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Management of late leaf spot (*Phaeoisariopsis personata*) and root rot (*Macrophomina phaseolina*) diseases of groundnut (*Arachis hypogaea* L.) with plant growth-promoting rhizobacteria, systemic acquired resistance inducers and plant extracts

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Abstract Greenhouse experiments were conducted to study the efficacy of plant growth-promoting rhizobacteria (PGPR) for control of root rot (RR) in groundnut (*Arachis hypogaea* L.) caused by *Macrophomina phaseolina* and to test the ability of plant systemic acquired resistance (SAR) inducers and plant extracts to protect groundnut plants from late leaf spot (LLS) caused by *Phaeoisariopsis personata*. Seed treatment and soil application of a talc-based formulation of *B. subtilis* strain G1 significantly reduced the incidence of root rot under greenhouse conditions. In experiments with SAR inducers, foliar application of salicylic acid (SA) (7 mM) on 45 days after sowing significantly reduced LLS incidence and increased the pod yield. Foliar application of aqueous extract (10%) from leaves of *Adhatoda vasica* and zimmu (*Allium sativum* x *A. cepa*) on 45 days after sowing significantly decreased the LLS incidence and increased the pod yield compared with the untreated control. Field experiments were conducted to develop an integrated method for the management of LLS and RR of groundnut using the best performing PGPR, SAR inducer and plant extract in combinations. Combined application of *B. subtilis* strain G1 through seed (10 g/kg) and soil (2.5 kg/ha) followed by foliar application of *A. vasica* extract (10%) on 30, 45 and 60 days after sowing significantly reduced LLS and RR diseases in groundnut and increased the pod yield under field conditions. The above treatment resulted in significant reductions in the area under the disease progress curve (AUDPC) for LLS compared with that of untreated control.

Keywords *Adhatoda vasica* · *Bacillus subtilis* · Peanut · Seed treatment · Soil treatment

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Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an important monoecious annual legume mainly grown for oilseed, food and animal feed all over the world (Upadhyaya et al. 2006). It is grown on 24.6 million ha worldwide with a total production of 38 million metric tons (FAOSTAT 2012). Groundnut production is constrained by several abiotic and biotic factors (Caliskan et al. 2008). Diseases pose a major threat to groundnut production worldwide. Among the fungal diseases, late leaf spot (LLS) caused by *Phaeoisariopsis*

personata (Berk. & Curt.) v. Arx [*Cercosporidium personatum* (Berk. & Curt.) Deighton] and root rot caused by *Macrophomina phaseolina*, (Tassi) Goid are the most important. Late leaf spot is prevalent wherever groundnut is grown, and however, the incidence and severity varies between localities and seasons. The disease is characterized by the appearance of circular dark spots on leaves. When the environmental factors are favourable for the development of the disease, individual lesions coalesce, resulting in premature senescence and shedding of affected leaves. The disease causes damage to groundnut by reducing leaf area available for photosynthesis because of defoliation of affected plants. The fungus survives from season to season on volunteer groundnut plants and on infected plant debris, which form important sources of primary inoculum. Secondary spread of the pathogen is through windborne conidia produced on the infected leaves. Yield losses up to 50% have been attributed to leaf spots (McDonald et al. 1985).

The fungus, *M. phaseolina*, causing root rot (RR) of groundnut is a warm dry weather pathogen. It causes lesions on hypocotyls of young plants near the soil surface resulting in girdling of hypocotyls and death of young plants. On older plants, lesions appear on the stem near the soil surface that extends to roots and causes root rot followed by death of plants. Also the infection causes discoloration of pods, reduction in oil content and alterations in fatty acid composition (Sharma and Bhowmik 1987). The pathogen causes yield losses to an extent of 80% depending on the stage of the crop (Sen 2000). The fungus is soil-borne and survives as sclerotia in diseased plant debris in the soil in the absence of host plants. Furthermore, *M. phaseolina* has a wide host range infecting about 500 plant species (Wyllie 1993). In general, soil-borne diseases are difficult to control by using fungicides because of the difficulty in dispersing fungicides through the soil profile (Thiessen and Woodward 2012). Moreover, fungicides are effective only on the active metabolic stage of the pathogen propagules and not on resting structures.

In order to control these diseases several strategies are continually explored. Development of resistant cultivars could be one of the best approaches. However, complete resistance to LLS and RR has not been identified in the cultivated species of groundnut. The current method of management of LLS relies on foliar application of synthetic chemical fungicides. It is well known that the use of fungicides increases the cost of production, environmental pollution and causes damage to the ecosystem. Moreover, frequent application of fungicides may lead to the development of fungicide resistance in the target organism (Smith and Littrell 1980). This necessitates soliciting alternative strategies for environmentally friendly management of LLS and RR of groundnut.

