



## Biological control of downy mildew of maize caused by *Peronosclerospora sorghi* under environmentally controlled conditions

Y. Sireesha and R. Velazhahan\*

Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore- 641 003 (Tamil Nadu), INDIA

\*Corresponding author. E-mail: [velazhahan@hotmail.com](mailto:velazhahan@hotmail.com)

Received: June 12, 2015; Revised received: October 20, 2015; Accepted: February 25, 2016

**Abstract:** Downy mildew disease, caused by *Peronosclerospora sorghi*, is one of the most serious diseases of maize. The disease is currently managed by seed treatment with metalaxyl fungicides. However, problems regarding environmental pollution resulting from the use of fungicides and development of fungicide resistance within populations of *P. sorghi* are of increasing concern. Assuming that biological control by means of using antagonistic microorganisms may be an alternative for the management of this disease, the efficacy of biocontrol agents viz., *Bacillus subtilis* G1, *Bacillus amyloliquefaciens* B2, *Brevibacillus brevis* 57 and *Pseudomonas fluorescens* Pf1 for the management of downy mildew of maize and for promoting plant growth was evaluated. The results indicated that seed treatment with *B. subtilis* G1 and *B. amyloliquefaciens* B2 significantly ( $P = 0.05$ ) increased the germination percentage and seedling vigour of maize as assessed by roll towel method. Among them, *B. subtilis* G1 was the most effective and recorded 9% and 31% increases in germination percentage and seedling vigour of maize respectively, as compared to the control. A talc-based powder formulation of *B. subtilis* G1 when applied through seed at the rate of 10 g/kg reduced the downy mildew incidence up to 54% under greenhouse conditions. Results of this study suggest that *B. subtilis* G1 is a promising bioagent for the management of downy mildew of maize and for promoting plant growth. This antagonist could be further exploited for commercial scale up for ecofriendly management of downy mildew of maize under localized climatic conditions.

**Keywords:** *Bacillus subtilis*, Biocontrol, Downy mildew, *Peronosclerospora sorghi*, *Zea mays*

### INTRODUCTION

Downy mildew, caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, is a disease of worldwide importance on maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench]. The fungus produces both local and systemic infection in both the crops (Bonman *et al.*, 1983) and cause significant yield losses under favourable environmental conditions. Soil-borne oospores or aerially disseminated conidia cause systemic infection, whereas local infection is caused by conidia (Cohen and Sherman, 1977; Ramalingham and Rajasab, 1981; Bock *et al.*, 1998). The local infection acts as a source of inoculum for subsequent systemic infection on young plants (Cohen and Sherman, 1977). This disease can occur at any stage of maize development from seedling to harvest, though it primarily infects soon after seedling emergence, until one month after planting. *P. sorghi* can cause significant yield losses under favourable environmental conditions and yields can be reduced up to 100% in susceptible cultivars depending on environmental conditions (Lukman *et al.*, 2013). Genetic variabilities among isolates of *P. sorghi* have been reported (Bock *et al.*, 2000; Perumal *et al.*, 2006; Mathiyazhagan *et al.*, 2008; Sireesha and Velazhahan, 2015). This disease is managed primarily with seed treatment with

systemic fungicide, metalaxyl (Anahosur and Patil, 1980). But the use of synthetic chemical fungicides may pose threat to the environment by polluting the ecosystem. Further, the seed treatment with fungicides may not protect the crop for a longer period. The development of fungicide resistance within populations of *P. sorghi* has already been reported (Isakeit and Jaster, 2005). Although few resistant genotypes have been identified against this disease (Yen *et al.*, 2004; Rashid *et al.*, 2013), currently there are no commercially acceptable maize cultivars with adequate level of resistance to *P. sorghi*. Biological control method, by using naturally occurring non-pathogenic, antagonistic microorganisms has been considered as an environmentally safe and sustainable alternative to the use of synthetic chemical fungicides for the management of soil borne diseases. The applied antagonistic microorganisms can compete with the pathogen for nutrients, inhibit multiplication of pathogens by secreting antibiotics or lytic enzymes or reduce pathogen population through hyperparasitism. Sadoma *et al.* (2011) reported that crude culture filtrates of *Trichoderma viride*, *T. harzianum*, *Gliocladium virens* and *Bacillus subtilis* inhibited germination of oospores and conidia of *P. sorghi*. Further, the authors reported that seed soaking and spray treatment with crude culture

