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Management of late leaf spot of groundnut (*Arachis hypogaea*) with chlorothalonil-tolerant isolates of *Pseudomonas aeruginosa*

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Fifteen groundnut-associated bacterial isolates that inhibited by > 90% the *in vitro* conidial germination of *Phaeoisariopsis personata*, causal agent of late leaf spot disease of groundnut, were applied as a prophylactic spray (10^8 cfu mL^{-1}) and tested for control of the disease in the glasshouse. Two groundnut seed-associated bacterial isolates, GSE 18 and GSE 19, identified as *Pseudomonas aeruginosa*, reduced the lesion frequency (LF) by up to 70%. A 90-day-old peat-based formulation of *P. aeruginosa* GSE 18 reduced LF measured 15 days postinoculation by up to 60%. Both *P. aeruginosa* GSE 18 and GSE 19 were tolerant to chlorothalonil (Kavach®) up to 2000 μg mL^{-1} in LB broth. In glasshouse trials, GSE 18 and GSE 19 tested in combination with reduced concentrations of chlorothalonil were highly efficient in management of the disease. The disease was completely controlled by chlorothalonil (> 250 μg mL^{-1}), and in the presence of GSE 18 or GSE 19, 100 μg mL^{-1} chlorothalonil was equally effective. Application of rifamycin-resistant mutants of GSE 18 or GSE 19 together with chlorothalonil significantly increased the survival of these isolates in the groundnut phylloplane. In the field, a combination of GSE 18 and 500 μg mL^{-1} chlorothalonil reduced disease severity comparable to 2000 μg mL^{-1} chlorothalonil alone. Use of chlorothalonil-tolerant pseudomonads together with a quarter concentration of the recommended field dose of chlorothalonil doubled pod yield compared with the untreated unsprayed control.

Keywords: *Arachis hypogaea*, integrated disease management, peanut, *Phaeoisariopsis personata*

Introduction

Biological control of foliar diseases has been less successful compared to soilborne diseases in a range of food and commercial crops. Biocontrol agents for the control of foliar diseases are available, but inconsistent performance of the introduced agents on aerial plant parts poses a limitation for their extensive adoption (Andrews, 1992). Modification of the delivery systems or supplementation of nutrients and other additives is likely to enhance the performance of biocontrol agents in the phylloplane (Knudsen & Spurr, 1988; Yuen *et al*., 2001; Guetsky *et al*., 2002). Supplementation with specific compounds may provide a competitive advantage for the establishment of the introduced biocontrol agents and improve the biocontrol.

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Fungicide tolerance of selected biocontrol agents has been utilized for their use as key components of integrated disease management (IDM). Combining fungicide-tolerant biocontrol agents with fungicides improved the degree of disease control attained and also reduced the quantity of fungicides required for effective disease management (Frances *et al*., 2002; Buck, 2004). Integrated use of biocontrol agents and chemical fungicides was effective against damping-off of tomato (Kondoh *et al*., 2001), rhizoctonia root rot and take-all of spring wheat (Duffy, 2000), and postharvest diseases of fruits (Chand-Goyal & Spotts, 1996), compared with the individual components of disease management.

Late leaf spot (LLS) caused by *Phaeoisariopsis personata* is a disease of groundnut or peanut (*Arachis hypogaea*), and is of major economic interest in the semi-arid tropics (SAT) of Asia and Africa (McDonald *et al*., 1985). Management of LLS is highly dependent on chemical fungicides, as adequate levels of host plant resistance with desirable agronomic traits are scarce in cultivated germplasm. However, the need for repeated application of fungicides coupled with uncertain rainfall remains an obstacle for the wide adoption of chemical management

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Table 1 *In vitro* antifungal activity of selected bacterial isolates against *Phaeoisariopsis personata* and their glasshouse evaluation for control of late leaf spot of groundnut