Biological control of soil-borne diseases using antagonistic microorganisms has become a critical component of integrated disease management (Patel and Anahosur 2001). Bioprotectants provide unique opportunity for crop protection, since they grow, proliferate, colonize and protect the newly formed plant parts to which they were not initially applied. Several strains of *Pseudomonas fluorescens, Bacillus subtilis, Burkholderia* sp. have been successfully used for the biological control of *M. phaseolina* in groundnut (Meena et al. 2001; Karthikeyan et al. 2006) and other crops (Satya et al. 2011). Some of the antagonistic microorganisms also act as plant growth-promoting rhizobacteria (PGPR) as they promote plant growth and yield (Baker et al. 1986). Several strains of PGPR have been reported to induce systemic resistance in plants to fungal (Wei et al. 1991), bacterial (Vidhyasekaran et al. 2001) and viral diseases (Raupach et al. 1996).

Several studies have demonstrated that resistance in plants can be induced by certain avirulent pathogens, non-pathogens, biocontrol fungi, mycorrhizal fungi, PGPR and chemicals (Walters et al. 2013). In addition, some of the plant extracts are also known to trigger defense mechanisms in plants and render the susceptible cultivars resistant to infection by pathogens (Doubrava et al. 1988; Daayf et al. 1995; Fofana et al. 2002; Satya et al. 2007). For instance, Milsana, a commercial product of the leaf extract of the giant knotweed (*Reynoutria sachalinensis*), has been reported to have a protective effect against powdery mildew on cucumber (Fofana et al. 2002). Leaf extracts of spinach or rhubarb were shown to induce SAR in cucumber plants against anthracnose caused by *Colletotrichum lagenarium* (Doubrava et al. 1988). Satya et al. (2007) demonstrated that leaf extract of zimmu (*Allim cepa* x *Allium sativum*) when applied to first and second leaves of cotton plants induced systemic resistance in third and fourth leaves against bacterial leaf blight, caused by *Xanthomonas campestris* pv. *malvacearum*. Research by Karthikeyan et al. (2007) found that foliar application of 50EC formulation of zimmu at a concentration of 0.3% (*v*/v) on

60, 75 and 90 days after sowing significantly reduced the incidence of sorghum grain mold and increased the grain weight and grain hardness. Hence exploitation of induced resistance may be an alternative strategy for the management of crop diseases. In the present study, the efficacy of PGPR, plant extracts and SAR inducers against LLS and RR of groundnut was determined under greenhouse conditions and attempts were made to develop an integrated method for the management of LLS and RR of groundnut using the best performing PGPR, plant extract and SAR inducer under field conditions.

Materials and methods

Microorganisms

The fungus, *M. phaseolina* was isolated from root rot infected groundnut plants and maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) medium under laboratory conditions.

An antagonistic bacterium *Bacillus subtilis* strain G1, isolated from the rhizosphere of groundnut showing a marked inhibition (28%) of mycelial growth of *Macrophomina phaseolina* (Hassen et al. Unpublished) in dual culture assay on PDA medium was used in the present study since this isolate also inhibited *Sclerotium rolfsii* and promoted growth of groundnut plants (Shifa et al. 2015a).

Development of formulation of *B. subtilis* strain G1

A loopful of *B. subtilis* strain G1 was inoculated into the nutrient broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28 ± 2 °C). After 48 h of incubation, the broth containing 9×10^8 cfu/ml was used for the preparation of talc-based formulation. To the 400 ml of bacterial suspension, 1 kg of the sterile talc powder, 15 g of calcium carbonate and 10 g of carboxymethyl cellulose (CMC) were added and mixed under sterile conditions (Vidhyasekaran and Muthamilan 1995). The product was shade dried to reduce the moisture content to 35% and then packed in white polypropylene bag and sealed. The prepared formulation was tested for its ability to suppress root rot of groundnut under greenhouse conditions. At the time of application, the population of bacteria in the talc-based powder formulation was 2.5×10^8 cfu/g.

Greenhouse studies

Evaluation of *B. subtilis* strain G1 for biological control of groundnut root rot

The root rot susceptible groundnut cultivar, cv. TMV7 (Bunch type; duration 115–120 days) obtained from the Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India was used. The fungus, *M. phaseolina*, was multiplied in sand-maize medium (Riker and Riker 1936) for 15 days and the sand–maize inoculum was mixed with the sterilized soil in the ratio of 1:19 in polyethylene bags. The polyethylene bags were shaken vigorously to ensure uniform distribution of the inoculum. Earthen pots (30 cm diameter) were filled up with 5 kg of infested soil and arranged on the greenhouse benches. The pots were incubated for three days before planting. Seeds were treated with the powder formulation of *B. subtilis* strain G1 at the rate of 10 g/kg of seeds and the treated seeds were sown in the infested soil. Five groundnut seeds were planted in each pot. In another set of pots, seed treatment was followed by soil application of talc-based powder formulation at the rate of 5 g/ pot at the time of sowing. Seeds mock-treated with the talc powder formulation without *B. subtilis* strain G1 were kept as control. Carbendazim (0.2%) was used as a positive check. Each pot served as a replicate and each treatment was replicated five times. The percentage of root rot incidence was recorded 30 days after sowing. The experiment was repeated three times.

