J. Biol. Control, 14(1): 25-29, 2000

Integration of soil solarization and biological control with a fluorescent *Pseudomonas* sp. for controlling bacterial wilt *Ralstonia solanacearum* (E. F. Smith) Yabuuchi *et al.* of ginger

K. N. ANITH, T. P. MANOMOHANDAS, M. JAYARAJAN
K.VASANTHAKUMAR and K. C. AIPE
Regional Agricultural Research Station, Kerala Agricultural University
Ambalavayal, Wayanad 673 593, Kerala, India
E-Mail: kauhqr@ren.nic.in

ABSTRACT: **Soil solarization after irrigation, 45 days prior to planting, is found to be effective in reducing bacterial wilt disease incidence in a wilt sick field in Wayanad district of Kerala (16.02 % compared to 21.10 % in control). The maximum mean difference in temperature taken at 14.00 h. was 12.2° C in plots mulched after irrigation. Significant reduction in the soil microbial population was observed during solarization. Seed treatment with *Pseudomonas fluorescens* strain EM 85 along with solarization decreased the wilt incidence to 7.42 per cent and increased the yield to 29.42 t/ ha compared to 19.51 t/ha in control. Soil amendment with neem cake before solarization provide no additional advantage in controlling the disease.**

KEY WORDS: Bacterial wilt of ginger, biological control, *Ralstonia solanacearum*, soil solarization

Ginger is an important spice crop of Kerala and the state contribute around 40 per cent of the total ginger production of the country. Bacterial wilt caused by *Ralstonia solanacearum* is the most important constraint in the production of ginger. The pathogen is both soil and seed borne in nature. The absence of any resistant variety and effective plant protection chemicals, necessitates exploitation of various non-chemical methods for disease management. Another hurdle is the non-setting of seeds in ginger under natural conditions which limits the possibility of conventional breeding programmes for incorporation of disease resistance.

Soil solarization has been found to be an effective method for reducing the inoculum of many soil borne diseases (Katan and DeVay, 1991; Keinath, 1995; Ristaino *et al.*, 1996). The use of bacterial biological control agents, especially fluorescent pseudomonads was reported to have positive effect on the management of wilt diseases caused by *Ralstonia solanacearum* in many crops (Anuratha and Gnanamanickam, 1990; Trigalet and Trigalet 1990; Arwiyanto *et al.*, 1994; Mulya *et al.*, 1996). Soil solarization alone and in combination with application of a bacterial biological control agent for the management of bacterial wilt disease of ginger is evaluated in the present study.

MATERIALS AND METHODS

A disease sick field in Wayand district of Kerala which showed heavy incidence of bacterial

wilt in the previous year was selected for the study. Transparent white polythene sheets of 200 gauge thickness were used for mulching in solarization process. The treatments included polythene mulching (wet and dry), polythene mulching (wet and dry) with neemcake amendment, polythene mulching (wet) with seed bacterization and control (without solarization). All the wet treatments plot were irrigated to field capacity prior to covering with polythene sheet. Neem cake was amended at the rate of one kg / m^2. Solarization was carried out for 45 days starting from the 1st week of March, 1998 and planting was done thereafter. Soil temperature at a depth of 5cm was recorded at 14.00h at weekly interval. The population dynamics of total bacteria and fungi during solarization and after planting the crop was observed by serial dilution and plating of soil from surface and at a depth of 5 cm. *Pseudomonas fluorescens* strain EM 85 obtained from the Division of Microbiology, IARI, New Delhi, which is reported to have biological control property against many soil borne plant pathogens (Anith, 1997; Anith *et al.*, 1998; Anith *et al.*, 1999) was used for seed bacterization. Seed rhizomes were dipped in a bacterial suspension of strain EM 85 cultured in Kings B broth (approximately 10^8 cfu /ml) for 20 minutes just before planting. Cultural practices and manuring were done as per the Package of Practices recommendation of Kerala Agricultural University (Anon, 1996). The incidence of bacterial wilt was recorded at fortnightly interval by counting the number of wilted plants till harvest.

RESULTS AND DISCUSSION

Temperature builds up during soil solarization

The maximum difference in the mean temperature namely 12.2 ^{0}C was observed between the control plot and polythene mulching (wet) plots (Table 1). Wetting of the field before solarization has been found to be effective than dry treatment. The maximum mean soil temperature during the period was recorded in wet mulched plots (44.5 ^{0}C), whereas the minimum (32.4 ^{0}C) was recorded in control plots (Table 1). The mean ambient temperature (maximum) during the period of solarization was 31.4 0C. The study shows that, though the area of experiment, Wayanad, enjoys a cool subtropical climate (altitude 974 m above MSL), it is possible that solarization could be used as a method for building up higher soil temperature. Solarization can be started after a few summer showers normally received during the first fortnight of March to reduce the cost of irrigation.

