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Soil solarization and amelioration with calcium chloride or *Bacillus licheniformis* - an effective integrated strategy for the management of bacterial wilt of ginger incited by *Ralstonia pseudsolanacearum*

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Abstract Bacterial wilt (BW) incited by *Ralstonia pseudosolanacearum* (Rps), is one among the most economically important and devastating disease prevalent in all the ginger growing countries. Several strategies encompassing cultural, physical and chemical means have been reported to manage bacterial wilt but with limited success. In the present study, a technology integrating physical (soil solarization), chemical (soil amelioration with calcium chloride) and biological (ginger apoplastic bacterium-*Bacillus licheniformis*) methods has been developed to manage BW efficiently, economically and eco-friendly. The results indicated that, $CaCl_2$ (2 to 4%) is inhibitory to *R. pseudosolanacearum* under in vitro conditions. *In planta* evaluation under challenge inoculation showed 71%, 98% and 100% reduction in BW with *B. licheniformis*, 3% and 4% $CaCl_2$, respectively. Subsequent field evaluation involving soil solarization followed by soil amelioration with $CaCl_2$ or with *B. licheniformis* resulted in significant reduction in the population of *R. pseudosolanacearum* from 10^8 to 10^3. Further field evaluation in farmer's plot in BW endemic regions also resulted in 100% disease suppression adopting the technology. The results emanated from the present study indicated that the technology developed which includes soil solarization along with soil amelioration with either $CaCl_2$ 3% or *B. licheniformis* would serve as a viable and effective integrated strategy for the management of BW in ginger.

Keywords Bacterial wilt · Calcium chloride · Ginger · *Ralstonia pseudosolanacearum* · Disease management · Spices · *Zingiber officinale*

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Introduction

Ginger (*Zingiber officinale* Rosc.), the rhizomatous member representing Zingiberaceae family is one among the most valued and widely cultivated herbaceous spice crops in the world. Globally, commercial cultivation of ginger is concentrated in Hawaii, Jamaica, China, Indonesia, Japan, Malaysia, Nigeria, Queensland, Sierra Leone and The Philippines. However, India is the major producer contributing to approximately 32.75% of the world production (https://www.nabard.org/english/ginger.aspx). In India, Kerala, Karnataka, Himachal Pradesh, Sikkim, Meghalaya, Assam and certain north-eastern states cultivate ginger extensively. Among the diseases, bacterial wilt (BW) and rhizome rot /soft rot are the most dreaded diseases prevalent in all ginger growing tracts. Bacterial wilt (BW), referred as '*Mahali*' or 'ginger blast' caused by *R. solanacearum* (Rs) (renamed as *R. pseudosolanacearum* (Rps.) Safni et al. 2014), is the most devastating pathogen reported from all the ginger growing countries (Hayward 1994; Elphinstone 2015) with a wide host range (Wicker et al. 2007; Hayward 1994). Mathew et al. 1979 reported 100

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% bacterial wilt incidence in Wayanad, Kerala, India whereas yield loss up to 98–100% latent infection in seed rhizomes were reported in Ethiopia (Kifelew et al. 2015).

Among the *Rps* strains, *g*inger is infected only by race 4 biovar 3 strain, which induce characteristic green wilt symptoms during early hours of the day followed by yellowing and wilting leading to complete collapse of the plants (Fig. 1). Wilting is due to the entry of bacterium into the roots either through points of root formation or through injured roots and occupy the vascular system (Vasse et al. 1995). It further move along with transpirational flow and cause vascular wilt (Denny 2000). The bacterium survives without the host or in nutrient-depleted soils (Genin and Boucher 2002; Grey and Steck 2001). It also persists at low inoculum levels in naturally infested soil without a host and the population can reach a threshold level with the advent of host (Dittapongpitch and Surat 2003). Even though several chemical and non-chemical strategies have been employed to manage BW, none of them showed satisfactory control at field levels (Ciampi-Panno Fernandez et al. 1989; Yamazaki 2001; Hacisalihoglu et al. 2007; Liu et al. 2013) making it compulsive to search for alternative management strategies.

Amending soil with calcium oxide (CaO) and urea were reported to be effective in managing tomato BW (Michel et al. 1997) possibly by altering soil pH and nitrite accumulation in the soil (Michel and Mew 1998). Dannon and Wydra (2004) reported that soil application of silicon reduces tomato BW whereas, Kai et al. (2014) reported $CaCO_3$ as a better option in reducing BW since $CaCO_3$ serves as a soil amendment to alter soil pH and also increased Ca^{2+} content in the soil thereby imparting resistance against BW. (Jiang et al. 2013; Yamazaki and Hoshina 1995; Conway et al. 1992, 1988; Volpin and Elad 1991; Bateman and Lumsden 1965; Forster and Echandi 1975; Yamazaki and Hoshina 1995). Calcium plays a vital role in plant disease resistance and is supposed to be the central regulator for plant growth and development (Hepler 2005).

From the time course different areas of biological control were exploited for BW management in solanaceous crops including endophytic bacteria, rhizospheric bacteria, phages etc. *Stenotrophomonas maltophila* has been found effective against potato brown rot in Egypt (Messiha et al. 2007). An avirulent bacteriocin producing *R. solanacearum* strain was also found effective against tomato BW in Brazil (de Araujo et al. 2004). Similarly, an antagonistic *Pseudomonas fluorescence* was reported as effective against BW of *Solanum melongena* (Ramesh et al. 2009). In due course, phages were exploited for the control of BW (Yamada et al. 2007; Prameela et al. 2012). Baptista et al. (2006 and 2007) reported that by adopting soil solarization, the soil pH, K, Na, B and Zn contents, microbial biomass and respiration in soil was reduced without affecting the soil chemical properties. In light of the above information, the present study was focussed on investigating an integrated management strategy combining both soil solarization and amelioration with either calcium chloride or bioagent *Bacillus licheniformis* in suppressing BW of ginger. The outcome of the study is expected to provide a simple and environmentally feasible technology for managing BW of edible ginger both under organic and inorganic system of cultivation.

Materials and methods

In the present study, a series of six experiments were undertaken to delineate the effect of $CaCl_2$ in managing BW of which two experiments are under in vitro conditions and two experiments under green house conditions maintaining a temperature of 24-28 °C and final validation and demonstration was done under field conditions.

