



Bioagent mediated management of citrus canker

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ABSTRACT

Citrus canker caused by *Xanthomonas citri* pv. *citri* (Xcc) is one of the most destructive diseases of citrus. We investigated the citrus canker disease management in Experimental Farm of AAU, Jorhat, Assam (2017–19) using three microbial bioformulations, viz. Biogreen-5 (Combination of *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae* and *Trichoderma viride*), Bioveer (*Trichoderma viride*) and Biosona (*Beauveria bassiana*) in Assam lemon (*Citrus limon* L. Burm. f) as a test crop, a premier commercial citrus cultivar of Assam. These bioformulations were observed highly effective in suppressing citrus canker incidence and enhancing fruit yield. Biogreen-5 applied as soil application as well as foliar spray showed maximum reduction in canker incidence (35.55–20.96%) coupled with an enhanced fruit yield (12.43–41.93%). Enhancement of the population count of phylloplane as well as rhizosphere microbes were conspicuously observed in plants treated with microbial bioformulations, offering an environmentally benign alternative of canker management of Assam lemon.

Keywords: Bioformulation, Citrus canker, Disease management, Leaf miner infestation, Phylloplane and rhizospheric population, Yield enhancement

Citrus canker is one of the most important disease of citrus in Assam appearing in severe form in Kagzi lime (*Citrus aurantifolia* Swingle), Rangpur lime (*Citrus limonia* Osbeck) and Assam lemon (*Citrus limon* L. Burm. f) (Das and Dubey 1989). Assam lemon considered to be one of the major commercial citrus cultivars of northeast India and Assam is severely affected with this disease. The cultivar as an air layered vegetatively propagated planting material is subjected to this major disease caused by *Xanthomonas citri* pv. *citri*, on account of round the year warm temperature coupled with high rainfall, ultimately causing huge socio-economic loss to the citrus industry of Assam (Das 2003). In recent years, some potential fungal and bacterial antagonists in controlling foliar diseases have been identified (Gnanaminickam and Mew 1992).

Hazardous nature of chemical pesticides have diverted disease management approach in recent times addressing both, environmental and human health safety along with economic feasibility (Bora *et al.* 2013, 2016). Biological control agents, including antagonistic microbes, plant growth-promoting bacteria have proved to be a good option in this regard (Ghazanfar *et al.* 2018, Jhadhav *et al.* 2018). Certain strains of plant growth promoting *Pseudomonas* sp.,

have already been used as biocontrol agents to suppress citrus canker, and offer an attractive way to improve crop growth and development (Pandaya 2015). However, the microbial formulations that control pests and diseases by non-toxic mechanisms are regarded as more preferred technologies (Bora *et al.* 2019). Biopesticides being living organisms (natural enemies) or products there of pose less threat to the environment and human health, hence can be used for safe management of pests and diseases (Kumar 2015). Applications of microbial formulations have been observed to enhance yield by reducing disease incidence and increase population of phylloplane and rhizospheric microbes (Andrews 1992, Bora *et al.* 2016). In this background, a study was carried out to evaluate the efficacy of few bioformulations in suppressing citrus canker incidence, enhancement in fruit yield and changes in phylloplane and rhizospheric microbes of Assam lemon predominantly grown under humid tropical conditions of Assam.

MATERIALS AND METHODS

Isolation of pathogenic bacteria: The pathogenic bacterium, *Xanthomonas citri* pv. *citri* (Xcc) was isolated from diseased citrus leaves (Assam lemon), and was characterized culturally, morphologically and biochemically following standard protocols. The Electron Microscopy of the bacterium was done under a Field Emission Scanning Electron Microscope (FESEM), while molecular characterization was done using 16SrDNA sequencing.