filtrates of a combination of *T. viride* with *T. harzianum* or *B. subtilis* effectively reduced the incidence of downy mildew in maize under field conditions. Amin *et al.* (2013) demonstrated that seed treatment followed by soil application at 20 days after sowing with endophytic fungus *Beauveria* sp. effectively controlled downy mildew of maize. In this study, the efficacy of various bacterial antagonists *viz.*, *Bacillus subtilis* G-1, *Bacillus amyloliquefaciens* B2, *Brevibacillus brevis* (Bbv) 57 and *Pseudomonas fluorescens* Pfl selected based on previously determined antagonistic activity against other soilborne fungal pathogens (Meena *et al.*, 2001; Shifa *et al.*, 2015a) was assessed for suppression of downy mildew of maize caused by *P. sorghi* under environmentally controlled conditions. The aim of the present study was to find out the efficacy of biocontrol agents *viz.*, *Bacillus subtilis* G1, *Bacillus amyloliquefaciens* B2, *Brevibacillus brevis* 57 and *Pseudomonas fluorescens* Pfl for the management of downy mildew of maize and for promoting plant growth was evaluated.

## MATERIALS AND METHODS

**Bacterial cultures:** The bacterial antagonists *viz.*, *Bacillus subtilis* G-1, *Bacillus amyloliquefaciens* B2, *Brevibacillus brevis* (Bbv) 57 and *Pseudomonas fluorescens* Pfl were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The cultures of *Bacillus* spp. were maintained on nutrient agar (NA) medium and *P. fluorescens* Pfl was maintained on King's medium B (KMB) (King *et al.*, 1954) under laboratory conditions.

**Seed germination and seedling growth bioassay:** *B. subtilis* G1, *B. amyloliquefaciens*, *B. brevis* were grown on nutrient broth and *P. fluorescens* Pfl was grown on Kings' B broth with constant shaking at 150 rpm for 48 hrs at room temperature ( $26 \pm 2^\circ\text{C}$ ). The bacterial cells were harvested by centrifugation at 6,000 rpm for 15 min and the bacterial pellet was suspended in 0.01 M phosphate buffer (pH 7.0). The final concentration of the suspension was adjusted to approximately  $10^8$  CFU/ml ( $\text{OD}_{595} = 0.3$ ) in a spectrophotometer (Thompson, 1996) and amended with 0.2% sterilized carboxymethyl cellulose (CMC) as a sticker. Maize seeds (cv. CM 500) were surface sterilized with 0.1% mercuric chloride for 5 min and rinsed thoroughly in sterile distilled water. The bacterization of seed was done by soaking surface sterilized maize seeds in bacterial suspensions @ 5 g/25 ml and incubating in a rotary shaker for 6 hrs to facilitate attachment of bacterial cells to the seed coat. The seeds were then dried in shade for 2 hrs and the plant growth promoting activity of the antagonists was assessed based on the seedling vigour following the standard roll towel method (International Seed Testing Association, 1996). The bacterized seeds were placed on coarse blotter paper sheets and covered with a moistened blot-

ter and rolled. The roll was kept on a butter paper sheet and rolled as a bundle and incubated in a growth chamber at  $25^\circ\text{C}$  with 80 % RH. Four replications were maintained for each treatment. The root and shoot lengths of seedlings were measured and the germination percentage was calculated after 7 days. The vigour index was calculated as suggested by Baki and Anderson (1973).

Vigour index = Per cent germination x seedling length (shoot length + root length)

**Greenhouse experiments:** The talc-based powder formulations of the antagonists were prepared by mixing 400 ml of the bacterial suspension ( $9 \times 10^8$  CFU  $\text{mL}^{-1}$ ) with 1 kg of sterilized talc powder amended with 10 g of carboxymethylcellulose under aseptic conditions (Vidhyasekaran and Muthamilan, 1995). Surface-sterilized maize seeds (cv. CM 500) were treated with talc formulation at a rate of 10 g/kg of seed and sown in 30 cm diameter plastic pots filled with autoclaved soil and sand (2:1). Seeds mock-treated with the powder formulation without bacteria were kept as control. As a check, seeds were treated with Metalaxyl at 2 g  $\text{kg}^{-1}$  seed. Five seeds were sown in each pot and five pots were kept as one replication. The trial was conducted in a completely randomized design (CRD) with four replications.

