Journal of Biological Control, 26 (3): 255–259, 2012

Research Article

Bioefficacy of peat formulation of bacterial antagonists on growth promotion and disease suppression in cardamom (*Elettaria cardamomum* Maton)

G. SIVAKUMAR*[1], A. JOSEPHRAJKUMAR and R. RANGESHWARAN[1]

Cardamom Research Station, Pampadumpara 685 566, Idukki, Kerala, India

[1] National Bureau of Agriculturally Important Insects, HA Farm Post, Bellary Road, Hebbal, Bangalore 560 024, Karnataka, India

** Corresponding author: E-mail: spicessiva@yahoo.co.in*

ABSTRACT: Among the 90 rhizobacterial isolates screened against capsule rot pathogen of cardamom *Phytophthora meadii* Mc Rae two bacterial strains *viz., Pseudomonas fluorescens* Pf 51 and *Bacillus subtilis* Bs were found highly inhibitory. Strain Pf 51 exhibited highest inhibition (40.2%) against *P. meadii.* Similarly *B.subtilis* strain Bs also exhibited highest inhibition (39.7%) against *P. meadii*. *P. fluorescens* strain Pf51 was found compatible with strain *B. subtilis* Bs. Application of antagonists both Pf 51 and Bs in combination with rhizome bacterization and soil application resulted in 60% reduction of capsule infection over control as compared to single methods such as rhizome bacterization (53%) and soil application (46%). Application of copper oxy chloride resulted in 73% reduction of capsule infection. Maximum height (169.7cm) and number of tillers (36.3) were recorded due to the application of mixture of both the strains through rhizome bacterization and soil application.

KEY WORDS: Cardamom, capsule rot, bacterial antagonists, peat formulation

(Article chronicle: Received: 15-12-2011 Revised: 9-8-2012 Accepted: 1-9-2012)

INTRODUCTION

Capsule rot (Azhukal) caused by *Phytophthora meadii* Mc Rae of A2 mating type is a serious threat and causing extensive damage to cardamom during south west monsoon in many areas of south India. Pathogen affects capsules of all ages and panicles. In severe cases, infection spreads over to the rhizomes and tillers also. Decayed tillers break and fall off at the collar region. The loss in productivity due to this disease was up to 30%. Biological control was projected as an alternative to the chemical control and cultural practices due to various advantages. Exploitation of antagonistic microorganisms against capsule rot pathogen is an alternative approach to produce cardamom in a sustainable basis and also protect the cardamom ecosystem. Plant growth promoting rhizobacteria (PGPR) such as *viz., Pseudomonas* spp. and *Bacillus* spp. have been widely used for the biological control of several fungal, bacterial and viral pathogens. Some of the antagonistic *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn also act as inducers of systemic resistance in plants. The growth promotion and biocontrol activity of *P. fluorescens* and *B. subtilis* against *P. meadii* and *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen were earlier proved (Thomas and Vijayan, 2003). Although, there is a potential for managing the disease using biocontrol agents, no comprehensive work has been attempted in depth to exploit bacterial antagonists against the capsule rot disease. In the present study, the bio efficacy of a peat formulation containing either a single strain or mixture of strains of bacterial antagonists was evaluated against capsule rot of cardamom.

MATERIALS AND METHODS

Collection of pathogen and rhizobacteria

The studies were undertaken at Cardamom Research Station, Pampadumpara, Kerala during the period from 2007 & 2008. Capsule rot pathogen of cardamom *P. meadii* was isolated from infected cardamom capsules collected during monsoon season using PVPH medium (Tsao and Guy, 1977). The grown culture was further transferred to carrot agar medium and maintained in slants for further use. The pathogenicity was established with pure cultures of the pathogens under sterile environment. Rhizobacteria were isolated on King's B and Nutrient Agar medium from fresh roots of cardamom, black pepper, coffee, vanilla, ginger from cardamom hill reserves of Idukki district of Kerala, Theni and Dindigul districts of Tamil Nadu. The Rhizobacteraia were characterized based on colony morphology, gram and

endospore staining techniques. All the pure cultures of rhizobacteria were maintained on King's B and Nutrient Agar medium. The identity of highly promising strains *P. fluorescens* Pf51 and *B. subtilis* Bs were confirmed with Project Directorate of Biological control (PDBC), Bangalore.