Ginger plants Ginger variety, IISR Rejatha was used for the experiment. The seed rhizomes were treated with a mixture of mancozeb (0.25%) and quinalphos

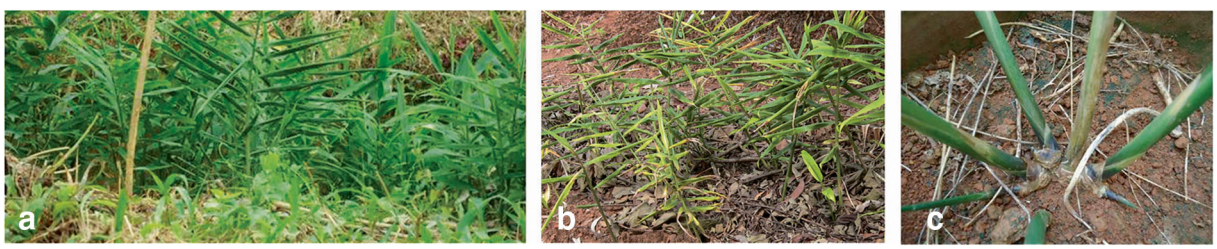

Fig. 1 Field symptoms of bacterial wilt (**a**) -Green wilt symptoms, (**b**) Advanced stage of infection (**c**) Pseudostem infection

(0.075%) and stored in a cool place with sawdust till planting (Ginger Extension Pamphlet 2015).

Bioagent The bioagents viz., *Bacillus licheniformis* strain GAP 107, an apoplastic bacterium isolated from ginger and found inhibitory to *Rps* both *in planta* and in the field (Prameela 2016), *B. putida* strain BP25, an endophyte isolated from black pepper effective against ginger rot pathogens (Aravind et al. 2012) and *B. amyloliquefaciens* strain GRB 35, effective against rhizome rot of ginger (Dinesh et al. 2015) were used for the study. The bioagents grown in NB broth culture for 48 h was used for the experiments. *Rps* race 4 strain was inoculated in CPG broth (Kelman 1954). and incubated in an orbital shaker at a speed of 180 rpm for 24 h at 28 °C. The absorbance of culture was recorded and 100 ml of OD 600 = 0.1 (10^8 CFU/ml) culture was used for inoculating ginger plants.

Effect of $CaCl_2$ on survival of *Rps*

To study the direct effect of $CaCl_2$ on race 4 Biovar 3 strain of *Rps*, CPG broth was prepared by amending four different concentrations of $CaCl_2$ viz., 1, 2, 3 and 4% and inoculated with *Rps*. CPG broth inoculated with *Rps* alone served as control. The tubes were kept at 28 °C for 48 h and the absorbance was recorded at OD 600 using biophotometer plus (Eppendorf, Germany). The growth was indicated as increase in turbidity of the medium. Simultaneously, plate assay was carried out by streaking the broth culture on CPG medium to assess the growth of *Rps*. The experiment was repeated twice for confirmation.

Green- house experiments

Pot culture experiment was undertaken for the evaluation of $CaCl_2$ against BW incidence under challenge inoculated conditions and compared with promising bioagents and recommended chemicals.

a) **Experiment 1:** Pilot experiment was conducted to study the effect of $CaCl_2$ on *Rps* infection under challenge inoculated conditions where inorganic salt like sodium chloride (NaCl) was used as a comparison. Here NaCl was included in the study since it was already reported inhibitory to *Phytophthora capsici*, the foot rot pathogen of black pepper (Bhai et al. 2009). In this experiment, briefly 45 day old ginger plants in polybags (size 20 × 10 cm holding 500 g potting mixture containing soil: sand: farmyard manure in the ratio 1:1:1) were drenched with 250 ml each of NaCl (3%) and $CaCl_2$ (3%) and the plants were challenged with 50 ml of *Rps*. (10^8 CFU/mL) after two days. A positive control was maintained with *Rps* alone. The experiment was repeated twice for confirmation of the result. Based on the results of this experiment, pot experiment was conducted under greenhouse conditions.

b) **Experiment 2:** The experiment was designed in CRD with 10 treatments and 3 replications with three pots per replication. Pots (20 cm diameter) were filled with potting mixture (10 kg mixture/pot) containing soil, sand and farm yard manure in 1:1:1 proportion. The ten treatments (Table 1) included one positive control with *Rp*s and one negative control without *Rps* also. Approximately 25 g ginger rhizome was planted in each pot and mulched with green leaves and watered twice a week. One week after planting, the treatments were imposed. After 45 days of planting, the plants were challenged with the virulent strain of race 4 strain of *Rps* (GRs Mnt2**).** The pseudostem count in each pot was recorded on the day of pathogen inoculation and further the plants were monitored for green wilt symptoms due to BW. The symptoms due to BW were recorded at 15, 30 and 45 days after inoculation by counting the number of pseudostems infected and the percent pseudostem infection was computed using the formula:

$$\text{Per cent incidence} = \frac{\text{No.of pseudostems infected}}{\text{Total no.pseudostems}} \text{x } 100$$

Based on the results of greenhouse experiments, field trial was conducted.

Isolation of culturable microbes

The total culturable bacteria were isolated using dilution plate technique in nutrient agar. Briefly 1 g of the air dried soil was suspended in 9 ml of sterile distilled water and from this serial dilutions were made up to 10^5 dilution and 1 ml from this 10^3 and 10^5 were plated by pour plate method and incubated at 48 h at 28-30 °C.The bacterial colonies appeared were counted manually and represented as cfu/g soil.

Table 1 Details of green house evaluation against bacterial wilt

Treatments	Particulars
T1 Absolute control	No treatments
T2 Pathogen control	*Rps* strain GRs Mnt 2 alone
T3 *Peudomonas putida* strain BP 25	Seed treatment and soil application (100 ml having 10^9 CFU/ml)
T4 Copper oxychloride (0.25%)	Soil application (100 ml/pot)
T5 *Bacillus licheniformis* (strain IISRGAP 107)	Seed treatment and soil application @100 ml having 10^9 CFU/ml
T6 *B. licheniformis* (strain IISRGAP 107) + $CaCl_2$ (3%)	Seed treatment with GAP and soil application @100 ml having 10^9 CFU/ml
T7 $CaCl_2$ (3%)	Soil application (100 ml/pot)
T8 *B. amyloliquefaciens* (strain GRB 35)	Seed treatment and soil application @100 ml having 10^9 CFU/ml
T9 Bleaching Powder [Ca $(ClO)_2$]	Soil application @10 g/pot
T10 $CaCl_2$ (4%)	Soil application (100 ml/pot)
Application (at the time of planting and at 30, 45 and, 60 days after planting)	

Field trial

Field trial for the evaluation of $CaCl_2$ in managing BW was conducted at the ICAR-IISR experimental farm, Peruvannamuzhi, Kozhikode, India during 2016–17. The plot selected was having the history of severe BW incidence during 2015–16 with high density of residual inoculum of *Rps*. The experiment was carried out with split plot design where the main treatments were with and without solarization with seven sub-treatments (Table 2). Beds of 3 × 1 m dimension were prepared during January 2016 and required number of beds were subjected to solarization using polythene sheets of 100 μm for 65 days where the temperature remain 50-58 °C during noon at 11 am to 3 pm. Planting was done during first week of May 2016. In treatments (T1 and T3) seed treatment was carried out with GAP107 before planting (being an apoplastic bacteria it would require short duration to colonize the internal vasculature) as well as soil application. Briefly, required quantity of seed rhizomes were immersed in the suspension of GAP 107 with an inoculum density of 10^9 CFU /ml for 30 min. All other treatments were imposed as soil drenching at the time of planting as depicted in Table 2.