In order to prepare pathogen inoculum, maize leaves showing symptoms of downy mildew were collected from the experimental farm of Tamil Nadu Agricultural University, Coimbatore, India. The infected leaves were wiped out with wet absorbent cotton, cut into small pieces of 4-5 cm lengths and placed with their abaxial side facing up in 9 cm diameter Petri dishes lined with wet filter paper on both the sides. The plates were incubated in the dark for 6-7 hrs at  $20^\circ\text{C}$  for sporulation (Narayana *et al.*, 1995). Conidia were harvested from the surface of leaves by gently washing them into cold distilled water using a camel hair brush. The concentration of conidia was adjusted to  $1 \times 10^8$  conidia  $\text{mL}^{-1}$  and used as inoculum. At two leaf stage, the seedlings were challenge inoculated by spraying with conidial suspension of *P. sorghi* ( $4 \times 10^4$  spores  $\text{mL}^{-1}$ ). The inoculated seedlings were maintained at  $20 \pm 2^\circ\text{C}$  temperature with >95% relative humidity. At 30 days after sowing (DAS), plant height and percent disease incidence were measured. The inoculated plants were examined for leaf reaction 7 days after inoculation. Numerical values of 1, 2 and 3 were assigned to resistant (R), intermediate (I) and susceptible (S) leaf reaction classes, respectively as described by Craig (1982).

**Data analysis:** Arc sine transformation of data on percentage of downy mildew incidence was done and Duncan's multiple range test (DMRT) was first applied to the transformed values and then transferred to the original means (Gomez and Gomez, 1984). The data were analyzed using AgRes statistical software, version 3.01 (Pascal Int1 Software solutions).

## RESULTS AND DISCUSSION

In the present study the biocontrol agents viz., *B. subtilis* G1, *B. amyloliquefaciens* B2, *B. brevis* 57 and *P. fluorescens* Pfl were tested for their plant growth promoting activity and efficacy in controlling downy mildew of maize under greenhouse conditions. The results indicated that seed treatment with *B. subtilis* G1 and *B. amyloliquefaciens* B2 significantly increased the percent germination and seedling vigour compared to control. Among the biocontrol agents, *B. subtilis* G1 recorded the maximum percent germination (96%) and seedling vigour (4339.2) (Table 1; Fig. 1). However, the fungicide check Metalaxyl recorded the highest germination percentage (97%) and seedling vigour (4573.5). Talc-based powder formulations of the biocontrol agents were prepared and evaluated under greenhouse conditions for their potential in the management of downy mildew of maize. The results indicated that all the tested antagonists significantly ( $P=0.05$ ) reduced the incidence of downy mildew compared to control (Table 2). Among the biocontrol agents tested, *B. subtilis* G1 was found to be the most effective in reducing the downy mildew disease when used as seed dresser@ 10 g/kg followed

by *B. amyloliquefaciens* B2, *B. brevis* 57 and *P. fluorescens* Pfl. Seed treatment with the commercial fungicide Metalaxyl completely eliminated the development of downy mildew in maize seedlings. Several strains of *B. subtilis* are widely used in agriculture as biopesticides for the management of plant diseases especially those caused by soil-borne plant pathogens (Jayaraj *et al.*, 2005; Furuya *et al.*, 2011; Yanez-Mendizabal *et al.*, 2012; Hu *et al.*, 2014; Khabbaz and Abbasi, 2014; Zhao *et al.*, 2014). *Bacillus* spp. offer several advantages over other antagonistic microorganisms because they are capable of growing in different environmental conditions due to their ability to produce endospores that can tolerate extreme pH, temperature, and osmotic conditions (Earl *et al.*, 2008). Many *B. subtilis* strains when applied through seed bacterization rapidly colonize plant roots and suppress the growth of phytopathogens (Beauregard *et al.*, 2013). The mechanisms of plant disease control by *B. subtilis* includes production of antibiotic substances (Stein 2005; Shifa *et al.*, 2015b), production of cyclic lipopeptides like iturins, fengycins and surfactins (Ongena and Jacques, 2007), production of hydrolytic enzymes, including  $\beta$ -1,4-*N*-acetyl glucosaminidase (NAGase), chitinase and  $\beta$ -1,3-glucanase (Manjula and Podile, 2005; Liu *et al.*, 2011),