Evaluation of SAR inducers for control of late leaf spot

An experiment was conducted under greenhouse conditions to determine the effect of SAR inducers on groundnut late leaf spot. Groundnut (cv. TMV-7) plants were raised in pots (30 cm diameter) filled with a potting mixture (sand/red soil, 1:1 *v*/v) in a greenhouse at 26 ± 2 °C and 60–85% RH under cycles of 12 h light/12 h darkness. Plants were sprayed with salicylic acid (7 mM) (sd fiNE-CHEM Ltd. (Mumbai, India)), DL-β -amino-n-butyric acid (BABA) (15 mM) (Sigma-Aldrich, Missouri, USA) and Bion 50WG (1 mM), a product of acibenzolar-S-methyl (ASM) (Syngenta India Ltd., Goa, India) at 45 d after planting until run-off. Solutions of the inducers were prepared with sterile deionized water to which Tween 20 (0.01%, *v*/v) was

added. The plants sprayed with deionized water served as control.

The pathogen *P. personata* was obtained from infected leaves of the susceptible groundnut cultivars collected in a field near Aliyarnagar, Tamil Nadu, India and incubated at (20 ± 2 °C) in moist chamber overnight. Conidia were gently washed off the leaves into sterile distilled water, filtered through muslin cloth, and the concentration of conidia was adjusted to approximately 5×10^4 conidia/ml of solution using a haemocytometer under a microscope.

The inducer-treated plants were challenge inoculated with the pathogen three days after pre-treatment by spraying the conidial suspension onto the leaf surfaces until runoff. Leaf spot intensity was assessed by using 1–9 scale (Subrahmanyam et al. 1995) three weeks after inoculation, where, 1 = no disease, all leaves healthy; 2 = lesions present largely on lower leaves, no defoliation; 3 = lesions present largely on lower leaves, very few on middle leaves, defoliation on some leaflets evident on lower leaves; 4 = lesions on lower and middle leaves but severe on lower leaves; 5 = lesions present on all lower and middle leaves, over 50% defoliation of lower leaves; 6 = severe lesions on lower and middle leaves, lesions present but less severe on top leaves, extensive defoliation of lower leaves, defoliation of some leaflets evident on middle leaves; 7 = lesion on all leaves but less severe on top leaves, defoliation of all lower and some middle leaves evident; 8 = defoliation of all lower and middle leaves, severe lesions on top leaves, some defoliation of top leaves evident; and 9 = almost all leaves defoliated leaving bare stems, some leaflets may remain, but show severe leaf spots. The experiment was arranged in a completely randomized design (CRD) with five replications per treatment considering one pot with five plants as a replication.

Evaluation of plant extracts and *B. subtilis* strain G1 for control of late leaf spot

The medicinal plants viz., *Adathoda vasica, Andrographis paniculata* and Zimmu (*Allium cepa* x *Allium sativum*) were collected from the Department of Medicinal Plants, Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The leaves were washed in running tap water to remove dirt if any, ground in a sterile mortar and pestle by adding 100 ml of sterile distilled water for every 100 g of leaf tissue (100% concentration) and finally filtered through two layers of muslin cloth. The extract was then centrifuged at 10,000 x g for 20 min and the supernatant was transferred to a fresh tube for further studies. The plant extracts were diluted further to a 10% concentration (*v*/v) and applied as foliar sprays until run-off at 45 days after planting. *B. subtilis* strain G1 cell suspension was sprayed onto groundnut leaves until run-off at a concentration of 10^8 CFU/ml. The treated plants were challenge inoculated with the pathogen three days later as described above. Leaf spot intensity was rated three weeks after challenge using a 1–9 scale as described earlier.

Integrated management of late leaf spot and root rot in groundnut with selected bacterial antagonist, SAR inducer and plant extract under field conditions

Experimental sites

Field experiments were conducted at the experimental field of Tamil Nadu Agricultural University, Coimbatore (trial I) and farmers' field at Kavilipalayam (trial II), Tamil Nadu, India where the incidence of root rot and late leaf spot occur regularly every year. The experiments were conducted during the Kharif (June to October)(trial I) and Late-Kharif (August to December)(trial II) seasons in 2014. The soil at both experimental plots was sandy loam and slightly alkaline.

