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Biological management of citrus canker on acid lime through *Bacillus subtilis* (S-12) in West Bengal, India

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ABSTRACT

Citrus canker incited by *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin *et al.* is a serious disease of acid lime [*Citrus aurantifolia* (Christm.) Swingle] all over the world including West Bengal, India. The disease depends much upon its secondary spread through rain splash, mechanical contact in stormy weather and leaf damage by citrus leaf minor (*Phyllocnistis citrella* Stainton). For controlling citrus canker the usual recommendation includes antibiotics and some agrochemicals in the form of spraying. Little work has been reported on biological management of the disease. An experiment was set up in a farmer's field (acid lime orchard), Nadia, West Bengal, using an inhibitory strain of *Bacillus subtilis* (S-12) during 2009-2010. Single spray of aqueous suspension (2.7 x 10^9 cells/ml) of bacterial cells was spread on 5 batches (6 numbers of plants/batch) of plants keeping 4 batches unsprayed. Per cent Disease Index (PDI) was recorded throughout the year at every month using 0-4 scale from both treated and untreated plants. Initial PDI was also taken before one week of spraying. A single spray of the bacterial suspension during the peak season for disease that is in July has resulted in a satisfactory decline of the disease. A sharp decline of the disease was recorded at 20 days after treatment indicating that the spore forming bacteria might have taken over on the leaf surfaces of the plants.

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INTRODUCTION

Citruses are distributed throughout the world and cultivated almost in all the states and union territories of India. Several species and varieties of citruses and limes are subjected to different diseases caused by fungi, bacteria, viruses and phytoplasma. Bacteria cause some of the most serious diseases of citrus in the world. Citrus canker (c.o. - *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin *et al.*) is one of the most important diseases of acid lime. The disease has been studied worldwide for its endemic nature. Among the commercially grown citrus and lime, *Citrus aurantifolia* is the most susceptible one (Das and Dubey, 1989). In India, citrus canker was first recorded from Punjab and subsequently from different citrus growing states (Kalita *et al.*, 1995). Damage by citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), has been reported to exacerbate citrus canker in different parts of the world including Australia, Brazil, India, Florida and Yemen (Sohi and Sandhu, 1968; Sinha *et al*., 1972; Cook, 1988; Gottwald *et al*., 1997; Chagas *et al*., 2001; Christiano *et al*., 2007; Hall *et al.*, 2010), and it is difficult to locate any acid lime orchard completely free from canker infection. Das *et al.* (2012) recorded the year round disease intensity on acid lime during 2002-2003, and stated different associated biotic and abiotic factors that include citrus leaf minor and weather variables. They observed peak disease intensity in July followed by September whereas in March disease intensity was the lowest. Hence, the present work was undertaken to manage the disease with a spore forming bacterium in peak period of disease infection.

MATERIALS AND METHODS

An experiment was set up in a farmer's field (acid lime orchard), Nadia, West Bengal, using an inhibitory strain of *Bacillus subtilis* (S-12) during 2009-2010. The bacterial isolates *Bacillus subtilis* (S-12) was obtained from Department of Plant

Table 1. Year-round incidence of citrus canker on acid lime

Month	Avg. Temp. (°C)		Total Rainfall (mm)	Avg. RH (%)		PDI (Avg.)
	T_{max}	T_{min}		RH_{max}	RH_{min}	
April, 2009	37.3	25.5	0.2	89.77	41.03	4.70
May, 2009	34.6	25.7	241.2	89.06	61.10	8.62
June, 2009	35.6	27.4	66.3	89.5	64.5	16.90
July, 2009	32.8	26.5	227.4	94.16	76.33	29.81
August, 2009	32.3	26.2	387.3	96.19	80.13	27.63
September, 2009	32.9	26.3	211.1	95.2	76.77	22.98
October, 2009	32.4	22.0	91.1	94.81	63.94	13.86
November, 2009	30.3	18.4	0.00	93.3	54.83	7.98
December, 2009	26.8	11.8	0.00	93.87	49.42	3.89
January, 2010	23.7	9.3	0.00	96.0	51.0	1.29
February, 2010	29.6	14.7	7.2	92.0	43.0	0.88
March, 2010	35.8	22.4	0.5	89.0	38.0	0.85

PDI = Percent disease index

Pathology, Bidhan Chandra Krishi Viswavidyalaya. The isolate was maintained on PDA for this work. Subsequently the bacterium was grown in nutrient broth. After 7days incubation in BOD at 28±1°C the bacterial population was measured by turbidometry method using McFarland scale (Kiraly *et al.,* 1974). Through addition of sterile water bacterial suspension was prepared containing 2.7 x 10^9 cells/ml. The suspension was spread on 5 batches (A, C, D, F, G) of plants keeping 4 batches (B, E, H, I) unsprayed. There were 6 numbers of plants/batch. Per cent Disease Index (PDI) was recorded throughout the year at every month using 0-4 scale (0=No incidence, 1=1-5% incidence, 2=6-30% incidence, 3=31-60% incidence, 4=61-100% incidence) from both treated and untreated plants (Das *et al.,* 2012). Initial PDI was also taken before spraying. For estimating PDI, three plants were randomly selected from each batch and tagged. Forty leaves were sampled out randomly from each tagged plant and observed minutely for the magnitude of the disease. The causal bacterium was isolated from infected leaves in NA medium and confirmed as per standard characterization in Bergey's Manual (Holt *et al.,* 2000).