Approximately 20–25 g seed rhizomes were planted in each pit (40 pits/bed) with basal application of farmyard manure. After planting, cultural operations like mulching, fertilizer application and earthing up adopted as per package of practice recommendations of ICAR-IISR (Ginger Extension Pamphlet 2015). and the plants were monitored for germination and BW incidence at regular intervals. Further, the soil was also analyzed for nutrient status, pH, EC and soil microbial load of *Rps* and total culturable bacteria.

Study on soil physical and biological properties

The soil samples collected from the rhizosphere of treated plants (Field trial 1) were analyzed for soil

Table 2 Details of field treatments

Treatments	Modes of application
T1- IISRGAP 107	Seed treatment and soil application (@ 5 l/bed with 10^9 CFU/ml) at the time of planting and at 30, 45, 60 and 90 days
T2- $CaCl_2$ (3%)	Soil application (@5 l/bed having 10^9 CFU/ml) at the time of planting and at 30, 45, 60 and 90 days
T3-IISRGAP 107 + $CaCl_2$ (3%)	Seed treatment and soil application (@5 l/bed having 10^9 CFU/ml) at the time of planting and at 30, 45, 60 and 90 days
T4-GRB 35(Rhizobacteria)	Soil application (@5 l/bed with 10^9 CFU/ml) at the time of planting and at 30, 45, 60 and 90 days
T5- Copper oxychloride (0.25%)	Soil application at the time of planting and at 30,45,60 and 90 days
T6-Bleaching Powder [Ca $(ClO)_2$]	Soil application @100 g/bed at the time of planting and at 30,45,60 and 90 days
T7-Absolute control	No treatments

physical properties such as pH and electrical conductivity (EC) adopting standard protocols. The soil nutrient analysis was done by standard analytical methods to assess the available calcium content and other essential nutrients in the soil. The pH was measured using a calibrated pH meter (Mettler Toledo) and EC was measured using Cyberscan Con II conductivity/TDS/°C meter (Eutech Instruments, Singapore). The dehydrogenase activity of soil was done according to Casida et al. (1964) besides enumerating the soil population of *Rps* and total bacterial count.

After confirming the results in both pot and field trials, the technology was demonstrated in two farmers plot in Wayanad where farmers left ginger cultivation in the area due to heavy incidence of bacterial wilt.

Field level demonstration trials

The demonstration trials were undertaken in two farmer's plots during 2017–18 in Manathavady (Plot 1) and Kenichira (Plot 2) in Wayanad district, Kerala, India. The two promising treatments from the field trial were demonstrated here along with control i.e. farmer's practice. The treatments were *B. licheniformis* GAP107 MTCC12725 (T1), and $CaCl_2$ (3%) (T2) and control (T3). The beds were prepared and subjected to solarization for >53 days (ie from 16 March to 8 May, 2017) with an illumination intensity of 1500 μE for 8 h daily (9.30 am to 4.30 pm) as described earlier and the planting was undertaken with the onset of monsoon during May, 2017. Control was maintained as farmer's practice without solarization. The treatments were imposed at the time of planting and at 30, 45, 60 and 90 days after planting. However, in case of *B. licheniformis* (T1) seed priming was done for 30 min before planting as described earlier. During the crop season, four doses of fertilizers (Ginger Extension Pamphlet 2015). were applied. Besides, one spray of quinalphos (0.075%) against shoot borer and carbendazim (0.2%) against *Phyllosticta* leaf spot was done during August. No other plant protection chemicals were applied to the crop till harvest. The observations on growth parameters like germination, pseudostem production and percent disease incidence were recorded periodically. At harvest (February, 2018) fresh yield of rhizomes was recorded and B:C ratio calculated.

Statistical analysis

The data analysis was performed using SAS software version 9.3 (SAS Institute Inc., Cary NC) as well as PROC ANOVA and PROC mixed procedure and means were separated according to Fisher's least significant difference (LSD) test ($p \leq 0.5$).

Results

The in vitro study with inorganic salts, NaCl (3%) and $CaCl_2$ (3%) showed growth suppression of *Rps* only with Calciun chloride and clearly revealed the inhibitory effect of $CaCl_2$ (3%) on *Rps*. The plants treated with NaCl and control showed typical green wilt symptoms in seven days of inoculation however, BW symptoms was not observed in $CaCl_2$ (3%) treated plants. These results provided an indication on the suppressive effect of $CaCl_2$ on *Rps* Based on this result, the second experiment was done with three different concentrations of $CaCl_2$ viz., 2, 3 and 4%. In *in planta*, when 3% and 4% of $CaCl_2$ were applied to ginger plants and challenged with *Rps*, 100% suppression of infection was observed compared to control without $CaCl_2$ (Table 3). This preliminary observation clearly indicated that 3 and 4% $CaCl_2$ are highly effective in suppressing *Rps* population. Based on these findings, subsequent field experiments were formulated.

Parallely, the direct effect of various concentrations of $CaCl_2$ on the growth of *Rps* was studied using CPG broth as indicated by degree of absorbance. At different concentrations of $CaCl_2$ either the growth was negligible or totally absent indicating the lysis of bacteria whereas uniform turbidity/dense growth were observed in control where CPG broth was not amended with $CaCl_2$. In plate assay using SMSA, growth was obtained only in control (3.4×10^9 CFU/ml) where as there was no growth in different tested concentrations of $CaCl_2$.

Green house evaluation

The greenhouse experiment was designed based on the results of preliminary experiments in which efficacy of $CaCl_2$ 3–4% was assessed in comparison with promising biocontrol agents as well as other recommended practices for the management of BW viz., application of copper oxychloride or bleaching powder [$Ca(ClO)_2$], seed treatment and soil application of

Table 3 Direct effect of different conc. of $CaCl_2$ on the growth of *Rps*. in CPG medium

Treatments	Absorbance	Plate assay	*In planta* suppression (%) of BW
CPG broth+*Rps*	4.868	Lawn of *Rps*	0.0
CPG broth+$CaCl_2$ 1%	0.426	Lawn *of Rps*	0.0
CPG broth+ $CaCl_2$2%	0.295	Faint growth	0.0
CPG broth+ $CaCl_2$3%	0.274	No growth	100
CPG broth+ $CaCl_2$4%	0.109	No growth	100