Experimental design and treatments

The susceptible groundnut seeds (cv.COGn4) obtained from the Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India were used in the field trials. The experiments were conducted in plots measuring 5 × 3 m with a spacing of 30 × 10 cm *B. subtilis* strain G1, salicylic acid and *A. vasica* leaf extract that demonstrated efficacy in reducing disease or promoting plant growth in greenhouse trials were tested under field conditions. *B. subtilis* strain G1 was applied as seed treatment and soil application or in combination with *A. vasica* leaf extract or salicylic acid. A randomized complete block design was arranged with the following treatments:

T1- Seed treatment (10 g/kg seeds) and soil application (2.5 kg/ha) of talc formulation of *B. subtilis* strain G1 at the time of sowing

T2- Foliar spray of *A. vasica* plant extract (10%) on 30, 45 and 60 DAS
T3- Foliar spray of salicylic acid (7 mM) on 30, 45 and 60 DAS
T4- Seed treatment (10 g/kg seeds) and soil application (2.5 kg/ha) of talc formulation of *B. subtilis* strain G1 at the time of sowing followed by foliar spray of *A. vasica* plant extract (10%) on 30, 45 and 60 DAS
T5- Seed treatment (10 g/kg seeds) and soil application (2.5 kg/ha) of talc formulation of *B. subtilis* strain G1 at the time of sowing + foliar spray of salicylic acid (7 mM) on 30, 45 and 60 DAS
T6- Seed treatment with tebuconazole (0.1%)
T7- Foliar spray with chlorothalonil (0.2%) on 30, 45 and 60 DAS
T8- Seed treatment with tebuconazole (1.5 kg/ha) plus soil application of *Trichoderma viride* 1 (Tv1) @ 4 kg/ha at the time of sowing and two sprays with tebuconazole (0.1%) after appearance of the symptom and 15 days later (Recommended practice)
T9-Untreated control.

Disease assessment

The percentage of root rot incidence was recorded 45 days after sowing by counting the number of infected plants. Leaf spot intensity rating, which accounted for severity and defoliation, was assessed by using 1–9 scale (Subrahmanyam et al. 1995) on 45, 60, 75 and 90 DAS at both locations on 20 randomly selected plants from each plot. Area under the disease progress curve (AUDPC) was calculated according to method of Shaner and Finney (1977) using the formula:

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1} [0.5(X_{i+1} + X_i)(t_{i+1} - t_i)]$$

Where, X_i is the leaf spot intensity ratings of disease at i^{th} assessment, t_i is the time of the i^{th} assessment in days from the first assessment date and n is the total number of disease assessments (Campbell and Madden 1990).

Data analysis

The combinations of the two locations and the three blocks in the field were used as six blocks to filter out differences due to the locations and the spots in the fields (Zheljazkov et al. 2011). For each response, the validity of model assumptions (normal distribution and constant variance of the error terms) was verified by examining the residuals as described in Montgomery (2013). Independence of the error terms assumption was validated through randomization of the treatments within each block. When treatment effect was significant ($p < 0.05$), multiple means comparison was completed by using Duncan's multiple range test (DMRT).

Disease progression of leaf spot from each treatment was calculated by transforming the percent disease severity values to the logistic model as ln(y/1-y), where 'y' is disease severity scores in proportion (Van der Plank 1963). The transformed data were then regressed over time (as DAS) so as to get the disease progress rate, which is the coefficient of the regression line. The logistic model was chosen because it had the best fit to the data based on coefficients of determination and standard errors for y. AUDPC values were used in the analysis of variance to compare amount of disease among plots with different treatments. Plant height, pods per plant and pod yield was also recorded after harvesting. The data were analyzed using Statistical Analysis System (SAS) software version 9.2 (SAS Institute 2008).

Results

Greenhouse testing of *B. subtilis* strain G1 for biological control of roor rot

A talc-based formulation of *B. subtilis* strain G1 was prepared and tested for its efficacy in controlling root rot under greenhouse conditions. The results showed that inoculation of *M. phaseolina* in groundnut caused 70% root rot disease incidence (Table 1). Seed treatment (or) soil application of powder formulation of *B. subtilis* strain G1 significantly reduced the incidence of root rot and increased the plant height. Seed treatment with the powder formulation of *B. subtilis* strain G1 alone was effective in controlling root rot disease compared to control; however combined application through seed and soil increased the efficacy. Complete protection of plants from root rot disease was noticed in pots treated with *B. subtilis* strain G1 through seed and soil. Control of root rot with application of *B. subtilis* strain G1 by seed treatment and soil application was not statistically different from that obtained with seed treatment and soil application with carbendazim (Table 1). Seed treatment

