4	48 x 10^8	10 x 10^5
Root	14 x 10^1	44 x 10^4	101 x 10^5	10 x 10^1	30 x 10^4	5 x 10^1
Stem	6x 10^1	28 x 10^3	3 x 10^1	5 x 10^1	20 x 10^3	3 x 10^1

Treatment means with same alphabets in superscript do not differ significantly

Table 6. Field evaluation of endophytic microbial consortium against bacterial wilt disease

Treatment	Per cent wilt incidence 30 DAP	Per cent reduction over control	Per cent wilt incidence 45 DAP	Per cent reduction over control	Per cent wilt incidence 60 DAP	Per cent reduction over control
			PKM-1			
+En. con	13.89[a] (0.381)	70.58	54.17[a] (0.828)	44.28	58.33[a] (0.869)	40.85
Rhi. Con	16.67[a] (0.399)	64.70	72.22[ab] (1.036)	25.71	83.33[bc] (1.157)	15.49
P. f	20.84[a] (0.473)	55.88	84.72[ab] (1.175)	12.86	87.50[bc] (1.213)	11.27
CoC	08.33[a] (0.287)	82.35	52.78[a] (0.817)	45.71	66.67[ab] (0.979)	32.39
Control	47.22[b] (0.757)	--	97.22[b] (1.365)	--	98.61[c] (1.365)	--
CD(*P*=0.05)	0.194		0.364		0.274	
			COTH-3			
+En. con	06.94[a] (0.277)	68.75	34.72[ab] (0.628)	35.90	36.11[a] (0.644)	46.94
Rhi. Con	13.89[ab] (0.381)	37.50	36.11[ab] (0.644)	33.34	48.61[a] (0.772)	28.57
P. f	08.33[a] (0.277)	62.50	43.06[bc] (0.716)	20.51	47.22[a] (0.758)	30.61
CoC	06.94[a] (0.286)	68.75	20.83[a] (0.473)	61.54	41.67[a] (0.701)	38.77
Control	22.22[b] (0.486)	--	54.17[c] (0.829)	--	68.06[b] (0.970)	--
CD(*P*=0.05)	0.170		0.152		0.152	
			Anagha			
++En. con	1.39 (1.192)	49.98	2.78(1.462)	50.02	2.78 (1.462)	50.02
Rhi. Con	0.00 (0.707)	100.00	4.17 (1.947)	25.03	4.17 (1.947)	25.03
P. f	1.39 (1.192)	49.98	4.17 (1.947)	25.03	4.17 (1.947)	25.03
CoC	1.39 (1.192)	49.98	1.39 (1.192)	75.01	1.39 (1.192)	75.01
Control	2.78 (1.462)	--	5.56 (2.431)	--	5.56 (2.431)	--
CD(*P*=0.05)	0.170		0.152		0.152	

+ Figures in paranthesis are Arc sine transformed values
+ + Figures in paranthesis are square root transformed values
En.con - Endophytic consortium
Rhi.con - Rhizospheric consortium
P.f - Pseudomonas fluorescens
CoC - Copper hydroxide 2g/l

22.22 per cent in control. At 45 DAP, minimum incidence (20.83 %) was noticed in copper hydroxide treated plots with 61.54 per cent wilt reduction followed by endophytic consortium showing 34.72 per cent wilt incidence and was on par with rhizosphere consortium. However, no significant difference was observed among the treatments at 60 DAP and all treatments were on par. Treatment with endophytic consortium showed lowest wilt incidence of 36.11 per cent with 46.94 per cent reduction over control. Moreover, this treatment showed only slight increase in infection from 45th to 60th day of planting whereas copper hydroxide showed drastic increase in incidence from 20.83 to 41.67 per cent.

Application of consortium is better than individual application because the multiple modes of action and synergistic effect of more number of organisms in endophytic consortium could be reasons for reduction in wilt incidence as compared to rhizospheric consortium and *P. fluorescens.* Moreover, the endophytic organisms reside within the plants that, competition with other microorganisms is less, and the environment is congenial for the multiplication of endophytes within the plant. The phenomenon of ISR is more pronounced in the case of endophytic microbes as compared to other rhizospheric organisms.