GAP 107, soil application of Rhizobacteria viz., *B. amyloliquefaciens* strain GRB 35 and *P. putida* strain BP 25. Data on germination percentage and pseudostem count were recorded 45 days after planting (Fig. 2). The maximum pseudostem count was noticed in absolute control without any treatments followed by $CaCl_2$ (4%). The treatment with GRB 35 and BP 25 showed minimum pseudostem production. In general, BW symptoms manifests within 7–10 days of challenge inoculation with the most virulent strain (GRsMnt2). In the present study, infection started within 7 days in almost all the treatments except T1, T7 and T10. The disease advanced in all the treatments and at 15 days after challenge inoculation the incidence ranged between 0 and 35% and within 20 days the incidence increased upto 69%. Further, 40 days after inoculation, 99% incidence was noticed and among the treatments, $CaCl_2$ (4%) showed 100% suppression followed by 3% $CaCl_2$ (98.28%), bleaching powder (82.58%) and GAP 107 (70.97%) (Figs. 3, 4 and 5). Finally, except T1, T7, T9 and T10 all the plants completely succumbed to the disease. The results clearly indicated the effect of $CaCl_2$ in suppressing *Rps*. The yield data indicated that, T7 registered maximum yield (CaCl2 3%) which was at par with T1 (absolute control) and T10 ($CaCl_2$ 4%) (Fig. 4) while, the yield levels were negligible in other treatments viz., T2, T3, T4 and T8.

Field trial

Since greenhouse evaluation showed $CaCl_2$ (3%) as effective as $CaCl_2$ (4%), lower concentration was selected for field evaluation. In the field, integrated strategy was adopted by incorporating soil solarization along with application of either $CaCl_2$ (3%) or *Bacillus licheniformis*.

With respect to germination, it is noticed that there is no significant difference between solarized or non-solarized plots or between sub-treatments, (Table 4). But there was a clear indication of the effect of solarization with either $CaCl_2$ or *Bacillus licheniformis* in reducing the disease in severely affected area. Disease symptoms appeared 50 days after planting during the month of June. At 60 days the incidence in both solarized and non-solarized plots ranged from 0 to 16.5%. The disease advanced in all the treatments except T1 and T2 under solarization. At 120 days, 0–91% incidence was recorded in all the treatments except T1, T2 and T3 where complete suppression of the disease was observed. In treatment with bleaching powder the disease was 31.84%, however, at 150 days all the plants collapsed (100% incidence) in all the treatments except T1 (*B. licheniformis* strain IISRGAP107), T2 ($CaCl_2$ (3%) and T3 (IISRGAP 107 + $CaCl_2$ 3%) (Table 5). Since the inoculum pressure of *Rps* was very high (beyond the limit of control) (10^8 CFU/g) in the non-solarized plot, the incidence was also high in all the treatments (Tables 5 and 6). Where as in the solaraized plot, there is celar cut difference between different treatments

Fig. 2 Greenhouse experiment T1-T9 (Table 1)

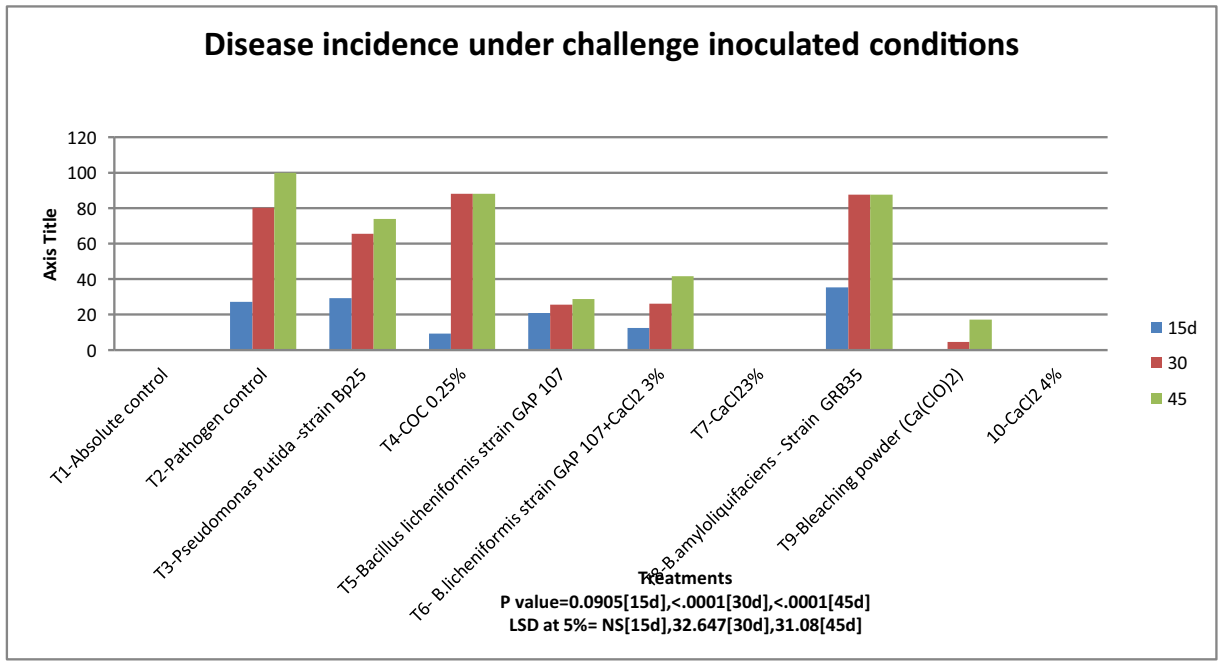

Fig. 3 Disease incidence under challenge inoculated conditions

showing the additive effect of solarization in controlling the disease. Similarly the total culturable population of bacteria was also high in the non-solarized plot indicating the effect of solarization in reducing the microbial population (Table 7).

The analysis of nutrient status of the soil indicated that, organic carbon, nitrogen and phosphorous were higher in the treatments T1-T3 where BW was completely suppressed. Similarly, other nutrients including manganese, boron, magnesium and calcium were also higher in these treatments (Table 8). The EC was also higher in treatments T1, T2 and T3 (Table 9). Similarly, pH of the soil also showed significant variation. $CaCl_2$ and COC treated plots showed pH below 6.0. But no correlation could be noticed between pH and disease reduction (Table 8). Similarly