Table 1 Efficacy of *Bacillus subtilis* strain G1 in root rot management and yield of groundnut under greenhouse conditions

Treatment	Root rot (%)	% reduction over control	Plant height (cm)	% increase over control	Pod yield (g/pot)	% increase over control
Soil application (SA) with *B. subtilis* (5 g/pot)	20^{bc}(26.6)	71.4	68.7^{d}	8.27	102.5^{c}	203.7
Seed treatment (ST) with *B. subtilis* (10 g/kg)	15^{bc}(22.8)	78.5	72.2^{c}	13.7	105.0^{b}	211.1
ST + SA with *B. subtilis*	0^{c} (1.3)	100.0	75.2^{a}	18.5	115.2^{a}	241.4
Soil application with Carbendazim (0.2%)	15^{bc} (22.8)	78.5	67.5^{e}	6.3	101.0^{c}	199.2
Seed treatment with Carbendazim (2 g/kg)	25^{b} (30.0)	64.2	68.0^{de}	7.0	103.0^{c}	205.1
SA + ST with Carbendazim	15^{bc}(22.8)	78.5	73.7^{b}	16.1	114.2^{a}	238.5
Control	70^{a} (56.8)	–	63.5^{f}	–	33.7^{d}	–

The data are mean of five replications

Root rot incidence was recorded 30 days after sowing

and soil application with the powder formulation of *B. subtilis* strain G1 significantly increased the pod yield besides controlling root rot disease.

Greenhouse evaluation of SAR inducers for control of late leaf spot

The ability of SAR inducers viz., SA, Bion and BABA to potentiate resistance in groundnut to *P. personata* was evaluated under greenhouse conditions. The results indicated that foliar sprays of all tested SAR inducers significantly reduced the incidence of late leaf spot compared to the untreated control (Table 2). SA (7 mM) when applied as foliar spray significantly ($P = 0.05$) reduced leaf spot severity by 60% as compared to control and increased the pod yield by 16% (Table 2).

Greenhouse testing of plant extracts and *B. subtilis* strain G1 for control of late leaf spot

The results of the experiment showed that foliar spray of groundnut with 10% aqueous leaf extract of *A. vasica,* zimmu, *A. paniculata* and and cell suspension of *B. subtilis* strain G1 (10^8 CFU/ml) significantly reduced the leaf spot incidence by 56, 56, 38 and 51% respectively compared to untreated control (Table 3). Foliar application of *A. vasica* leaf extract recorded the maximum pod yield compared to control.

Field evaluation of selected bacterial antagonist, SAR inducer and plant extract for integrated management of late leaf spot and root rot of groundnut

B. subtilis strain G1, salicylic acid and *Adathoda vasica* leaf extract that demonstrated their effectiveness in reducing disease incidence and/or promoting plant growth in greenhouse trials were evaluated in combination under field conditions.

Root rot incidence

The efficacy of *B. subtilis* strain G1, SAR inducer and *A. vasica* extract in the control of root rot was assessed under field conditions in two different locations and the results are given in Table 4. Data showed that there were significant differences

Table 2 Efficacy of foliar application of SAR inducers on the control of late leaf spot of groundnut under greenhouse conditions

Treatment	Disease score (1–9)	% decrease over control	Plant height (cm)	% increase over control	Pod yield (g/pot)	% increase over control
Salicylic acid (7 mM)	1.8^{c}	60	64^{a}	3.2	95^{a}	16
Bion (1 mM)	3.0^{b}	33	63^{a}	1.6	91^{b}	11
BABA (15 mM)	3.0^{b}	33	64^{a}	3.0	91^{b}	11
Control	4.5^{a}	–	62^{b}	–	82^{c}	–

Each value is the mean of five replicates

Means followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to DMRT

Table 3 Efficacy of foliar application of botanicals and *B. subtilis* strain G1 on the control of late leaf spot of groundnut under greenhouse conditions

Treatment	Disease score (1–9)	% decrease over control	Plant height (cm)	% increase over control	Pod yield (g/pot)	% increase over control
Adhatoda vasica (10%)	2.0 ^{c}	56	64.8 ^{a}	3.7	99 ^{a}	21
Zimmu (*Allium cepa* x *Allium sativum*) (10%)	2.0 ^{c}	56	64.8 ^{a}	3.7	97 ^{a}	18
Andrographis paniculata (10%)	2.8 ^{b}	38	63.5 ^{bc}	1.6	91 ^{b}	11
Bacillus subtilis strain G1 (10^{8}cfu/ml)	2.2^{c}	51	64.5^{ab}	3.2	97^{a}	18
Control	4.5 ^{a}	–	62.5 ^{c}	–	82 ^{c}	–