**Table 1.** Effect of seed treatment with biocontrol agents on seed germination and seedling vigour of maize.

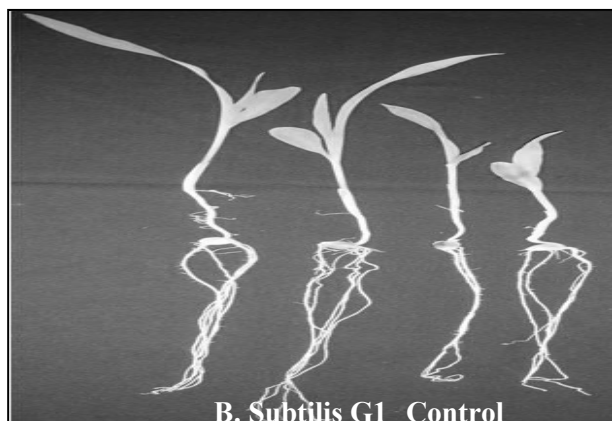
Treatments	% Germination*	Shoot length (cm)	Root length (cm)	Seedling vigour
<i>B. subtilis</i> G1	96(78.5) <sup>a</sup>	24.2 <sup>a</sup>	21.0 <sup>a</sup>	4339.2 <sup>a</sup>
<i>B. amyloliquefaciens</i> B2	94(75.82) <sup>a</sup>	23.9 <sup>a</sup>	20.3 <sup>a</sup>	4163.2 <sup>a</sup>
<i>Br. brevis</i> (Bbv) 57	89(70.65) <sup>b</sup>	22.6 <sup>b</sup>	19.7 <sup>b</sup>	3775.3 <sup>b</sup>
<i>P. fluorescens</i> (Pfl)	89(70.65) <sup>b</sup>	20.7 <sup>c</sup>	18.2 <sup>b</sup>	3471.8 <sup>b</sup>
Metalaxyl	97(80.17) <sup>a</sup>	23.9 <sup>a</sup>	20.3 <sup>a</sup>	4573.5 <sup>a</sup>
Untreated Control	88(69.73) <sup>b</sup>	20.3 <sup>c</sup>	12.2 <sup>c</sup>	3307.0 <sup>c</sup>
CD (0.05)	4.48	2.15	1.55	381.5

The root and shoot lengths of seedlings were measured and the germination percentage was calculated 7 days after treatment; \*Arc sine transformation of data was done prior to analysis; Four hundred seeds were used per treatment in four replications of 100 seeds each. The data are mean of four replications. Means within a column followed by a common letter are not significantly different ( $P=0.05$ ) by Duncan's multiple range test.

**Table 2.** Effect of seed treatment with talc based formulations of biocontrol agents on the incidence of downy mildew of maize under greenhouse conditions.

Treatment	Number of leaves in each reaction class			% of leaves in reaction class			Mean leaf reaction score	% disease incidence
	R	I	S	R	I	S		
<i>Bacillus subtilis</i> G1	40	23	17	50	29	21	1.7	46(42.71) <sup>b</sup>
<i>Bacillus amyloliquefaciens</i> B2	10	16	26	19	31	50	2.3	64(53.13) <sup>c</sup>
<i>Brevibacillus brevis</i> (Bbv) 57	8	22	38	12	32	56	2.4	69(56.17) <sup>c</sup>
<i>Pseudomonas fluorescens</i> Pfl	16	18	24	28	31	41	2.1	73(58.69) <sup>d</sup>
Metalaxyl	85	0	0	100	0	0	1.0	0(0.29) <sup>a</sup>
Untreated Control	7	16	35	12	28	60	2.5	100(89.72) <sup>e</sup>

The bacterial antagonists were applied as seed treatment (10 g/kg) at the time of sowing. The bacterized plants were inoculated with *P. sorghi* at two leaf stage and the incidence of downy mildew was recorded 30 days after sowing; Numerical values of 1, 2, and 3 were assigned to resistant (R), intermediate (I) and susceptible (S) reactions respectively. A mean score was calculated for the leaf reactions of each treatment.; Data are mean of four replications; Data followed by the same letter in a column are not significantly different ( $P=0.05$ ) from each other according to DMRT.