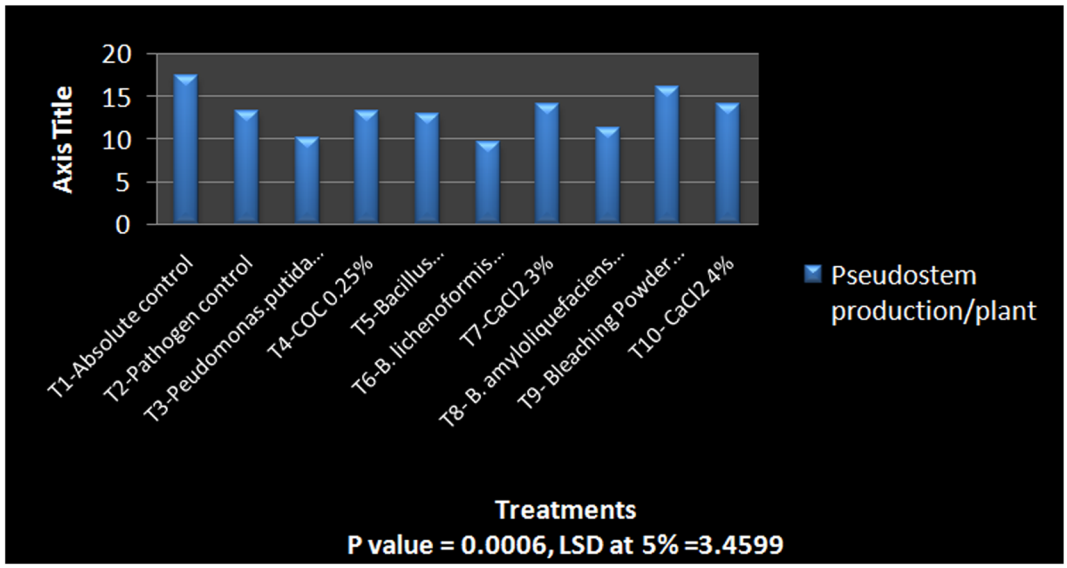

Fig. 4 Pseudostem production /plant

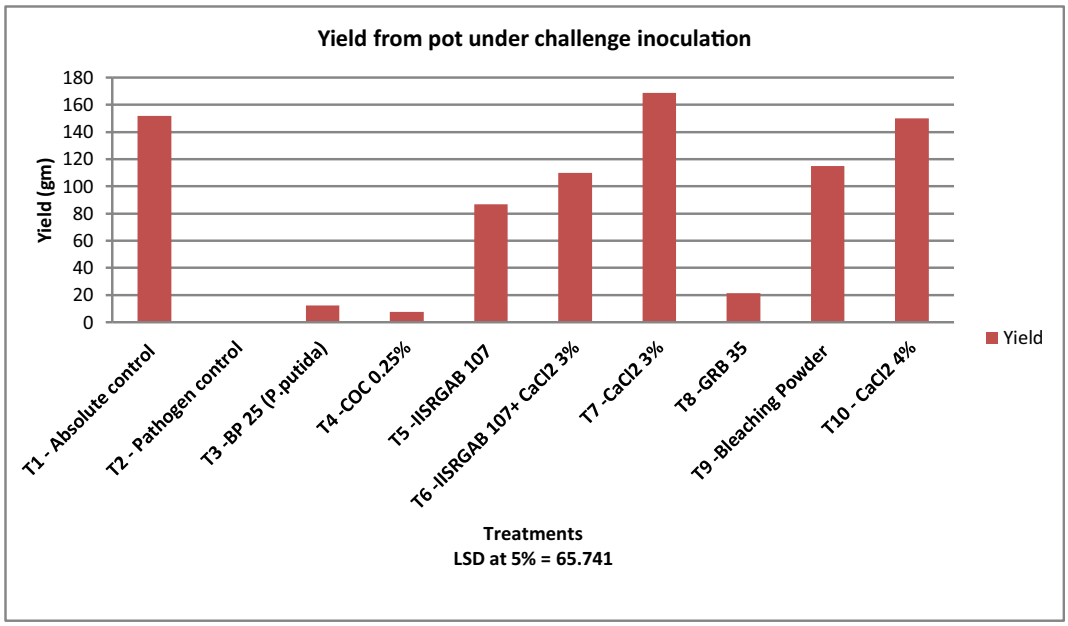

Fig. 5 Yield from pots under challenge inocualtion

except zinc and copper all the other micro and macro nutrients were significantly higher with both GAP 107 and $CaCl_2$ treatments. The results clearly indicated the role of calcium in suppressing the disease. The yield data clearly revealed the effect of first three treatments in realizing better yield (Fig. 6) in which the maximum yield was recorded in T1 and T2 followed by T3.

Demonstration trial

The filed level demonstration was conducted in two hot spot areas in Wayanad, Kerala, India where farmers abandoned ginger cultivation due to severe incidence of BW over the years. In the two experimental plots, the average pseudostem production was significantly superior over the control (Fig. 7). During the period, BW was not noticed in both the treatments in the two plots, indicating efficacy of the treatments in suppressing the disease. Nevertheless, the control plot and adjacent ginger plots showed heavy incidence of BW (>30%) (Fig. 8). On harvest, the rhizomes were absolutely healthy and disease-free. From Plot1 the farmer harvested 1822.7 kg from $CaCl_2$ treatment (45.6% more than control) and 1245 kg from *B. licheniformis* treatment (20.4% more than control). Similarly, from Plot 2 the farmer harvested 695 kg from $CaCl_2$(82% more than control) and 413 kg from *B. licheniformis* treated beds (69.5% more than control) (Table 10). Usually in ginger cultivation, plant protection chemicals to manage diseases especially the BW are applied monthly. While the present study clearly indicated that, BW can be managed effectively and economically adopting the technology resulting in the production of healthy disease-free rhizomes with a B:C ratio of 1:3.

Table 4 Effect of Treatments on germination of ginger seed

Treatments	% Germination	
	Solarized	Non solarized
T1- IISRGAB 107	80.63(32.25)*	68.75(27.50)
T2- $CaCl_2$ (3%)	81.25(32.50)	75.63(30.25)
T3- IISRGAB 107+ CaCl2 (3%)	80.63(35.25)	80.63(32.25)
T4- GRB 35	78.13(31.25)	80.63(32.25)
T5- COC(0.25%)	86.88(34.75)	84.38(33.75)
T6- Bleaching Powder (10 g/pot)	88.75(35.50)	81.88(32.75)
T7- Absolute control	84.38 (33.75)	75.0(30.00)

LSD at 5% = Main Plot- NS, Sub Plot- NS Main plot x Sub Plot – NS

Figures in brackets are transformed values (Angular transformation)

Discussion

The results of in vitro and green house experiments confirmed that, $CaCl_2$ and *B. licheniformis* strain GAP107 are

Table 5 Disease incidence at different intervals(Field %)

Treatments	60dai		120dai		150dai	
	Solarized	Non solarized	Solarized	Non solarized	Solarized	Non solarized
T1- IISRGAB 107	0.0	9.01	0.00c	100	0.00e	100
T2- $CaCl_2$ (3%)	0.0	16.50	0.00c	100	0. 00 g	100
T3- IISRGAB 107+ $CaCl_2$ (3%)	0.0	8.02	0.00c	100	0.00f	100
T4- GRB 35	0.0	4.35	86.30a	59.91	100c	100
T5- COC(0.25%)	11.29	11.62	78.90a	64.44	100b	100
T6- Bleaching Powder (10 g/pot)	0.72	2.42	31.84b	57.62	100a	100
T7- Absolute control	1.45	3.24	90.83a	68.82	100d	100
LSD at5%	NS	15.202	19.018	28.429	0.0	Ns

effective in suppressing bacterial wilt in ginger. In the field, integrated strategy incorporating either $CaCl_2$ or *B. licheniformis* along with pre-plant solarization of the soil is promising in controlling bacterial wilt as evidenced by the absence of bacterial wilt in the treated plots when compared to control and other treatments. This is the first instance where bacterial wilt is found satisfactorily controlled by a chemical or bioagent at the field level. The field evaluation undertaken in the present study undoubtedly proved that BW in ginger can be managed effectively with a three pronged approach amalgamating physical, chemical and biological components.