Each value is the mean of five replicates

Means followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to DMRT

between the treatments in the efficacy. Seed treatment and soil application with the powder formulation of *B. subtilis* strain G1 was effective in reducing root rot incidence from 22 (in control) to 9%. Control of root rot by application of *B. subtilis* strain G1 through seed and soil was not statistically different from that obtained with application of *B. subtilis* strain G1 as seed treatment and soil application followed by foliar sprays of *Adhatoda* extract (10%) on 30, 45 and 60 DAS and with positive control, i.e. seed treatment with tebuconazole plus soil application of *Trichoderma viride* @ 4 kg/ and 2 sprays with tebuconazole (0.1%) up on appearance of the symptoms and 15 days later (Table 4).

Table 4 Integrated management of root rot and late leaf spot of groundnut under field conditions

Treatment	Root rot incidence (%)	Late leaf spot			Plant height (cm)	No. of Pods per plant	Pod yield (kg/ha)
		Final severity rating[a]	AUDPC[b]	Disease progress rate (units/day)[c]			
T1- Seed treatment (10 g/kg) and soil application (2.5 kg/ha) with *B. subtilis* G1	9.6^{d}	3.8^{cd}	116^{d}	0.032^{cd}	44.5^{d}	28.4^{cd}	2125^{c}
T2- Foliar spray with *A. vasica* extract (10%) on 30, 45 and 60 DAS	14.5^{c}	3.7^{cd}	114^{d}	0.031^{cd}	43.5^{de}	28.0^{cd}	2080^{cd}
T3- Foliar spray with salicylic acid (7 mM) on 30, 45 and 60 DAS	15.7^{b}	4.5^{b}	139^{b}	0.045^{b}	42.3^{g}	26.3^{e}	1989^{d}
T4 = T1 + T2	9.0^{d}	3.0^{e}	100^{f}	0.024^{d}	48.9^{a}	32.8^{a}	2406^{a}
T5 = T1 + T3	9.8^{d}	3.8^{cd}	115^{d}	0.033^{cd}	45.6^{c}	30.1^{b}	2259^{b}
T6- Seed treatment with tebuconazole (0.1%)	14.5^{c}	4.0^{c}	128^{c}	0.035^{c}	42.5^{fg}	27.9^{d}	2116^{c}
T7- Foliar spray with chlorothalonil (0.2%) at 30, 45 and 60 DAS	15.3^{bc}	3.6^{d}	110d^{e}	0.033^{cd}	43.4^{ef}	28.9^{c}	2094^{cd}
T8- Seed treatment with tebuconazole (1.5 ml/kg) + soil application of *Trichoderma viride* 1 (Tv1) @ 4 kg/ha and foliar sprays (2 times) with tebuconazole (0.1%) first spray after appearance of the symptom and second 15 days later (Recommended practice)	9.2^{d}	3.0^{e}	101^{ef}	0.029^{cd}	47.7^{b}	32.8a	2303^{ab}
T9- Untreated control	22.0^{a}	7.6^{a}	214^{a}	0.084^{a}	36.1^{h}	16.8^{f}	1253^{e}

Means followed by the same letter in a column are not significantly different according to DMRT

[a] Scale 1 to 9: 1 = no disease, all leaves healthy and 9 = almost all leaves defoliated leaving bare stems, some leaflets may remain, but show severe leaf spots

[b] AUDPC was calculated using 1–9 scale rating (Subrahmanyam et al. 1995)

[c] Disease progress rate was calculated using logistic model ln(l/1-y) (Van der Plank 1963)

Late leaf spot severity

Late leaf spot was the predominant foliar disease during the later part of the season in both the locations. Seed treatment and soil application of the talc-based powder formulation of *B. subtilis* strain G1 followed by foliar application of *A. vasica* leaf extract on 30, 45 and 60 days after sowing significantly ($P = 0.05$) reduced the leaf spot severity in field trials (Table 4). The unsprayed control plots recorded a maximum leaf spot severity of 7.6. Integration of biocontrol agent with plant extract was found to be equivalent to, or better than, the recommended fungicide application in controlling this disease.

The AUDPC calculated for disease severity rating was significantly different ($P = 0.05$) among treatments. The maximum AUDPC value of 214 was estimated on the untreated plot and the lowest AUDPC value 100 was obtained from plots treated with *B. subtilis* strain G1 as seed treatment and soil application and *A. vasica* leaf extract as foliar spray (Table 4).

Similarly, disease progress rate of late leaf spot was significantly different ($P = 0.05$) among the treatments. The disease progress rate in untreated plots (0.084 units day^{-1}) was about 3.5 times more than in plots treated with *B. subtilis* strain G1 as seed treatment and soil application followed by foliar spray with *A. vasica* leaf extract (0.024 units day^{-1}) (Table 4).