Microbial population dynamics during soil solarization

Significant reduction in the population of bacteria and fungi in soil was observed during

Table 1. Temperature builds up during solarization at a depth of 5cm

Treatment	Temperature ^{0} C					
	1st week	2nd week	3rd week	4th week	6th week	Mean
Mulching (dry)	36.0c	36.0b	38.5b	36.8b	37.0b	36.9
Mulching (wet)	43.8b	43.8a	47.8a	44.5a	44.0a	44.6
Mulching(dry) + Neemcake	43.8b	43.8a	39.8b	37.0b	39.0b	40.6
Mulching(wet) + Neemcke	48.3a	45.3a	39.8b	42.0a	39.0b	42.9
Control	32.0b	32.0c	33.5c	31.5c	33.0c	32.4

Figures followed by same letter in a column are not significantly different (P= 0.05).

the process of solarization. It was observed that the initial bacterial population of the sub-surface soil was higher compared to the surface soil. However, at the end of soil solarization, surface soil showed higher bacterial population than the sub-surface soil. This shows that the sub-surface bacterial flora is more vulnerable to temperature build up. The reduction in the bacterial population in wet mulching, mulching (dry or wet) with neem cake amendment was found to be on par for surface as well as sub-surface level of soil at the end of solarization (Table2). The fungal population of both surface and sub surface soil also showed gradual reduction in all the treatments, except for surface soil with dry mulching, with the progress of solarization. However, the rate of decline was not as drastic as that of bacterial population (Table 2).

Bacterial wilt incidence and yield of ginger

Combination of mulching (wet) and seed bacterization recorded the lowest incidence of disease (Table3). Survival and establishment of the introduced biological control agent at the target site is an important factor determining the efficiency of biological control. *Pseudomonas fluorescens* strain EM 85 has been reported to be an efficient rhizosphere colonizer in maize, cotton and efficiently controlled many soil borne fungal diseases (Anith, 1997). It is observed that the introduced strain was able to inhibit the ginger wilt pathogen, *Ralstonia solanaciarum*, under *in vitro* conditions. Many bacterial antagonists including fluorescent pseudomonads trigger Induced Systemic Resistance (ISR) in the host crop, besides inhibiting the pathogen directly and

Table 2. Microbial population dynamics in soil during solarization

Treatment	Bacterial Population (x 10^6 cfu/g soil) [a] days of solarization				Fungal population (x 10^4 cfu/g soil) [a] days of solarization			
	0	20	45	15	0	20	45	15
Surface soil								
T1	25.00 a	7.50 b	4.43 b	13.00 c	17.50 a	5.50 b	8.50 b	14.66 b
T2	27.00 a	2.00 c	0.27 bc	3.50 d	11.50 a	6.25 b	2.75 c	10.50 b
T3	26.00 a	1.88 c	0.16 c	9.25 c	16.60 a	4.50 b	4.00 c	16.50 b
T4	27.50 a	1.00 c	0.28 c	29.25 b	17.75 a	15.50 a	3.25 c	19.00 b
T5	26.50 a	24.50 a	17.00 a	65.00 a	18.50 a	17.50 a	11.50 a	30.75 a
Sub surface soil								
T1	43.00 ab	2.30 b	5.75 b	25.00 b	15.00 ab	15.25 a	14.20 b	14.00 b
T2	41.00 ab	1.83 b	0.02 c	4.00 d	11.00 bc	2.50 c	1.75 c	4.50 c
T3	48.00 a	2.23 b	0.03 c	16.25 c	17.50 a	9.00 b	4.20 c	30.00 a
T4	39.90 ab	2.25 b	0.18 c	6.50 d	11.20 bc	2.50 c	2.20 c	3.90 c
T5	36.00 b	17.50 a	7.75 a	35.20 a	9.50 c	12.50 a	26.50 a	17.00 b

T1: Mulching (dry) T2: Mulching (wet) T3: Mulching (dry) + Neem cake T4: Mulching (wet) + Neem cake T5: Control

[a] Figures followed by same letter in a column do not differ significantly (P = 0.05) according to Dunkans Multiple Range Test.

Table 3. Wilt disease incidence and yield of ginger

Treatment	Germination (%)	Disease incidence* (%)	Yield of green rhizomes (t/ha)
Mulching (dry)	88.78	21.88 (27.85)	20.31
Mulching (wet)	92.58	16.02 (21.98)	26.39
Mulching (dry) + Neemcake	84.37	26.96 (31.07)	19.92
Mulching (wet) + Neemcake	81.25	30.47 (32.88)	19.12
Mulching(wet) + seed bacteriazation	92.58	7.42 (14.94)	29.42
Control	85.94	21.10 (26.98)	19.51
CD (P= 0.05)	NS	11.28	6.30

* Figures in parentheses are arcsine- transformed values.

there by reducing the disease severity (Liu *et al.*, 1995a, b; Wei *et al.*, 1996). Use of biological control agents along with solarization have been reported to provide synergistic effect in reducing incidence of plant diseases (Mohapatra and Dash, 1990; Rao and Krishnappa, 1995; Ristaino *et al.*, 1996). The population of total bacteria in the surface and sub-surface soil showed an increasing trend after the planting of the crop, with the application of organic manure and chemical fertilizers (Table2). Solarization together with amendment of soil with neem cake or organic matter has been reported to reduce diseases caused by soil borne fungi and nematodes (Lodha, 1995). However, under wilt sick conditions soil amendment with neem cake along with solarization had no added advantage over solarization alone in reducing the incidence of bacterial wilt disease in ginger.