In resistant variety Anagha, the wilt incidence was comparatively very less, hence, no significant difference was noticed among the treatments in all the three intervals of observations. At 30 DAP, plants treated with rhizospheric consortium were free of wilt disease and other treatments also showed less incidence of 1.39 per cent against 2.78 per cent in control. At 45 DAP, slight increase was noticed in all treatments except copper hydroxide recording lowest incidence of 1.39 per cent with 75.01 per cent reduction over control which is closely followed by endophytic consortium with 2.78 per cent against 5.56 per cent in control. Same trend was observed at 60 DAP, as no disease progression was noticed in any of the treatments. The effect of endophytic consortium in a resistant variety like Anagha is not so pronounced as in susceptible variety.

In moderately resistant variety Mukthi, drastic reduction in wilt incidence was observed with the application of endophytic consortium recording 16.67, 19.44, 23.33 as against 36.11, 42.78, 49.44 per cent in control at 30, 45 and

Table 7. Effect of endophytic microbial consortium on biometric characters of tomato

Treatment	Plant height (cm)	Days to flowering (DAP)	Days to first harvest (DAP)	Average number of fruits / plant	Average weight of fruits (g)	Per plant yield (g)	Yield per plot (kg)
			PKM-1				
En. con	74.99[a]	39.60[a]	72.48[a]	10.97	29.20	310.5[a]	2.67[a]
Rhi. Con	72.81[a]	40.04[a]	73.69[a]	10.20	31.25	285.2[ab]	1.63[ab]
P.f	73.60[a]	41.75[b]	74.08[a]	09.58	30.18	279.5[ab]	0.87[ab]
CoC	70.40[a]	40.02[a]	74.90[a]	11.12	26.90	308.7[a]	1.92[a]
Control	61.02[b]	42.55[b]	78.96[b]	09.00	26.30	246.3[b]	0.25[b]
CD (0.05)	4.756	1.34	2.768	NS	NS	7.353	1.52
			COTH-3				
En. con	81.33[a]	36.79[a]	70.13[a]	17.04	33.72	563.80[a]	8.62[a]
Rhi. Con	79.79[ab]	39.05[ab]	71.03[a]	15.67	31.13	395.15[bc]	5.53[bc]
P. f	78.31[ab]	38.66[ab]	71.47[a]	16.80	28.47	476.23[b]	5.90[b]
CoC	74.39[ab]	39.37[ab]	70.44[a]	13.80	29.03	491.82[ab]	6.00[ab]
Control	70.99[b]	40.10[b]	75.70[b]	13.57	28.78	382.55[c]	3.25[c]
CD (0.05)	7.89[1]	2.295	3.445	NS	NS	8.238	3.15
			Anagha				
En. con	86.87[a]	35.00[a]	69.00[ab]	19.80	29.12	556.97[a]	12.50[a]
Rhi. Con	83.00[ab]	36.90[b]	70.23[b]	19.67	28.82	490.20[b]	10.42[b]
P.f	80.87[ab]	37.03[b]	69.05[ab]	18.57	29.14	534.38[ab]	11.33[ab]
CoC	78.55[b]	36.03[ab]	68.66[a]	18.40	26.10	565.73[a]	12.98[a]
Control	77.03[b]	37.30[b]	71.97[c]	15.38	24.94	394.54[c]	10.25[c]
CD (0.05)	7.315	1.158	1.147	NS	NS	10.258	1.659

En.con - Endophytic consortium
Rhi.con - Rhizospheric consortium
P.f - *Pseudomonas fluorescens*
CoC - Copper hydroxide 2g/l

60 days after planting respectively and also recorded 52.81 per cent efficiency over control at 60 DAP (Table 8).