Though bleaching powder, the existing recommended control measure showed comparatively lesser incidence upto 40 days, later there was total deterioration of the rhizome due to disease. This phenomenon is because, if one plant/pseudostem (in visual observation) in a bed or pot is infected, the xylem residing bacteria will be discharged from the infected plant into the soil and will reach a level beyond the manageable level, any recommended chemical or bioagents failed. Moreover, subsequent spread of the pathogen under field conditions is facilitated by incessant rain, rain splash, run-off water and even through the farm implements. Hence, it is highly imperative to prevent colonization of bacteria in the soil by proper amelioration of the soil with suitable chemical/bioagent as a precautionary measure. In the present study, it was found that in the first two treatments (B. *licheniformis* (T1), and $CaCl_2$ 3% (T2) in solarized plot, *Rps* population was only at the level of 10^3 CFU/g which is below the threshold inoculum level to incite disease where it was as higher as 10^5 CFU/g in other treatments where the disease incidence was 100% (Table 6**)**. There was significant difference between *Rps* populations in treatments T1-T3 compared to other treatments, highlighting the effect of $CaCl_2$ and *B. licheniformis* in reducing *Rps* population which is indicated by the total bacterial count (Table 7).

Table 6 Influence of treatments on *Ralstonia* population in the soil (log cfu/g)

Treatments	*Rps* population (Initial)		*Rps* population(At the end of the experiment)	
	Solarized	Non solarized	Solarized	Non Solarized
T1- IISRGAB 107	6.05×10^2	3.16×10^6	$1.50(0.5 \times 10^3)$b	$8.71(15.68 \times 10^8)$
T2- $CaCl_2$ (3%)	1.106×10^3	1.92×10^7	$1.70(1.75 \times 10^3)$b	$8.75(3.225 \times 10^8)$
T3- IISRGAB 107+ $CaCl_2$ (3%)	2.15×10^3	4.26×10^6	$1.70(1.75 \times 10^3)$b	$8.51(4.41 \times 10^8)$
T4- GRB 35	2.3×10^3	9.2×10^4	$5.67(9.7 \times 10^5)$a	$6.24(1.98 \times 10^6)$
T5- COC(0.25%)	3.9×10^4	2.56×10^5	$5.14(2.5 \times 10^5)$a	$5.92(1.08 \times 10^6)$
T6- Bleaching Powder	2.36×10^4	5.73×10^5	$6.38(2.25 \times 10^7)$a	$5.72(5.5 \times 10^5)$
T7- Absolute control	3.16×10^4	4.8×10^5	$5.85(2.25 \times 10^7)$a	$6.92(1.26 \times 10^8)$

S-Solarized, NS-Non-solarized, LSD at 5% = Main Plot- 0.94, Sub Plot-Non significant

Main plot x Sub Plot – 1.42

Table 7 Effect of Treatments on total culturable bacteria (log cfu/g)

Treatments	Solarized	Non solarized	Sub plot mean
T1- IISRGAB 107	7.25	8.55	7.9
T2- $CaCl_2$ (3%)	7.91	8.60	8.26
T3- IISRGAB 107+ $CaCl_2$ (3%)	7.12	8.69	7.91
T4- GRB 35	8.06	8.48	8.27
T5- COC(0.25%)	8.56	7.95	8.26
T6- Bleaching Powder	8.68	8.46	8.57
T7- Absolute control	8.09	8.39	8.24
Main plot mean	7.95B	8.45A	

LSD at 5% = Main Plot- 0.23, Sub Plot-0.41 Main plot x Sub Plot – 0.58

The nutrient status of the soil was also found higher in the treatments with $CaCl_2$ and GAP 107 as compared to control and other biological as well as chemical treatments (Table 8). However the same treatments could not control the disease under non-solarized condition due to the high inoculum density which was more than 10^8 g^{-1} soil. Usually it is not advisable to cultivate ginger in the same area in consecutive years as it significantly depletes nutrient status and facilitates the build-up of residual pathogen inoculum. Though the beds are subsequently treated with recommended chemicals, the residual inoculum surviving on collateral weed hosts and adjacent infested soil would serve as primary sources of inoculum for the spread of disease for succeeding season.

Many strategies such as biological, physical, chemical or cultural means were experimented for managing BW (Fortnum and Martin 1998; Ji et al. 2005; Hong et al. 2011; Wagura et al. 2011). Similarly, from the time course different areas of biological control were exploited including endophytic bacteria, rhizospheric bacteria, phages etc. for BW management in different solanaceous crops. *Stenotrophomonas maltophila* has been found effective in controlling potato brown rot in Egypt (Messiha et al. 2007) whereas an avirulent bacteriocin producing *R. solanacearum* strain was found effective against tomato BW in Brazil (de Araujo et al. 2004). An antagonistic *Pseudomonas fluorescence* was found effective against BW of *Solanum melongena* (Ramesh et al. 2009). So also, phages were exploited for the control of BW (Yamada et al. 2007; Prameela et al. 2012). However, all these strategies met with limited success under field conditions. However in the present study, *B. licheniformis* GAP107 isolated as an apoplastic bacteria from ginger showed significant disease suppression both *in planta* and under field conditions.