**Fig. 1.** Maize seedlings showing enhanced growth upon seed treatment with *Bacillus subtilis* G1.

which causes lysis of fungal cell walls and membranes and induction of systemic acquired resistance in plants (Choudhary and Johri, 2009; Lahlali *et al.*, 2013; Sivasakthi *et al.*, 2014). In addition to direct antagonistic activity against several soil borne fungal pathogens, *B. subtilis* is known to promote plant growth and yield (Kloepper *et al.*, 2004; Perez-Garcia *et al.*, 2011). The genome of *B. subtilis* strain BAB-1 has been fully sequenced and annotated and genes encoding the antifungal active compound have been identified in the genome (Guo *et al.*, 2014). A number of commercial products based on *B. subtilis* including Kodiak (Gufstafson Biologicals, Plano, TX), Serenade (Agraquest Inc., Davis, CA), Subtillex (Becker Underwood, Ames, IA) have been developed for the control of various plant diseases (Schisler *et al.*, 2004). Shifa *et al.* (2015a) recently reported that seed treatment and soil application with the powder formulation of *B. subtilis* strain G1 effectively reduced the incidence of stem rot of groundnut caused by *Sclerotium rolfsii* and increased the pod yield. This strain was compatible with other beneficial rhizobacteria including *Bacillus megaterium* var *phosphaticum* strain PBS, *Rhizobium* strain BMBS, *Azospirillum brasiliense* strain 204 and *Azotobacter chroococcum* strain AC1. Furthermore, production of 22 different kinds of antibiotics including aldehydes, fatty acids, alkanes, esters and sulphur containing compounds by this antagonistic strain was demonstrated (Shifa *et al.*, 2015b). The increase in plant growth due to application of *subtilis* G1 in the present study may be due to plant growth promoting characteristics of *B. subtilis* G1 and the reduction in the incidence of downy mildew may be due to direct antagonistic activity of *B. subtilis* G1 against *P. sorghi*.

## Conclusion

In the absence of downy mildew resistant maize cultivars, biological control by means of using antagonistic microorganisms will definitely reduce the environmental pollution and the cost of plant protection meas-

ures. The results of this study suggest that *B. subtilis* G1 could be considered as a promising alternative to synthetic chemical fungicides in the management of maize downy mildew and could be successfully exploited as a biocontrol agent within the framework of integrated disease management system.

## ACKNOWLEDGEMENTS

This study was supported by the INSPIRE Fellowship (Grant No.IF120724), Department of Science and Technology, Government of India, New Delhi to the first author.

## REFERENCES

- Amin, N., Daha, L., Nasruddin, A., Junaed, M. and Iqbal, A. (2013). The use of endophytic fungi as biopesticide against downy mildew *Peronosclerospora* spp. on maize. *Acad. Res Int.* 4: 153-159.
- Anahosur, K. H. and Patil, S. H. (1980). Chemical control of sorghum downy mildew in India. *Plant Dis.* 64: 1004-1006.
- Baki, A.A.A. and Anderson, J.D. (1973). Vigour determination in soy-bean seed by multiple criteria. *Crop Sci.* 31: 630-633.
- Beauregard, P.B., Chai, Y., Vlamakis, H., Losick, R. and Kolter, R. (2013). *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proc. Natl. Acad. Sci USA* 110: 1621-1630.
- Bock, C.H., Jeger, M.J., Mughogho, L.K., Cardwell, K.F., Mtisi, E., Kaula, G. and Mukansabimana, D. (2000). Variability of *Peronosclerospora sorghi* isolates from different geographic locations and hosts in Africa. *Mycol Res.* 104: 61-68.
- Bock, C.H., Jeger, M.J., Mughogho, L.K., Mtisi, E. and Cardwell, K.F. (1998). Production of conidia by *Peronosclerospora sorghi* on sorghum crops in Zimbabwe. *Plant Pathol.* 47: 243-251.
- Bonman, J.M., Renfro, B.L. and Singburadom, N. (1983). Correlation between resistance in maize to local and systemic infection by *Peronosclerospora sorghi* in Thailand. *Plant Dis.* 67: 219-220.
- Choudhary, D.K. and Johri, B.N. (2009). Interactions of *Bacillus* spp. and plants with special reference to induced systemic resistance (ISR). *Microbiol. Res.* 164: 493-513.
- Cohen, Y. and Sherman, Y. (1977). The role of airborne conidia in epiphytotics of *Sclerospora sorghi* on sweet corn. *Phytopathology* 67: 515-521.
- Craig, J. (1982). Identification of sorghum downy mildew resistance in corn by leaf reaction to conidial inoculum. *Phytopathology* 72: 351-352.
- Earl, A.M., Losick, R. and Kolter, R. (2008). Ecology and genomics of *Bacillus subtilis*. *Trends Microbiol.* 16: 269-275.
- Furuya, S., Mochizuki, M., Aoki, Y., Kobayashi, H., Takayanagi, T., Shimizu, M. and Suzuki, S. (2011). Isolation and characterization of *Bacillus subtilis* KS1 for the biocontrol of grapevine fungal diseases. *Biocontrol Sci. Technol.* 21: 705-720.
- Gomez, K.A. and Gomez, A.A. (1984). Statistical procedure for agricultural research. New York, USA.
- Guo, Q., Li, S., Lu, X., Zhang, X., Wang, P. and Ma, P.