RESULTS AND DISCUSSION

Weather parameters

The pattern of citrus canker disease development and its progress was observed through year round disease monitoring studies. The highest PDI was recorded in July followed by August and September whereas, moderate PDI was observed in June and October. After October, the disease intensity gradually lowered down with a minimum in March. The above findings corroborated with earlier workers (Kalita *et al.,* 1995, Das *et al.,* 2012). It appears clearly from the Table 1 that the disease in the orchard started to increase in April, 2009 with some amount of pre-monsoon rainfall during March of the same year. There was a sharp increase of the disease intensity in June and July again due to a high degree of precipitation in the previous month i.e. in May (241.2 mm). The increase of the disease intensity in this period was due to warm temperature and high relative humidity (RH), which helped in greater multiplication of the bacteria (Table 1). With pre-monsoon showers during March - May the bacterial cells that mostly survived on cankers lesion started multiplying (Goto *et al.,* 1978, Malavotra *et al.,* 1987). The disease intensity gradually increased and reached maximum in July and the result was similar with the findings of earlier workers (Broadbent, 1992; Kalita *et al.,* 1995, Das *et al.,* 2012). The intensity of disease sharply declined after September with the decline in rainfall and RH. The disease remained in the experimental plants up to March in a very insignificant intensity (Table 1). This insignificant intensity in case of perennial host plants perhaps helps in carrying over the pathogen to the next favourable season. The lower intensity of disease was recorded during November to May. Eventually the bacterial pathogen was in a very vulnerable position during February to March. The disease may spread through rain splash and mechanical contact of branches of plants, as well as infestation by citrus leaf miner during monsoon, when new flush emerges. More emphasis has to be given to determine the precise time of bacterial multiplication on host surface as well as exact sources of inoculum for citrus canker in this area in order to develop suitable management practices.

Table 2. Biological control of citrus canker with a bacterial inhibitor

Batch	Average per cent disease index (PDI) on different dates			Change of disease situation (PDI) i.e. B-A
	Initial PDI	PDI at 7 days after spraying (A)	PDI at 20 days after spraying (B)	
A (Treated)	22.82	27.22	22.25	(-) 4.97
B (Untreated)	22.87	28.60	32.43	(+) 3.83
C (Treated)	23.41	26.34	20.46	(-) 5.88
D (Treated)	23.40	27.43	20.62	(-) 6.81
E (Untreated)	21.57	24.65	28.79	(+) 4.14
F (Treated)	16.55	19.18	14.75	(-) 4.43
G (Treated)	23.22	28.20	24.22	(-) 3.98
H (Untreated)	19.69	23.83	28.45	(+) 4.62
I (Untreated)	23.44	26.09	29.26	(+) 3.17
Average PDI of 5 treated and 4 untreated batch				
Untreated batch (B, E, H, I)	21.89	25.79	29.73	(+) 3.94
Treated batch (A, C, D, F, G)	21.88	25.67	20.46	(-) 5.21

Initial PDI estimated on 3rd July, 2nd observation on 11th July and 3rd observation on 31st July 2009, (+) = disease increment, (-) = disease reduction

Biological control of citrus canker

Results as presented in Table 2 clearly depicted the more or less similar increasing trend of the disease intensity after 7 days of spraying in both treated (A, C, D, F and G) and untreated (B, E, H and I) plants whereas after 20 days of spraying only treated batches of plants showed distinct reduction of the disease. When the data of the entire orchard was compiled, the summary picture was more clear showing distinct increase of disease (3.94%) in untreated plants of batches B, E, H and I with decline in disease intensity (5.21%) on treated plants in batches A, C, D, F and G.

The entire results on protection provided by the antagonistic strain *Bacillus subtilis* (S-12) to citrus canker bacterium on single application is highly encouraging and deserves to be tried as a new recommendation for controlling citrus canker. Biological control of citrus canker using actinomycetes (Takeuchi *et al.*, 1988) and fungal inhibitors (Masroor and Chandra, 1989) has also been attempted. Present study on biological control using a bacterial inhibitor viz. *Bacillus subtilis* (S-12) has been quite successful. A single spray of the bacterial suspension during the peak season for the disease that is July has resulted in a satisfactory decline of the disease.

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