The highest yield and the lowest disease incidence was recorded in solarized (wet) plots planted with bacterized seeds (Table 3). The disease incidence in plots with wet mulching alone and wet mulching combined with seed bacterization was found to be on par. However, the later treatment recorded an increase in yield of rhizomes, though not statistically significant. One of the factors limiting the use of bacterial biocontrol agents is the lack of appropriate formulated products. We are currently engaged in the development of suitable carrier based inoculants of the biocontrol agent *Pseudomonas fluorescens* strain EM 85 for the seed rhizome treatment in ginger.

ACKNOWLEDGEMENT

We thank the Govt. of India for providing fund for this research project through its National Watershed Development Programme for Rainfed Areas (NWDPRA).

REFERENCES

Anith, K. N. 1997. Molecular basis of antifungal activity of a fluorescent Pseudomonad. Ph.D. thesis, IARI, New Delhi.

Anith, K. N., Tilak, K. V. B. R, Khanuja, S. P. S. and Saxena, A. K. 1998. Cloning of genes involved in the antifungal activity of a fluorescent *Pseudomonas* sp. *World Journal of Microbiology and Biotechnology*, **14**: 939-941.

Anith, K. N., Tilak, K. V. B. R, and Khanuja, S. P. S. 1999. Molecular basis of antifungal toxin production by fluorescent *Pseudomonas* sp. strain EM 85 – A biological control agent. *Current Scienc*, **77**: 671 - 677.

Anonymous, 1996. Package of Practices for Crops. Kerala Agricultural University, Vellanikkara, Thrisur, Kerala.

Anuratha, C. S. and Gananamanickam, S. S. 1990. Biological control of bacterial wilt caused by *Pseudomonas solancearum* in India with antagonistic bacteria. *Plant and Soil*, **124**: 109-116.

Arwiyanto, T., Goto, M., Tsuyamu, S. and Takikawa, Y. 1994. Biological control of bacterial wilt of tomato by a virulent strain of *Pseudomonas solanacearum* isolated from *Strelitzia reginae*. *Annals of Phytopathological Society of Japan*, **60**: 421-430.

Katan, J. and De Vay, J. E. 1991. Soil solarization. CRC press, Boca Raton, Florida.

Keinath, A. P. 1995. Reduction in inoculum density of *Rhizoctonia solani* and control of belly rot on pickling cucumber with solarization. *Plant Disease*, **79**: 1213-1219.

Lodha, S. 1995. Soil solarization, summer irrigation and amendments for the control of *Fusarium oxysporum* f.sp. *ciceri* and *Macrophomina phaseolina* in arid soil. *Crop Protection*, **14**: 215-219.

Liu, L., Kloepper, J. W. and Tuzun, S. 1995a. Induction of systemic resistance in cucumber against *Fusarium* wilt by PGPR. *Phytopathology*, **85**: 695-698.

Liu, L., Kloepper, J. W. and Tuzun, S. 1995b. Induction of systemic resistance in cucumber by plant growthpromoting rhizobacteria: duration of protection and effect of host resistance on population and root colonization. *Phytopathology*, **85**: 1064-1068.

Mohapatra, P. D. and Dash, S. C. 1990. Efficacy of different methods of controlling *Sclerotium rolfsii* in betelvine. *Orrisa Journal of Agricultural Research*, **3**: 126-129.

Mulya, K., Watanabe, M., Goto, M., Takikawa, Y. and Tsuyumu, S. 1996. Suppression of bacterial wilt disease of tomato by root dipping with *Pseudomonas fluorescens* pf G32: the role of antibiotic substances and siderophore production. *Annals of the Phytopathological Society of Japan*, **62**: 134-140.

Rao, V. K. and Krishnappa, K. 1995. Integrated management of *Meloidogyne incognita – Fusarium oxysporum* f. sp. *ciceri* wilt disease complex in chickpea. *International Journal of Pest Management*, **41**: 234-237.

Ristaino, J. B., Perry, K. B. and Lumsden, R. D. 1996. Soil solarization and *Gliocladium virens* reduce the incidence of southern blight (*Sclerotium rolfsii*) in bell pepper in the field. *Biocontrol Science and Technology*, **6**: 583-593.

Trigalet, A. and Trigalet, D. 1990. Use of a virulent mutants of *Pseudomonas solanecarum* for the biocontrol of bacterial wilt of tomato plants. *Physiological and Molecular Plant Pathology*, **36**: 27-38.

Wei, G., Kloepper, J. W. and Tuzun, S. 1996. Induced systemic resistance to cucumber disease and increased plant growth by PGPR under field conditions. *Phytopathology*, **86**: 221-224.