Field evaluation of bioformulations: The bioformulations,

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viz. Biogreen-5 (Combination of *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae* and *Trichoderma viride*), Bioveer (*Trichoderma viride*) and Biosona (*Beauveria bassiana*) were evaluated under field condition for their efficacy in suppression of citrus canker incidence and enhancement of citrus fruit yield. The experiment was conducted at Experimental Farm of AAU, Jorhat (2017–19) in randomized block design with 7 treatments, each carrying with 3 replications. Each replication consisted of 12 lemon plants naturally infected by XCC. Microbial bioformulations were applied as foliar spray alone or along with soil application. Microbial suspension were prepared with 2 kg of microbial Bioformulations (Biosona, Bioveer and Biogreen-5) mixed with 100 l of water and sprayed in the citrus leaves @1 l/plant. Moreover, 1 kg of these bioformulations was mixed with 100 kg of vermicompost and applied at the base of the plant @ 1 kg/plant. The spraying of bioformulations was carried out at an interval of 15 days for 5 months from April, 2017 (Total 10 sprays).

Disease incidence and yield assessment: The plants were observed individually and severity percentages are arrived and the numerical rating is given as 0, 1, 3, 5, 7 or 9 shown below. Normally, 50 or 100 leaves were observed and individual rating was given. Per cent Disease Index (PDI) was worked out as per the standard grade chart (Ravinder Reddy 1982) described as disease score chart: grade 0 (0-10% leaf area infected), grade 1 (11–15% leaf area infected), grade 3 (16–25% leaf area infected), grade 5 (16–25% leaf area infected), grade 7 (26–50% leaf area infected) and grade 9 (more than 50% leaf area infected). Canker incidence was recorded each before and after application of bioformulations continuously for 6 months as (Mc Kinney's 1923);

$$\frac{\text{Sum of numerical rating} \times 100}{\text{Total number of leaves observed} \times \text{maximum disease grade}}$$

The yield enhancement was calculated as;

$$\frac{\text{Yield enhancement: Number of fruits in treated plants minus no of fruits in control plants}}{\text{Number of fruits in control plants}} \times 100$$

Isolation and population assay of phylloplane and rhizospheric microbes: Phylloplane and Rhizospheric microbes were isolated from Assam lemon plants following leaf washing technique (Voznoyokoyskaya and Khudyakov 1960) and standard serial dilution technique respectively. Microbes isolated from both Phylloplane and Rhizosphere efficacy was checked against XCC following dual culture assay. The microbes found effective was characterized culturally, morphologically and biochemically following standard protocols. Molecular sequencing results revealed it to be *Trichoderma viride*, *Trichoderma harzianum* form rhizosphere and *Cladosporium cladosporoides*, *Geotricum candidum* from phylloplane.

Population dynamics of phylloplane and rhizospheric microbes of citrus (Assam lemon) plants was assayed before and after application of different microbial bioformulations

to understand the up-regulating dominance of these effective microbes.

RESULTS AND DISCUSSION

Field evaluation of microbial bioformulations: All the treatments showed a different degree of efficacy in controlling the disease incidence which translated an increase in fruit yield as compared to control (Table 1). The lowest disease (20.96%) incidence was recorded after 6th spray with Biogreen-5 (soil + foliar), i.e. after 180 days of the 1st spray. Similarly, highest disease reduction (71.28%) was also recorded after 6th spray with Biogreen-5 (soil + foliar), i.e. after 180 days of the 1st spray. Yield of citrus fruits also enhanced after 6th spray with Biogreen-5 (soil + foliar), i.e. after 180 days after of 1st spray. The lowest disease incidence (20.96%) and highest disease reduction (71.28%) were recorded after 6th spray with Biogreen-5 (soil + foliar). Similarly, yield of Assam lemon fruits enhanced after 6th spray with Biogreen-5 (soil + foliar) (12.43–41.93%).

Microbial population on phylloplane and rhizosphere: It was interesting to observe varying responses of application of different microbial bioformulations on the changes in phylloplane and rhizosphere-based microbial communities (Table 2). The use of microbe based bioformulations has been earlier reported for the control of various fungal and bacterial pathogens (Nandkumar *et al.* 2001). This concept has exploited the potential of microbe based bioformulations and resulted in the commercialization of a number of microbial biocontrol products. Earlier, Badiger *et al.* (2016) reported that bio-agents like, *B. subtilis* and *P. fluorescens* was effective in inhibiting the growth of *X. citri* pv. *citri*. Jadhav *et al.* (2018) also reported that *T. viride* is capable

Table 1 Efficacy of different microbe based bioformulations on canker disease incidence (%), disease reduction (%) and yield enhancement (Pooled data: 2017–19)