Pod yield and yield components

The maximum pod yield of 2406 kg/ha was obtained in plots treated with the talc-based powder formulation of *B. subtilis* strain G1 as seed treatment and soil application followed by the foliar spray of *A. vasica* leaf extract. Control plots recorded pod yield of 1253 kg/ha (Table 4). Plots treated with *B. subtilis* strain G1 as seed treatment and soil application plus foliar spray with *A. vasica* leaf extract on 30, 45 and 60 DAS recorded the highest plant height (48.9 cm) and number of pods per plant (32.8).

Discussion

An integrated approach for management of LLS and RR diseases of groundnut was undertaken by evaluating PGPR, SAR inducers and plant extracts. The results of the greenhouse experiments indicated that seed treatment or soil application of talc-based powder formulation of *B. subtilis* strain G1 significantly reduced the incidence of root rot and increased the plant height and pod yield. Under conditions of high disease pressure, complete protection from root rot incidence was observed when the antagonist applied through seed and soil and its effects were equal to or greater than those achieved with the commercial fungicide. The bacterial antagonist *B. subtilis* G1 has been previously shown to be effective in increasing the root length, shoot length and seedling vigour of groundnut (Shifa et al. 2015a). Furthermore, production of 22 different kinds of antibiotics by *B. subtilis* strain G1 has been reported (Shifa et al. 2015b). Different species of *Bacillus* viz., *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides* and *B. sphaericus* are known to reduce the incidence or severity of various diseases on a diverse host plants (Kloepper et al. 2004; Choudhary and Johri 2009). *Bacillus* spp. are capable of growing in diverse environments due to production of endospores that can tolerate extreme pH, temperature and osmotic conditions; therefore, they offer several advantages over other antagonistic microorganisms (Earl et al. 2008). *B. subtilis* is known to rapidly colonize plant roots and has the capacity to multiply on the roots (Dijkstra et al. 1987). It remains close to the root tip by passive displacement on the elongating cells. Furthermore several strains of *Bacillus* sp. are known to induce systemic resistance (Romeiro et al. 2010) and to promote plant and root growth through the production of phytohormones and extracellular enzymes (Lahlali et al. 2013). It has been reported that *B. subtilis* BN1 produced lytic enzymes, which are known to cause hyphal degradation and digestion of the cell wall components of *M. phaseolina* and *S. rolfsii* (Singh et al. 2008). Figueroa-Lopez et al. (2016) demonstrated that *Bacillus cereus* sensulato B25 which displayed antagonistic activity against *Fusarium verticillioides* produces glucanases, proteases or chitinases, as well as siderophores and auxins. It is possible that the antimicrobial compounds and lytic enzymes produced by *B. subtilis* strain G1 might be involved in the inhibition of *M. phaseolina* and the plant-growth promoting substances like auxins (Cameco et al. 2001) released by the *B. subtilis* strain G1 might have resulted in increased plant height and pod yield of groundnut.

Several studies have suggested that treatment of plants with various agents, including plant extracts and synthetic chemicals, can induce resistance to subsequent

pathogen attack (Walters and Fountaine 2009). This type of resistance is systemic and involves generation of signal molecules to activate diverse processes contributing to the development of resistance in plants (Walters et al. 2013). Such induced resistance seldom leads to complete pathogen control, instead to a reduction in lesion size and/or number (Kuc 1982). Salicylic acid (SA) is a natural phenolic compound present in many plants and is an important component in the signal transduction pathway and is involved in local and systemic resistance to pathogens (Delaney et al. 1995). An increase in SA levels precedes the onset of SAR in many plants (Malamy et al. 1990). Several studies have reported that treatment of plants with SA induces disease resistance and expression of genes associated with SAR in plants (Mills and Wood 1984; Malamy et al. 1990; Yalpani et al. 1991). It has been demonstrated that transgenic plants that are expressing a bacterial gene coding for salicylate hydroxylase which is involved in the degradation of SA, did not show SAR in response to pathogen infection (Gaffney et al. 1993). The results of the present study indicate that foliar application of SA on to groundnut leaves significantly reduced the intensity of LLS by 60% under greenhouse conditions. The results of this study are in agreement with those of Meena et al. (2001) and Jayaraj et al. (2004). Meena et al. (2001) demonstrated that foliar application of SA (1 mM) significantly reduced late leaf spot disease intensity and increased the pod yield in groundnut. Jayaraj et al. (2004) found that pre-treatment of wheat plants with salicylic acid significantly reduced the incidence of leaf blotch disease incited by *Stagonospora nodorum* up to 56% compared with untreated control plants and the induction of resistance was correlated with expression of two β-1,3-glucanases with apparent molecular weights of 31 kDa and 33 kDa and, a thaumatin-like protein with an apparent molecular weight of 25 kDa. Hence, the reduction in the intensity of LLS in groundnut due to exogenous application of SA might be due to induction of defense mechanisms.