A large body of evidence states the additive effect of fertilizers in reducing the incidence of BW of which calcium-based mixtures are the most reported one. Lower disease severity in tomato BW was correlated with an increase in Ca uptake (Yamazaki et al. 1996, 2000). Heyman et al. (2007) also reported the effect of Ca in suppressing Aphanomyces root rot in pea. The effect of Na and Ca salts in suppressing early blight of potato caused by *Alternaria solani* under laboratory, greenhouse and field conditions individually and combined with the yeast *Saccharomyces cerevisiae* has been reported (Nehal and Mokhtar 2009). In the present investigation, $CaCl_2$ was found highly effective in reducing disease severity than bleaching powder (Na_2CO_3). This may be due to the direct inhibition of polygalacturonase

Table 8 Nutrient status of solarized plot at the end of the experiment

Treatment s	Oc	pH	N	P	K	Ca	Mg	Mn	Zn	Cu	Bo
T1- IISRGAB 107	2.29^{A}	5.88^{DE}	243.75^{AB}	94.20^{AB}	183.00	991.00^{ABC}	94.75	13.73^{A}	1.96^{BCD}	1.03^{BC}	0.57^{A}
T2- $CaCl_2$ (3%)	2.26^{A}	6.78^{A}	248.50^{A}	104.90^{A}	230.00	1275.75^{A}	137.50	12.47^{AB}	2.04^{BC}	1.11^{B}	0.53^{AB}
T3- IISRGAB 107+ $CaCl_2$ (3%)	2.43^{A}	6.14^{CD}	253.25^{A}	66.00^{BC}	172.25	1208.25^{AB}	89.75	8.47^{BC}	2.87^{AB}	1.15^{B}	0.52^{ABC}
T4- GRB 35	1.90^{B}	5.64^{E}	211.00^{C}	44.13^{C}	233.00	741.50^{C}	89.00	10.03^{ABC}	3.54^{A}	0.71^{BC}	0.45^{BC}
T5- COC (0.25%)	2.16^{AB}	6.45^{ABC}	230.25^{ABC}	55.57^{C}	263.50	692.00^{C}	81.75	10.14^{ABC}	1.78^{CD}	4.28^{A}	0.44^{C}
T6- Bleaching Powder	1.85^{B}	6.28^{BCD}	215.50^{BC}	69.70^{BC}	222.50	845.50^{BC}	102.75	10.67^{ABC}	2.10^{BC}	0.88^{BC}	0.49^{ABC}
T7- Absolute control (with out any amendments)	1.88^{B}	6.66^{AB}	211.00^{C}	42.98^{C}	227.50	812.50^{C}	83.25	6.47^{C}	0.93^{D}	0.60^{C}	0.47^{BC}
LSD at 5%	0.32	0.48	29.66	33.54	NS	379.5	NS	4.30	1.08	0.45	0.08

Table 9 Effect of treatments on EC (μS/cm) of soil

Treatments	Solarized	Non -solarized	Sub-Plot Mean
T1- IISRGAB 107	339.80	250.23	295.01
T2- CaCl2 (3%)	379.78	245.78	312.78
T3- IISRGAB 107+ CaCl2 (3%)	186.85	293.25	240.05
T4- GRB 35	133.58	270.10	201.84
T5- COC(0.25%)	211.48	259.48	235.48
T6- Bleaching Powder	345.48	190.78	268.13
T7- Absolute control	161.50	216.00	188.5
Main plot mean	251.21 (NS)	246.51 (NS)	General Mean = 248.86

by Ca and other pectolytic enzymes produced by the pathogen or increased resistance in cell walls offered by Ca to these enzymes or due to inhibition of ethylene production by Ca (Conway et al. 1988; Raz and Fluhr 1992; Volpin and Elad 1991). Lemaga et al. (2005) reported that NPK and NP fertigation reduced BW by 29% and 50%, respectively with an increased yield in potato. This is supportive to the results obtained in the present study that in treatments (T1,T2,T3) where disease reduction was noticed, there was an increase of NPK and other essential nutrients.

A single application of rock dust effectively reduced BW in tomato which was attributed to higher soil pH and Ca content(Li and Dong 2013). In the present study, Ca content was found higher in treatments T1, T2 and T3 where disease incidence was also less. Nevertheless, pH was not high and was almost normal in case of bioagent while it was below 6.0 in case of $CaCl_2$ treated soil and could not be correlated with disease suppression. in vitro evaluation of $CaCl_2$, Na_2CO_3, $NaHCO_3$ and Ca $(ClO)_2$ at 0.25, 0.50, 0.75 and 1.0% concentrations against *Rs* causing BW in tomato showed minimum population of *Rs* with 1.0% bleaching powder followed by $CaCl_2$ (1.0%) Dinesh et al. (2012). Further, in vivo study with $CaCl_2$ @ 0. 1 and 0.25% and Ca $(ClO)_2$ @ 0.025 and 0.050% showed minimum disease incidence (47.5%) with $CaCl_2$ (0.1%) followed by Ca $(ClO)_2$ (0.025%) after 9th day of inoculation which is in confirmation with the present study. It was also reported that, soil amendment with N and CaO together with soil solarization is an effective IDM strategy in reducing BW of tomato with increased grower profit which is also adjudicating the present observations.

Soil solarization is effective in reducing the microbial load, however soil solarization alone is not sufficient to control BW as evinced from our studies that in the control plot with solarization alone showed 100% disease incidence indicating the effect of *B. licheniformis* as well as $CaCl_2$ in reducing the disease. According to Baptista et al. (2007) due to soil solarization microbial biomass and respiration in soil was reduced without affecting the soil chemical properties. In case of ginger cultivation solarization is viable because most of the farmers plant ginger only with the premonsoon rains in April/May. so they will get enough time to soalrize as recommended.