Bacterial isolate	Habitat	Inhibition of conidial germination (%)	Lesion frequency	Disease score
GGS 1	Geocarposphere	91·2 a	2·48 ± 0·29 f	7·6 ± 0·4 e
GGS 11	Geocarposphere	100·0 f	1·88 ± 0·19 bcd	6·4 ± 0·6 c
GPS 15	Phylloplane	92·8 bc	2·27 ± 0·29 ef	8·0 ± 0·2 g
P. aeruginosa GPS 21	Phylloplane	99·1 f	1·98 ± 0·20 cd	7·6 ± 0·5 ef
GRS 7	Rhizosphere	91·8 ab	1·97 ± 0·27 cd	7·4 ± 0·4 de
GRS 70	Rhizosphere	93·2 c	2·44 ± 0·34 f	8·0 ± 0·2 g
GRS 94	Rhizosphere	90·8 a	1·76 ± 0·22 bc	5·8 ± 0·4 b
GRS 100	Rhizosphere	92·8 bc	1·65 ± 0·34 b	5·6 ± 0·4 b
GRS 134	Rhizosphere	90·8 a	1·92 ± 0·22 bcd	5·6 ± 0·5 b
Pseudomonas sp. GRS 175	Rhizosphere	99·1 f	2·08 ± 0·23 de	7·3 ± 0·6 d
GRS 225	Rhizosphere	97·7 e	3·21 ± 0·30 g	8·6 ± 0·4 h
GSE 3	Seed	96·2 d	3·24 ± 0·24 g	8·8 ± 0·3 hi
P. aeruginosa GSE 18	Seed	97·7 e	1·06 ± 0·16 a	3·9 ± 0·4 a
P. aeruginosa GSE 19	Seed	97·7 e	1·15 ± 0·16 a	4·0 ± 0·2 a
P. aeruginosa GSE 30	Seed	100·0 f	2·36 ± 0·34 f	7·9 ± 0·4 fg
Control		0 g	3·51 ± 0·26 h	9·0 ± 0·0 i

Means in a column followed by the same letter do not differ significantly at $P \leq 0{\cdot}01$ by Duncan's multiple range test.

by the resource-poor farmers of the SAT. Thus, there exists a greater demand for economic and sustainable biocontrol technologies for LLS management. Initial attempts in this direction focused on the mycoparasitic fungi such as *Verticillium lecanii*, *Dicyma pulvinata* and *Acremonium obclavatum* (reviewed by Podile & Kishore, 2002). Field performance of these biocontrol agents is quite variable compared with performance in glasshouse studies.

The main objective of the present study was to identify an effective biocontrol system for management of late leaf spot of groundnut. Bacterial isolates associated with different habitats of groundnut were selected for their antifungal activity against *P. personata* and tested for their ability to control the disease in the glasshouse. Selected fungicide-tolerant biocontrol strains were tested both in the glasshouse and in the field, for their usefulness in controlling the disease with a reduced dose of chlorothalonil.

Materials and methods

Fungal inoculum

Conidia of *P. personata* harvested from a single-lesion culture maintained on detached groundnut leaves (Subrahmanyam *et al*., 1983) were suspended in sterile distilled water (SDW) containing 0·01% v/v Tween 20 (2×10^4 conidia mL^{-1}) and used as inoculum for artificial inoculation of groundnut plants.

Bacterial isolates

In an earlier study, 393 groundnut-associated bacterial isolates were evaluated for inhibition of *in vitro* conidial germination of *P. personata* (Kishore *et al*., 2005). In the present study, 15 of these bacterial isolates that inhibited the conidial germination by > 90% (Table 1) were chosen for evaluation as biocontrol agents of LLS. The selected bacterial isolates were identified at the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India, based on morphological, growth and biochemical characteristics.

Artificial inoculation and disease scoring

Thirty-day-old groundnut plants of cv. TMV 2 (highly susceptible to LLS) were used, grown in 15-cm-diameter pots (four plants/pot) in a potting mixture consisting of red alfisol, farmyard manure and sand (3:1:1) in the glasshouse at 28 ± 3°C. Plants were evenly sprayed to run off with *P. personata* inoculum, using a hand-operated atomizer. Inoculated plants were incubated in alternate wet (16 h) and dry (8 h) periods of leaf wetness up to 8 days after inoculation (DAI), by shifting between the dew chambers (Clifford, 1973) and the glasshouse with temperature maintained at 24 ± 2°C throughout. In all the glasshouse experiments, each treatment consisted of 24 plants in three replications and the experiments were repeated twice.

The severity of LLS in different treatments was measured as (i) lesion frequency (LF) – number of lesions per cm^2 leaf area at 15 DAI; and (ii) disease score (DS) of the whole pot based on a 1–9 rating scale (1, no disease, and 9, > 80% disease) at 30 DAI. For each plant the third quadrifoliate leaf from the top, tagged before inoculation, was used for measurement of LF.