Botanicals have long been considered as an attractive alternative to synthetic chemical fungicides for fungal disease management in crop plants because botanicals pose little threat to the environment or to human health. Antimicrobial substances are abundantly present in many higher plants (Fiori et al. 2000; Yamunarani et al. 2004; Satya et al. 2005). In addition to their direct antimicrobial activities several plant extracts are known to induce resistance in plants against various fungal and bacterial diseases (Srinivas et al. 1997; Kishore et al. 2001; Satya et al. 2007). There are few reports on management of LLS using plant extracts. Kishore et al. (2001) demonstrated that an extract from *Datura metel* sprayed on to the leaves of groundnut reduced the leaf spot disease severity by greater than 65% under greenhouse conditions. Foliar spray of 5% leaf extracts of *Calotropis procera* at 70 days after sowing proved to be highly effective in reducing the incidence of both early and late leaf spot diseases and increasing the yield of groundnut (Srinivas et al. 1997). Our studies demonstrated that foliar application of aqueous extract (10%) from leaves of *A. vasica* and zimmu significantly reduced LLS severity under greenhouse conditions. About 56% reduction in severity was recorded in treated plants compared to control plants. The antifungal activities of leaf extracts of zimmu (Satya et al. 2005) and *A. vasica* (Neela et al. 2014) have been demonstrated. Based on the availability of leaf materials for field trials and potentiality to increase pod yield, *A. vasica* was selected for subsequent experiments. *A. vasica* commonly known as Vasaka is an indigenous medicinal plant and is available in plenty in many tropical countries. It is frequently used as an ingredient in Ayurvedic medicine to treat cough, asthma and bronchitis (Claeson et al. 2000; Srivastava et al. 2001). The plant is a rich source of the quinazoline alkaloids, vasicine, vasicinone, deoxyvasicinone, vasicol and adhavasicinone (Claeson et al. 2000). The enhanced resistance of *A. vasica* leaf extract-treated groundnut plants against LLS might be due to triggering of biochemical defense responses.

In order to device an integrated method for management of both LLS and RR, the most effective biocontrol agent, SAR inducer and plant extract in the greenhouse studies were evaluated in combination under field conditions in hot spot areas where the incidence of these diseases occur every year. The results of our field experiments revealed that seed treatment and soil application of *B. subtilis* strain G1 followed by foliar spray of *A. vasica* leaf extract on 30, 45 and 60 DAS significantly reduced the incidence of RR and severity of LLS of groundnut and increased the pod yield. The maximum AUDPC value of 214 was estimated on the untreated control plot and the lowest AUDPC value of 100 was recorded from plots treated with *B. subtilis* strain G1 as seed treatment and soil application plus *A. vasica* leaf extract as foliar spray. The disease progress rate in the control plot was about 3.5 times more than in plots treated with *B. subtilis* strain G1 as seed treatment and

soil application plus foliar spray of *A. vasica* leaf extract. Several PGPR strains in combination with plant extracts are known to induce systemic resistance in plants against various diseases and to increase the plant growth (Latha et al. 2009; Muthukumar et al. 2010). Latha et al. (2009) demonstrated that combination of biocontrol agents with zimmu formulation was highly effective in reducing the disease incidence of *Alternaria* leaf spot of tomato. Muthukumar et al. (2010) demonstrated that combination of *Trichoderma viride*, *P. fluorescens* and zimmu leaf extract significantly reduced the growth of *P. aphanidermatum.*

In conclusion, an integrated approach viz., application of talc-based powder formulation of *B. subtilis* strain G1 as seed treatment and soil application followed by foliar spray of *A. vasica* leaf extract on 30, 45 and 60 DAS effectively controlled the late leaf spot and root rot of groundnut and increased the pod yield. Seed and soil treatment with *B. subtilis* strain G1 was shown previously to be more efficient for suppression of *Aspergillus flavus* population in the soil, *A. flavus* infection and aflatoxin B1 content in groundnut kernels (Shifa et al. 2016). Hence, this method may offer protection against multiple diseases and may be an environmentally safe and viable strategy for mitigating losses due to these diseases. Relatively low cost of the materials suggest that the above disease management strategy could be incorporated into groundnut production system to reduce the amount of synthetic fungicides introduced into the environment. Since, formulated plant extracts are best suited for use in organic crop production, further research is needed to develop a formulation of *A. vasica* for large scale field application.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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