Treatment	Incidence and reduction of citrus canker (%) and response on fruit yield		
	Disease incidence (%)	Disease reduction (%)	Fruit yield response (%)
Biogreen (Soil+Foliar)	20.96 (27.27)*	71.28 (57.61)	41.93 (40.40)
Biogreen (Foliar)	20.14 (28.11)	72.40 (58.31)	34.78 (36.15)
Bioveer (Soil+Foliar)	43.54 (41.32)	40.34 (39.47)	31.29 (34.02)
Bioveer (Foliar)	46.39 (42.94)	36.44 (37.17)	24.36 (29.60)
Biosona (Soil+Foliar)	58.51 (49.89)	19.83 (26.49)	23.07 (28.73)
Biosona (Foliar)	57.62 (49.37)	21.05 (27.35)	15.43 (24.73)
Control	72.99 (57.42)	0.00 (0.00)	0.00 (0.00)
SEd (±)	1.90	2.28	1.24
CD (P=0.05)	4.08	4.56	2.44

*Data in the parenthesis are angular transformed values, the basis for CD calculation

Table 2 Population assessment of net change in phylloplane and rhizospheric microbes in response to different bioformulations (Pooled data : 2017–19)

Treatment	Population rhizospheric and phylloplane microbes ($\times 10^7$ cfu/g)							
	<i>T. harzianum</i>		<i>T. viridie</i>		<i>C. cladosporoides</i>		<i>G. candidum</i>	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Biogreen (Soil+Foliar)	3	5	5	9	2	7	2	3
Biogreen (Foliar)	2	3	3	5	1	5	1	2
Bioveer (Soil+Foliar)	5	5	2	4	3	3	2	5
Bioveer (Foliar)	2	7	3	3	3	6	2	3
Biosona (Soil+Foliar)	1	4	2	2	2	3	1	2
Biosona (Foliar)	3	5	2	3	1	2	3	4
Control	1	1	2	2	1	1	1	2
CD (P=0.05)	NS	2	NS	3	NS	2	NS	2

of producing the cellulase enzymes, which shows strong inhibitory effect against *X. citri* pv. *citri*.

Galapon *et al.* (2017) reported reduction of bacterial blight of rice (BLB) incidence due to application of *T. harzianum* (5.34%), *M. anisopliae* (1.06%) and VAM (0.293%). Sharif *et al.* (2010) reported that destruxin isolated from five isolates of *M. anisopliae* caused mortality of citrus leafminer between 30–65%. According to Ghazanfar *et al.* (2018), entomopathogenic fungus *B. bassiana* possesses a significant potential to reduce the population of citrus leaf miner. Alvro *et al.* (2018) also reported that *T. harzianum* and *T. gamsii* along with *B. bassiana* achieved 65% larval mortality and 87.5% adult mortality of *Xylotrechus arvicola* an important pest of grapes in Spain. The benefits of biocontrol agents to the plant not only include suppression of pathogens but also yield enhancement of the crops. Pascale *et al.* (2017) reported that foliar spray or drenching of *T. harzianum* as well as their respective major secondary metabolites, harzianic acid (HA) and 6-pentyl-a-pyrone (6PP), increased yield in terms of weight (kg) of grape (63% and 97%), as compared to the untreated controls. Similar results were reported by Vivekananthan *et al.* (2006) in mango where pre-harvest application of *Pseudomonas fluorescens*; *Bacillus subtilis* and *Saccharomyces cerevisiae* resulted in 67.4% increase of fruits over control. Similar results were earlier reported shown Andrews (1992), where increase in the population dynamics of characteristic and cosmopolitan flora of microorganisms was assayed with application of PGPR in the phylloplane. Soil being the repertoire of both beneficial and pathogenic microbes, delivering of PGPR strains to soil was correlated to upregulated the population dynamics of augmented bacterial antagonists and thereby suppressed the establishment of pathogenic microbes on to the infection court (Vidhyasekaran and Muthamilan 1999).

These observations have provided database evidence in favour of effective control of citrus canker in Assam lemon using these microbial bioformulations. However, Biogreen (soil plus foliar application) proved their efficacy far better than rest of the bioformulations.

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