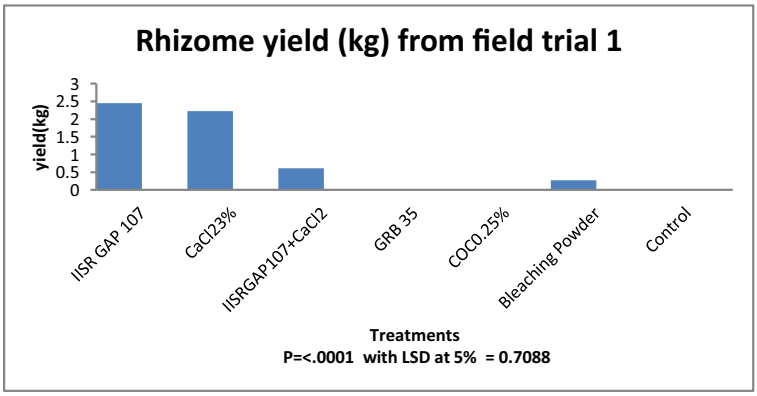

Fig. 6 Rhizome yield from field trial 1

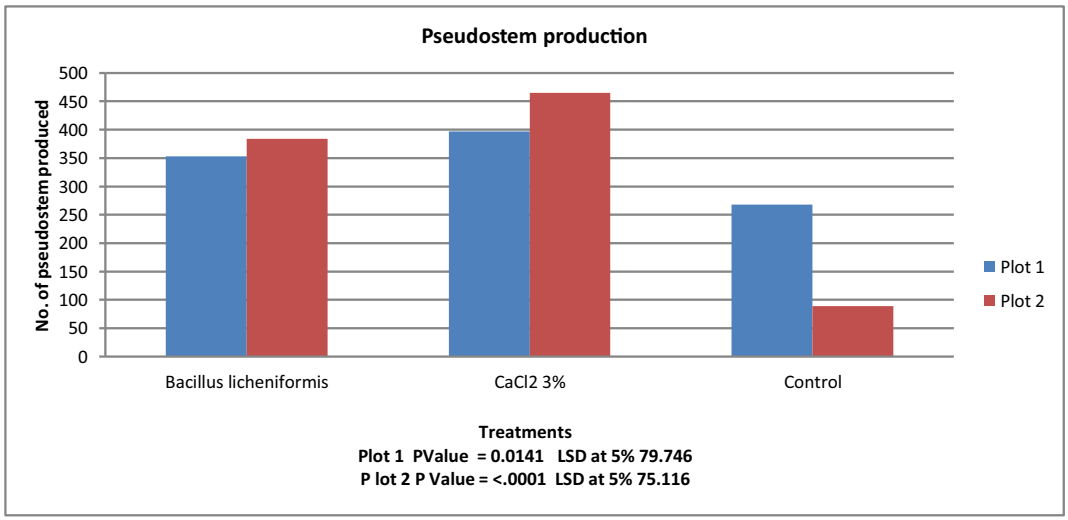

Fig. 7 Average pseudo stem production in the two plots

Pre-planting heat treatment of the infested soil at either 45 °C for 2 days or 60 °C for 2 h reduced the total bacterial as well as *Ralstonia* population (by 60 to 97%) and also the incidence of BW in tomato (Kongkiattikajorn and Thepa 2007). In the present study, solarization was carried out for more than 50 days for complete elimination of *Rps* population. However, complete elimination of the pathogen could not be achieved as the surrounding area served as a reservoir of residual inoculum of the pathogen.

Several researches have reported the inhibitory effect of inorganic elements on a wide range of soil-borne diseases *caused by Phytophthora* sp. such as potato pink rot, soybean stem rot and gerbera root rot etc. (Benson et al. 2009; Sugimoto et al. 2007, 2008; Toppe and Thinggaard 1998). Biggs et al. (1993), Biggs (1999) reported repeated sprays of $CaCl_2$ significantly reduced *Colletotrichum* as well as *Alternaria* infection in apple orchards. So also, enhanced pear fruit resistance to blue mould decay and side rot was achieved by spray

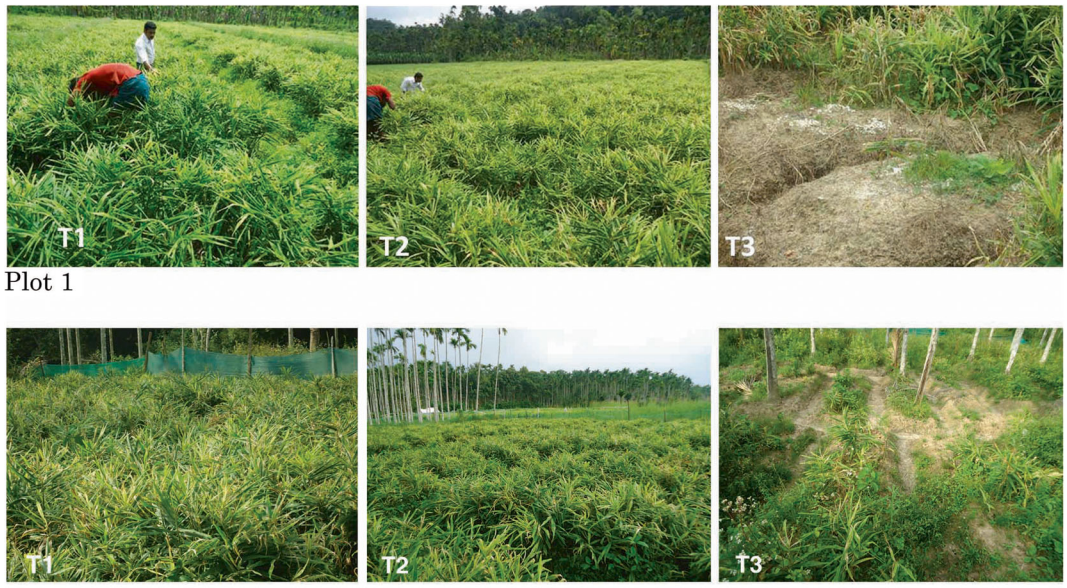

Fig. 8 Crop status in two field level demonstration plots 1 & Plot 2 T1-**B. *licheniformis*, T2-CaCl2,T3 Control**

Table 10 Average yield (kg/ 10 beds)

Treatment	Plot 1	Plot 2
T1- *B. licheniformis*	161.03 B	91.54 B
T2- Calcium Chloride	169.73A	110.50A
T3 -Control	113.28C	31.55C
p Value	0.1453	<.0001
CV(%)	24.96	12.25
SE(d)	26.124	6.744
LSD at 5%	NS	16.502

application of $CaCl_2$ in pear orchards (Sugar et al. 1991, 2003). Conway et al. (1992) attributed the mechanism by which $CaCl_2$ offers protection with improved structural integrity i.e. increased Ca content and enhanced production of pectolytic enzymes there by increased structural integrity. The present study shows that the concentration of calcium chloride used (3%) is on the safer side for ginger growth and development as it did not retarded the growth of plants. Moreover increased yield was also attributed to the amelioration of Calcium chloride.

Different methods of management of BW were discussed which include methods such as biological (54%), cultural (21%), chemical (8%) and physical methods (6%) and11% focused on IDM strategies. However, only 10% have reported the effect of such methods in increasing crop yield and chemicals offering better control than other biological or cultural means (Yuliar Nion and Toyota 2015). The present study reports the effect of prospective treatments in increasing the yield as revealed through B:C ratio which is economical.

Conclusion

The results emanated from the present study clearly indicated that *Bacillus licheniformis* as well as $CaCl_2$ 3% is effective in managing bacterial wilt of ginger caused by *Ralstonia pseudosolanacearum* and could reduce the population of *Rps* in the soil continuum considerably. $CaCl_2$ 3% and 4% were directly inhibitory to *Rps*. $CaCl_2$ was also found effective against *Rps in planta* when challenged, giving disease suppression of 98–100%. In the integrated system encompassing soil solarization and soil amendment with $CaCl_2$ (3%), BW was completely suppressed in a severely infested sick plot. *B. licheniformis* was also found as effective as $CaCl_2$ in reducing BW incidence. The technology developed in the present study which includes soil solarization along with soil amelioration with either $CaCl_2$ 3% or *B. licheniformis* would serve as an economically feasible and effective integrated strategy for the management of BW in ginger when imposed at the time of planting and repeated at 30, 45, 60 and 90 days after planting.

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Compliance with ethical standards

Ethical statement This research article is not submitted elsewhere for publication and this manuscript complies to the Ethical Rules applicable for this journal.

Conflict of interest None of the authors declare a conflict of interest, with all authors consenting to publication.

Human and animal studies This article does not contain any studies with human participants or animals performed by any of the authors.

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