Preparation of bacterial inoculum

For each treatment, bacterial cells grown in LB broth for 16 h at 30°C and agitated at 180 rpm were separated by

centrifugation for 5 min at 3600 ***g*** and 4°C. The cells were resuspended in 10 mM phosphate buffer, pH 7·0, at 10^8 cfu mL^{-1}, and used as a foliar spray 24 h before *P. personata* inoculation, with buffer alone as control. Plant growth conditions, pathogen inoculation and postinoculation conditions were as described above.

Glasshouse evaluation of peat-based formulations of *P. aeruginosa* GSE 18 and GSE 19

Initial pH of the peat (Biocare Technology Pvt. Ltd, Australia) was 6·1 and adjusted to 7·0 by adding $CaCO_3$. Ten grams of neutralized peat were packed in individual high-density polyethylene bags, and sterilized by autoclaving at 121°C for 20 min. Bacterial cells harvested from mid-log phase cultures in LB broth were resuspended in an equal volume of 10 mM phosphate buffer, pH 7·0. The cell suspension was diluted 100-fold, 5 mL aliquots aseptically added to an individual pack and the pack sealed. Inoculated packets were thoroughly kneaded to ensure uniform adsorption of the bacterial cells into the carrier material and incubated at 30°C for 180 days. Moisture loss from the packets was compensated by adding SDW at regular intervals, based on the reduction in initial weight. Bacterial viability in the peat formulations was determined at frequent intervals up to 180 DAI, by plating serial dilutions of 2 g of the formulation on LB agar. The plates were incubated at 30°C and the number of colonies recorded after 36 h was expressed as log cfu g^{-1}. The experiment was repeated twice with four replications in each treatment.

To test the efficacy of the peat-based formulations of GSE 18 and GSE 19 in LLS control, 10 g of a 90-day-old formulation was suspended in 90 mL of 10 mM phosphate buffer, pH 7·0, stirred for 30 min and filtered. The filtrate was applied as a foliar spray, 24 h before the pathogen inoculation. Foliar application of mid-log phase cell suspension (10^8 cfu mL^{-1}) was included as an additional treatment for comparison, and filtrate of sterile peat suspension served as control.

Management of late leaf spot with *P. aeruginosa* GSE 18 and GSE 19 in combination with chlorothalonil

Laboratory and glasshouse evaluation

The two selected biocontrol isolates, *P. aeruginosa* GSE 18 and GSE 19 were tested for their tolerance to chlorothalonil (Kavach) 78·1% a.i., a fungicide commonly used for control of the disease. Five hundred microlitres of cultures grown in LB broth for 16 h at 30°C and 180 rpm were added to 50 mL of LB broth in 250 mL flasks containing different concentrations of chlorothalonil ranging from 50 to 200 μg mL^{-1}. Inoculated flasks were incubated at 30°C and 180 rpm. Bacterial growth was observed at A_{600}, absorbance at 12, 24 and 48 h after incubation. Each treatment consisted of three flasks as individual replications and the experiment were repeated twice.

Pseudomonas aeruginosa GSE 18 and GSE 19 (10^8 cfu mL^{-1}), separately and in combination with chlorothalonil at a final concentration of 10, 50, 100, 250, 500, 1000 and 2000 μg mL^{-1}, were compared to the individual application of GSE 18, GSE 19 and chlorothalonil for control of the disease in the glasshouse. All the treatments were applied as a foliar spray, 24 h before *P. personata* inoculation, with 10 mM phosphate buffer, pH 7·0, as control.

Field evaluation

Experiments were conducted in farm fields of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, during 2001 and repeated in 2002 rainy season (July–October). Each treatment consisted of groundnut cv. TMV 2 grown in four rows, each of 9 m length, with intra- and interrow spacings of 15 and 60 cm, respectively. The treatments were arranged in a completely randomized block design with three replications. One row of groundnut cv. TMV 2 was planted on either side of the four rows of each treatment, as an infector and indicator row. Late leaf spot-infected crop debris of the previous season was applied at the base of all the infector rows 35 days after sowing (DAS), followed by spray inoculation with *P. personata* conidial suspension (2×10^4 mL^{-1}) at 40–45 DAS. Leaf wetness required for disease development was maintained with sprinkler irrigation on rain-free days. The following different treatments were individually applied as a foliar spray at 45, 60, 75 and 90 DAS (four applications) at 500 L ha^{-1} to test their efficacy for LLS control: (i) GSE 18 (10^8 cfu mL^{-1}) in phosphate buffer; (ii) GSE 19 (10^8 cfu mL^{-1}) in phosphate buffer; (iii) GSE 18 plus 500 μg mL^{-1} chlorothalonil; (iv) GSE 19 plus 500 μg mL^{-1} chlorothalonil; (v) chlorothalonil suspended in water at 500 μg mL^{-1} (w/v); (vi) chlorothalonil suspended in water at 2000 μg mL^{-1} (w/v) (recommended dose for field application); and (vii) phosphate buffer control.