- (2014). Complete genome sequence of *Bacillus subtilis* BAB-1, a biocontrol agent for suppression of tomato gray mold. *Genome Announc.* 2(4):e00744-14.
- Hu, X., Roberts, D.P., Xie, L., Maul, J.E., Yu, C., Li, Y., Jiang, M., Liao, X., Che, Z. and Liao, X. (2014). Formulations of *Bacillus subtilis* BY-2 suppress *Sclerotinia sclerotiorum* on oilseed rape in the field. *Biol. Control* 70: 54-64.
- International Seed Testing Association. (1996). International Rules for Seed Testing. *Seed Sci. Technol. Supp.* 24: 155-202.
- Isakeit, T. and Jaster, J. (2005). Texas has a new pathotype of *Peronosclerospora sorghi*, the cause of sorghum downy mildew. *Plant Dis.* 89: 529.
- Jayaraj, J., Radhakrishnan, N.V., Kannan, R., Sakthivel, K., Suganya, D., Venkatesan, S. and Velazhahan, R. (2005). Development of new formulations of *Bacillus subtilis* for management of tomato damping-off caused by *Pythium aphanidermatum*. *Biocontrol Sci. Technol.* 15: 55-65.
- Khabbaz, S.E. and Abbasi, P.A. (2014). Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. *Can. J. Microbiol.* 60: 25-33.
- King, E.O., Ward, M. K. and Raney, D. E. (1954). Two simple media for the demonstration of pyocyanine and fluorescein. *J. Lab. Clin. Med.* 44: 301-307.
- Kloepper, J.W., Ryu, C.M. and Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94: 1259-1266.
- Lahlali, R., Peng, G., Gossen, B.D., McGregor, L., Yu, F.Q., Hynes, R.K., Hwang, S.F., McDonald, M.R. and Boyetchko, S.M. (2013). Evidence that the biofungicide Serenade (*Bacillus subtilis*) suppresses clubroot on canola via antibiosis and induced host resistance. *Phytopathology* 103: 245-254.
- Liu, Y., Tao, J., Yan, Y., Li, B., Li, H. and Li, C. (2011). Biocontrol efficiency of *Bacillus subtilis* SL-13 and characterization of an antifungal chitinase. *Chin. J. Chem. Eng.* 19: 128-134.
- Lukman, R., Afifuddin, A. and Lubberstedt, T. (2013). Unraveling the genetic diversity of maize downy mildew in Indonesia. *J. Plant Pathol. Microb.* 4: 162.
- Manjula, K. and Podile, A.R. (2005). Production of fungal cell wall degrading enzymes by a biocontrol strain of *Bacillus subtilis* AF1. *Ind. J. Exp. Bot.* 43: 892-896.
- Mathiyazhagan, S., Karthikeyan, M., Sandoskumar, R. and Velazhahan, R. (2008). Analysis of variability among the isolates of *Peronosclerospora sorghi* from sorghum and corn based on restriction fragment length polymorphism of ITS region of ribosomal DNA. *Arch. Phytopathol. Plant Protect.* 41: 231-237.
- Meena, B., Marimuthu, T., Vidyasekaran, P. and Velazhahan, R. (2001). Biological control of root rot of groundnut with antagonistic *Pseudomonas fluorescens* strains. *J. Plant Dis. Protect.* 108: 368-381.
- Narayana, Y.D., Mughogho, L.K. and Bandyopadhyay, R. (1995). Evaluation of greenhouse inoculation techniques to screen sorghum for resistance to downy mildew. *Euphytica* 86: 49-53.
- Ongena, M. and Jacques, P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease control. *Trends Microbiol* 16: 115-125.
- Perez-Garcia, A., Romero, D., De Vicente, A. (2011). Plant protection and growth stimulation by microorganisms: biotechnological applications of *Bacilli* in agriculture. *Curr. Opin. Biotech.* 22: 187-193.