In the present investigation, a combination of host resistance and application of microbial consortium revealed better management of bacterial wilt disease thus supporting the findings of Manimala (2003). It is also in agreement with the earlier reports of Guetsky *et al*., (2002) and Jetiyanon and Kloepper (2002) that, consortial application of different bioagents is required for improved and stable control against a complex of disease and noticed reduction in population of pathogen and improvement in plant growth characters with co-inoculation of bioagents as compared to individual application and control. Consortial effect of rhizospheric antagonists against Phytophthora rot of black pepper and vanilla and bacterial wilt disease of chilli and ginger under field condition have also been studied by Mathew (2009).

Effect of endophytic microbial consortium on biometric characters of tomato

Biometric characters such as plant height, days to flowering, days to first harvest, number of fruits per plant, average weight of fruits, per plant yield and yield per plot were recorded and presented in Table 7.

In all the three varieties, significant difference was noticed with the different treatments compared to control with respect to plant height, with maximum of 74.99, 86.87 and 81.33 cm in treatment with endophytic consortium against 61.02, 77.03 and 70.99 cm in control, in PKM-1, Anagha and COTH-3 respectively.

No significant difference was noticed among the treatments with respect to days to flowering, days to first harvest and yield parameters in all the three varieties. However

Table 8. Effect of endophytic microbial consortium on bacterial wilt in moderately resistant variety (Mukthi)

Treatments	Per cent wilt incidence					
	30 DAP	Per cent reduction over control	45 DAP	Per cent reduction over control	60 DAP	Per cent reduction over control
Endophytic microbial consortium treated	16.67	53.84	19.44	63.17	23.33	52.81
Control	36.11	-	42.78	-	49.44	-

DAP: Days after planting

Table 9. Effect of endophytic microbial consortium on biometric characters of variety Mukthi

Treatments	Plant height (cm)	Days to flowering	Days to harvest	Yield/plant (g)	Yield (kg/plot)
Endophytic microbial consortium treated	58.40	50.5	81.0	623.5	6.380
Control	33.85	54.5	84.5	283.0	2.075

application of endophytic consortium had effect on increasing the number and weight of fruits in all the three varieties. Early flowering and fruit maturity were observed with application of endophytic consortium.

In case of per plant yield, all treatments were significantly superior to control in all the varieties and maximum was observed in consortium treatment with 310.5 g in PKM-1 and 563.8 g in COTH-3 against 246.3 g and 382.55 g in control respectively, whereas, in Anagha, treatment with copper hydroxide recorded maximum per plant yield with 565.73 g followed by treatment with consortium treatment with 556.97 g.

All treatments were significantly superior to control in all the varieties with respect to yield per plot. The treatment with endophytic consortium recorded significantly higher yield of 2.67 kg and 8.62 kg/plot against 0.25 kg and 3.25 kg/plot in control in varieties PKM-1 and COTH-3 respectively. In variety Anagha, copper hydroxide recorded maximum yield of 12.98 kg which was on par with endophytic consortium (12.5 kg) against 10.25 kg in control.

In moderately resistant variety Mukthi, endophytic consortium treated plants showed maximum plant height, early flowering, fruit maturity and yield compared to control. The yield per plot was three times higher than the control plot recording 6.38 kg against 2.08 kg (Table 9).

The effectiveness of a disease management strategy will be complete, when it coincides with the increase in crop yield. As a matter of fact, endophytic consortium contributes maximum towards the enhancement of crop yield in susceptible, resistant and moderately resistant varieties. In the present study, plots treated with endophytic microbial consortium recorded maximum yield of 2.67 kg, 8.62 kg and 6.38 kg against 0.25 kg, 3.25 kg and 2.08 kg in control in varieties, PKM-1, COTH-3 and Mukthi respectively. Therefore, considering the overall performances of various treatments, endophytic consortium was found effective in suppressing wilt disease and in promoting plant growth.

The application of microbial consortium could enhance the resistance considerably in highly susceptible, susceptible and moderately resistant varieties and to some extent in resistant ones and also promoted the plant growth, thereby increased the yield. Thus it revealed that, the microbial consortium had the ability to enhance the defense mechanism in tomato against the bacterial wilt pathogen.

ACKNOWLEDGEMENT

The authors greatly acknowledge the Department of Science and Technology, Govt. of India for providing financial assistance for conducting the research project.

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