Disease severity was scored on a 1–9 rating scale (Subrahmanyam *et al*., 1982) at 10-day intervals from 45 DAS up to harvest. At harvest, the plants were uprooted, and pods were hand-picked and sun-dried. The dry weight of pods in each treatment was recorded separately and calculated as yield ha^{-1}.

Phylloplane survival of rifamycin-resistant mutants of *P. aeruginosa* GSE 18 and GSE 19

The population dynamics of *P. aeruginosa* GSE 18 and GSE 19 applied alone and in combination with chlorothalonil (500 μg mL^{-1}), in the groundnut (cv. TMV 2) phylloplane, was determined using rifampicin resistance as a marker. Both the bacterial isolates were sensitive to rifampicin at > 5 μg mL^{-1}. Spontaneous mutants of these rifampicin-sensitive bacterial isolates, GSE 18-R_1 and GSE 19-R_1, were obtained by plating 100 μL of cell suspension (~10^9 cfu mL^{-1}) on LB agar containing 100 μg mL^{-1} rifampicin (LBR). The plates were incubated at 30°C for 96 h, and the observed mutants were subcultured. Among the several mutants obtained, GSE 18-R_1 and GSE 19-R_1 were subcultured 20 times on LBR to confirm the stability of their antibiotic resistance. These mutants were similar to the wild isolates in their morphology,

growth, antifungal activity, glasshouse control of LLS and other *in vitro* characteristics, namely production of siderophores, auxin and HCN (data not shown).

The two mutants, GSE 18-R_1 and GSE 19-R_1, were applied as a foliar spray (10^8 cfu mL^{-1}) at 60 DAS in the field. In different treatments, foliar application of each of GSE 18-R_1 and GSE 19-R_1 was combined with 500 μg mL^{-1} chlorothalonil. In each treatment, four randomly selected leaflets were excised from different plants at regular intervals of 24 h. The leaves were suspended in 50 mL of 10 mM phosphate buffer, pH 7·0, and incubated for 30 min at 180 rpm and 30°C. Serial dilutions of the suspension were plated on LBR with three plates for each dilution. The plates were incubated at 30°C and observed for the number of cfu after 48 h. Phylloplane survival of GSE 18-R_1 and GSE 19-R_1 was expressed as log cfu per g leaf. The experiment was conducted during the two crop seasons with four replications in each treatment.

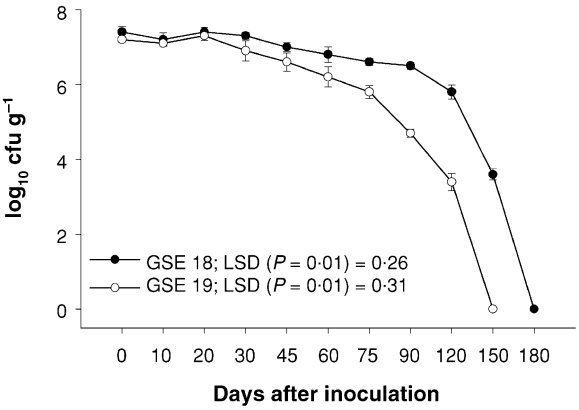

Figure 1 Survival of *Pseudomonas aeruginosa* isolates GSE 18 and GSE 19 in peat-based formulations. Data points are the mean of 12 replications.

Data analysis

Glasshouse and field experiments were conducted in a completely randomized block design. The data were subjected to analysis of variance (ANOVA) or Duncan's multiple range test using the Genstat 5 statistical package and compared at the 1% level of significance. Data values of bacterial survival in the formulations and phylloplane were log-transformed before subjecting to ANOVA/regression analysis. The mean values of disease severity and pod yields in each treatment were insignificant between the two crop seasons, and hence the data were pooled and analysed as six replications in each treatment.