- Perumal, R., Isakeit, T., Menz, M., Katile, S., No, E.G. and Magill, C.W. (2006). Characterization and genetic distance analysis of isolates of *Peronosclerospora sorghi* using AFLP fingerprinting. *Mycol. Res.* 110: 471-78.
- Ramalingham, A. and Rajasab, A.H. (1981). Epidemiology of sorghum downy mildew. VI. Relative importance of oospores and conidia in epidemics of systemic infection. *Proc. Indian Nat. Sci. Acad. Part B* 47: 625-630.
- Rashid, Z., Zaidi, P. H., Vinavan, M. T., Sharma, S. S. and Srirama Setty, T. A. (2013). Downy mildew resistance in maize (*Zea mays* L.) across *Peronosclerospora* species in lowland tropical Asia. *Crop Prot.* 43: 183-191.
- Sadoma, M.T, El-Sayed, A.B.B. and El-Moghazy, S.M. (2011). Biological control of downy mildew disease of maize caused by *Peronosclerospora sorghi* using certain biocontrol agents alone or in combination. *J. Agric. Res. Kafer El-Sheikh Univ.* 37 (1): 1-11.
- Schisler, D.A., Slininger, P.J., Behle, R.W. and Jackson, M.A. (2004). Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology* 94: 1267-1271.
- Shifa. H., Gopalakrishnan, C. and Velazhahan R. (2015a). Efficacy of *Bacillus subtilis* G-1 in suppression of stem rot caused by *Sclerotium Rolfsii* and growth promotion of groundnut. *International Journal of Agriculture, Environment and Biotechnology.* 8(1): 111-118.
- Shifa. H., Gopalakrishnan, C. and Velazhahan R. (2015b). Characterisation of antifungal antibiotics produced by *Bacillus subtilis* G-1 antagonistic to *Sclerotium rolfsii*. *Biochem. Cell. Archives.* 15(1): 99-104.
- Sireesha, Y. and Velazhahan, R. (2015). Assessing genetic diversity in *Peronosclerospora sorghi* causing downy mildew on maize and sorghum. *Indian Phytopath.* 68 (1): 73-77.
- Sivasakthi, S., Usharani, G. and Saranraj, P. (2014). Biocontrol potentiality of plant growth promoting bacteria (PGPR)- *Pseudomonas fluorescens* and *Bacillus subtilis*: A review. *African J. Agric. Res.* 9: 1265-1277.
- Stein, T. (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol. Microbiol.* 56: 845-857.
- Thompson, D.C. (1996). Evaluation of bacterial antagonist for re-reduction of summer patch symptoms in Kentucky blue grass. *Plant Dis.* 80: 856-862.
- Vidhyasekaran, P. and Muthamilan, M. (1995). Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.* 79: 782-786.
- Yanez-Mendizabal, V., Vinas, I., Usall, J., Torres, R., Solsona, C., Abadias, M. and Teixido, N. (2012). Formulation development of the biocontrol agent *Bacillus subtilis* strain CPA-8 by spray-drying. *J. Appl. Microbiol.* 112: 954-965.
- Yen, T.T.O., Prasanna, B.M., Setty, T.A.S. and Rathore, R.S. (2004). Genetic variability for resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*P. heteropogoni*) in the tropical/sub-tropical Asian maize germplasm. *Euphytica.* 138: 23-31.
- Zhao, P., Quan, C., Wang, Y., Wang, J. and Fan, S. (2014). *Bacillus amyloliquefaciens* Q-426 as a potential biocontrol agent against *Fusarium oxysporum* f. sp. *spinaciae*. *J. Basic Microbiol.* 54: 448-456.