Results

Selection of bacterial isolates for LLS control

Two isolates of *P. aeruginosa* GSE 18 and GSE 19 were the best of 15 bacterial isolates screened for control of late leaf spot of groundnut in a glasshouse evaluation (Table 1).

Glasshouse evaluation of peat-based formulations of *P. aeruginosa* GSE 18 and GSE 19

The initial populations of *P. aeruginosa* GSE 18 and GSE 19 in peat were log 7·4 and 7·2 cfu g^{-1} (Fig. 1). At 90 DAI, the populations were log 6·5 and 4·7 cfu g^{-1}, and declined to log 5·8 and 2·8 cfu g^{-1}, respectively, by 120 DAI. GSE 18 and GSE 19 failed to survive in peat beyond 120 DAI and 150 DAI, respectively.

In the glasshouse, peat formulations of GSE 18 were significantly better than GSE 19 controlling *Ph. personata*, reducing LF by 59 and 21%, respectively, in comparison to control. Similar reduction in the disease score (DS) was observed when measured on a 1–9 rating scale 30 DAI upon treatment with GSE 18 formulation, but treatment with the isolate GSE 19 formulation had a mean disease rating of 8·6 and was no better than the sterile peat formulation used as control (Table 2).

Management of LLS disease with *P. aeruginosa* GSE 18 and GSE 19 in combination with chlorothalonil

Laboratory and glasshouse evaluation

In vitro growth of *P. aeruginosa* GSE 18 and GSE 19 in LB broth containing chlorothalonil was comparable to LB broth alone (Fig. 2a and b). Growth of the two bacteria was observed even in the presence of 2000 μg mL^{-1} chlorothalonil, at all the incubation periods measured.

Disease severity was negligible in glasshouse plants treated with chlorothalonil at ≥ 250 μg mL^{-1} (Fig. 3). Application of either of the bacterial isolates in combination with chlorothalonil resulted in improved disease control, especially at the lower concentrations (10–100 μg mL^{-1}) of chlorothalonil. *Pseudomomas aeruginosa* GSE 18 or GSE 19 in combination with chlorothalonil at 100 μg mL^{-1} were equally effective in controlling disease as chlorothalonil alone at ≥ 250 μg mL^{-1}.

Field evaluation

Pseudomonas aeruginosa GSE 18 and GSE 19 significantly ($P \leq 0{\cdot}01$) reduced LLS severity up to 95 and 85 DAS, respectively (Fig. 4), in the field. Chlorothalonil (2000 μg mL^{-1}) was more effective than any other treatment and moderately effective at 500 μg mL^{-1} in the control of LLS. Application of GSE 18 or GSE 19 plus 500 μg mL^{-1} chlorothalonil was more effective than GSE 18 or GSE 19 or chlorothalonil (500 μg mL^{-1}) applied alone. GSE 18 plus 500 μg mL^{-1} chlorothalonil controlled the LLS disease up to 85 DAS, at a level equivalent to 2000 μg mL^{-1} chlorothalonil alone.

Highest pod yield, 1·21 t ha^{-1} (120% higher than the untreated control), was recorded for the chlorothalonil alone at 2000 μg mL^{-1} treatment (Table 3), and with 500 μg mL^{-1} chlorothalonil the yield (0·8 t ha^{-1}) was 46% higher than the control. GSE 18 and GSE 19, applied alone, increased the pod yields by 25 and 16%, respectively, compared with the control. Application of GSE 18 and GSE 19 along with chlorothalonil (500 μg mL^{-1}) resulted in pod yield increases of 96 and 66%, respectively, which

Table 2 Glasshouse evaluation of mid-log phase and peat-based formulations of *Pseudomonas aeruginosa* isolates GSE 18 and GSE 19 for control of late leaf spot of groundnut

Bacterial isolate	Formulation	Lesion frequency[a]	Disease score[b]
P. aeruginosa GSE 18	Mid-log phase cells	1·08 ± 0·09 a	3·8 ± 0·2 a
P. aeruginosa GSE 18	Peat formulation	1·59 ± 0·11 b	5·5 ± 0·3 b
P. aeruginosa GSE 19	Mid-log phase cells	1·11 ± 0·07 a	4·0 ± 0·3 a
P. aeruginosa GSE 19	Peat formulation	3·09 ± 0·28 c	8·6 ± 0·3 c
Control	Sterile peat	3·89 ± 0·24 d	9·0 ± 0·0 c

[a]Lesion frequency (number of lesions per cm^2 leaf area) was measured 15 days after inoculation.
[b]Disease score on a 1–9 rating scale was measured 30 days after inoculation.
Data points are the mean of nine replications from three sets of the experiment. Means in a column followed by the same letter do not differ significantly at $P \leq 0{\cdot}01$ by Duncan's multiple range test.

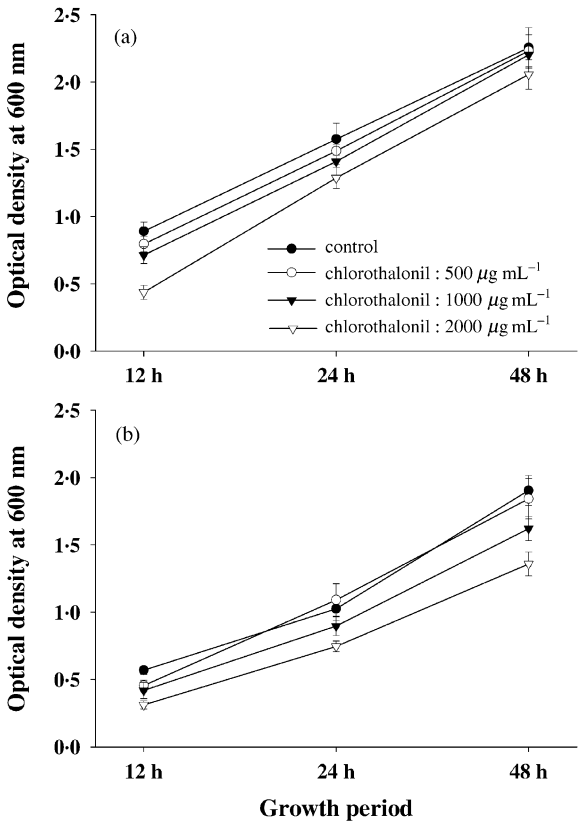

Figure 2 Growth of *Pseudomonas aeruginosa* GSE 18 (a) and *P. aeruginosa* GSE 19 (b) in LB broth with added chlorothalonil compared with LB broth alone. Treatments with < 500 μg mL^{-1} chlorothalonil are not shown, since the bacterial growth was the same as the control. Data points are the mean of nine replications.

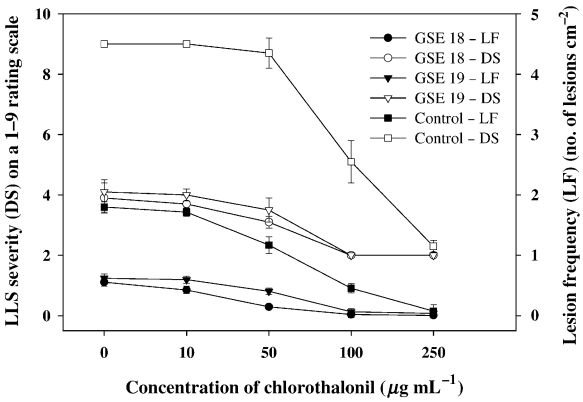

Figure 3 Glasshouse evaluation of chlorothalonil-supplemented *Pseudomonas aeruginosa* isolates GSE 18 and GSE 19 for control of late leaf spot of groundnut. Disease incidence in all the treatments with chlorothalonil at > 250 μg mL^{-1} was negligible, and is not presented. The data points are the mean of nine replications.

were significantly higher compared with the biocontrol agent or chlorothalonil (500 μg mL^{-1}) alone.

Survival of *P. aeruginosa* GSE 18-R_1 and GSE 19-R_1 in the phylloplane

In field tests, the populations of *P. aeruginosa* GSE 18-R_1 and GSE 19-R_1 drastically decreased in the groundnut phylloplane, from log 5·9 to ~3·0 cfu g^{-1} by 7 days after their application. The decrease in the phylloplane population of GSE 18-R_1 was less when these bacterial isolates were applied along with 500 μg mL^{-1} chlorothalonil. GSE 18-R_1 in the presence of chlorothalonil had the same populations up to 4 DAI compared with 1 DAI, and the population was $\log_{10}$ 3·8 cfu g^{-1} at 7 DAI. However, in all the treatments, the decline in bacterial population on the phylloplane with time is significant (Fig. 5a and b).

Discussion

Pseudomonas aeruginosa isolates GSE 18 and GSE 19 tolerant to chlorothalonil substantially reduced groundnut LLS in glasshouse trials. *Pseudomonas* spp. are known to have broad-spectrum antifungal activity (Haas & Keel, 2003) and are frequently identified as effective biocontrol agents of soilborne and foliar fungal diseases. In this context, groundnut seed treatment and foliar application with a talc-based powder formulation of *P. fluorescens* pf1 reduced the severity of LLS and rust (*Puccinia arachidis*), with a simultaneous increase in pod yield both in the glasshouse and in the field (Meena *et al*., 2002). Similarly, prophylactic foliar application of broad-spectrum antifungal *P. aeruginosa* isolates protected perennial ryegrass turf from grey leaf spot infection caused by *Pyricularia grisea* (Viji *et al*., 2003). Both isolates of *P.*

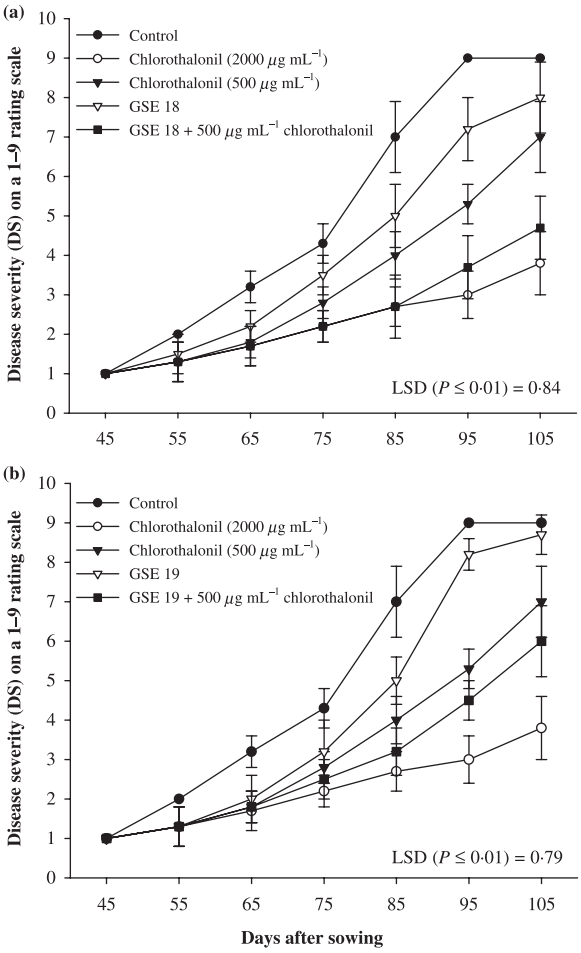

Figure 4 Field evaluation of chlorothalonil-tolerant *Pseudomonas aeruginosa* isolate GSE 18 (a) and *P. aeruginosa* isolate GSE 19 (b) for control of late leaf spot disease of groundnut. Data points are the mean values of six replications from a repeated field experiment.

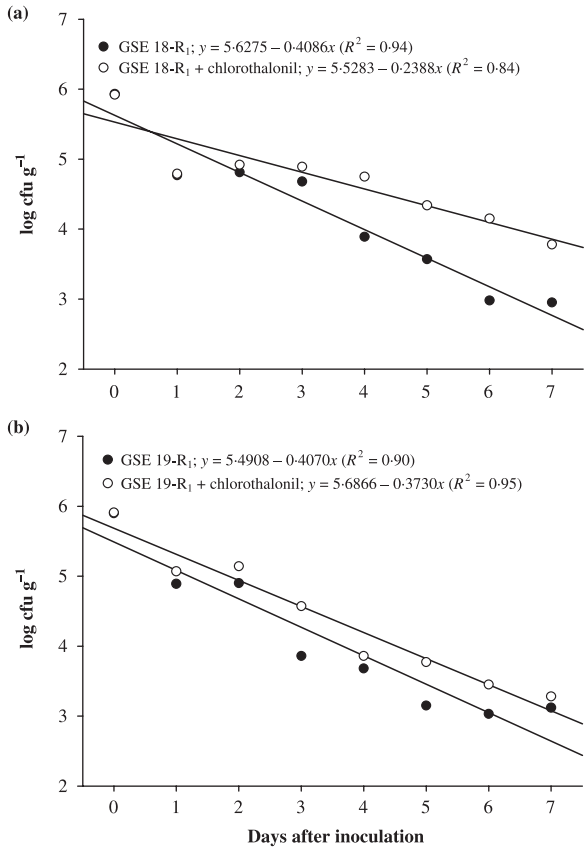

Figure 5 Survival and multiplication of rifamycin-resistant *Pseudomonas aeruginosa* GSE 18-R_1 with and without 500 μg mL^{-1} chlorothalonil (a) and *P. aeruginosa* GSE 19-R_1 (b) in groundnut phylloplane in the field. Data points are the mean of eight replications.

aeruginosa GSE 18 and GSE 19 have broad-spectrum antifungal activity against various fungal pathogens of groundnut: *Aspergillus flavus*, *A. niger*, *Cercospora arachidicola*, *Puccinia arachidis*, *Rhizoctonia bataticola*, *R. solani* and *Sclerotium rolfsii* (Kishore *et al*., 2005). Thus, these isolates may have an additional advantage in simultaneous control of other foliar diseases of groundnut in addition to LLS.

Pseudomonas aeruginosa GSE 18 and GSE 19 were tolerant to the recommended field application rate for chlorothalonil. Fungicide tolerance is not uncommon in *Pseudomonas* spp. In the study reported here, integrated use of chlorothalonil and pseudomonads reduced the fungicide requirement to a quarter of the normal for control of groundnut LLS. Similarly, *P. fluorescens* EPS288 and *B. subtilis* RB14-C, in combination with reduced doses of

Table 3 Effect of *Pseudomonas aeruginosa* isolates GSE 18 and GSE 19, chlorothalonil and combined treatments on the pod yield of groundnut during late leaf spot infection

Bacterial isolate	Foliar supplementation	Pod yield (t ha^{-1})[a]
P. aeruginosa GSE 18	–	0·69 ± 0·05 b
P. aeruginosa GSE 18	Chlorothalonil (500 μg mL^{-1})	1·08 ± 0·07 e
P. aeruginosa GSE 19	–	0·64 ± 0·04 ab
P. aeruginosa GSE 19	Chlorothalonil (500 μg mL^{-1})	0·91 ± 0·08 d
Chlorothalonil (500 μg mL^{-1})	–	0·80 ± 0·07 c
Chlorothalonil (2000 μg mL^{-1})	–	1·21 ± 0·06 f
Control	–	0·55 ± 0·03 a

[a]The dry pod yields are the mean of six replications of a repeated field experiment.
Means in a column followed by the same letter do not differ significantly at $P \leq 0·01$ by Duncan's multiple range test.

fungicides, were equally as effective as the standard fungicides alone in control of *Penicillium expansum* on pear fruits (Frances *et al*., 2002) and damping-off in tomato plants (Kondoh *et al*., 2001), respectively. Synergistic action of fungicides and fungicide-tolerant biocontrol isolates was reported to be beneficial in management of other phytopathogenic fungi (Conway *et al*., 1997; Buck, 2004).

In contrast to the observed disease control in a glasshouse environment, *P. aeruginosa* GSE 18 and GSE 19 were less effective during the advanced stages of LLS infection in the field, probably due to environmental fluctuations. However, improved control of LLS by the combined application of GSE 18 or GSE 19 and low doses of chlorothalonil corresponded with the enhanced phylloplane survival of these bacteria in the field. Although the microclimate remained the same in all the treatments, the presence of chlorothalonil might have reduced the microbial competition that leads to increased nutrient availability, as reported by Sigler & Turco (2002). Foliar application of GSE 18 or GSE 19 combined with a quarter of the recommended dose of chlorothalonil is of practical significance, since application of fungicide alone requires three to four sprays for effective control of LLS. This combination may also be effective against groundnut rust, commonly associated with LLS, as GSE 18 and GSE 19 were highly inhibitory to *Puccinia arachidis* (Kishore *et al*., 2005), and chlorothalonil is a recommended fungicide for control of rust as well.

Successful formulation of biocontrol agents is essential for their commercialization and is dependent on the carrier material, storage conditions and nutrient content. The biocontrol efficacy of peat formulations of *P. aeruginosa* GSE 18 and GSE 19 was related to the viable microbial populations in the formulation. Isolate GSE 18 formulated in peat was recovered in high numbers up to 90 DAI and was detected up to 120 DAI. The survival of bacteria in formulations can be improved by further testing of different carrier materials, nutrient supplements and storage conditions (Vidhyasekaran & Muthamilan, 1995).

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