In vitro screening of rhizobacteria

Ninety rhizobacterial isolates were tested *in vitro* for their antagonistic activity against *P. meadii.* The antagonistic potential of these bacterial isolates against the pathogen was tested by dual culture method and growth of the fungal mycelia towards the bacterial colony and the inhibition zone was recorded. The bacterial antagonists were streaked at one end of the PDA placed in sterilized plates 24 hr prior to the pathogen inoculation. Just opposite to the bacterial streak, a 9mm disc of the pathogen was placed. Three replications of each isolate including a control, i.e., with out the inoculation of the antagonists were maintained. The plates were incubated at 28±°C. The linear growth of the fungal mycelia towards the bacterial colony was measured after 72 hrs and the per cent inhibition was calculated using the formula I = C-T/Cx100, where I is the percent inhibition, C and T are radial growth of the pathogen in control and treatment respectively. The promising isolates of bacteria were maintained in glycerol stock.

Assessment of compatibility between *P. fluorescens* and *B. subtilis in vitro*

The compatibility of two antagonistic organisms were tested *in vitro* through two methods. The mutual compatibility of two antagonistic organisms was tested by dual culture method and the plates were assessed for inhibition zone after 48 h. In another method *P. fluorescens* culture was streaked on the King'B medium. After 2 days, the *B. subtilis* suspension was sprayed over the *P. fluorescens* colonies. Similarly, the *P. fluorescens* suspension was sprayed over *B. subtilis* colonies and the plates were assessed for the inhibition zone after 48 h (Bharathi *et al.,* 2004).

Shelf life in different formulations

P. fluorescens strain Pf51 and *B. subtilis* strain Bs were highly inhibitory against cardamom rhizome rot pathogens and these strains were further used for development of formulations. Strains Pf51 and Bs were grown in King'B broth and nutrient broth respectively for 48 h in shake culture at 150 rpm at room temperature (28 ± 2°C). Shelf life of bacteria was tested in four different carriers: peat, talc, vermiculite, lignite. Carboxy methyl-cellulose (10 g) was added to 1 kg of the carrier as a sticker and mixed well. The carriers were autoclaved for 45 min at 137.3 kPa pressure. Five hundred milliliters of bacterial suspension containing $9x10^8$ cfu/ml of broth was added to 1 kg of carrier and mixed well under aseptic conditions. The formulations were air-dried to 20% (w/v) moisture content, packed in three separate polythene bags (three replicates) and incubated at 28 ± 2°C. Samples were taken from each bag containing different carrier material at monthly intervals for up to 9 months. The population of *P. fluorescens* strain Pf51 and *B. subtilis* strain Bs were done on KBA and NA medium respectively using the serial dilution method. The experiment was set up as a completely randomized design using four replication. The populations were estimated at monthly intervals.

Efficacy of bioformulations in greenhouse conditions

The bioefficacy of effective strains of *P. fluorescens* Pf51 *and B. subtilis* Bs were evaluated individually as well as in combination against capsule rot in the green house using the best formulation. The experiment was conducted in a completely randomized block design in microplots with nine treatments and four replications using variety greengold. Cardamom clones were planted in the pits (75 x 75 x 45 cm) with the spacing of 1.5 x 1.5 m. Three tillers were planted in each pit. The following treatments were imposed T1: Rhizome bacterization with *P. fluorescens* strain Pf51 @ 50 g plant^{-1}, T2: Rhizome bacterization with *B. subtilis* strain Bs @ 50 g plant^{-1}, T3: Soil application with strain Pf51 @ 50 g plant^{-1}, T4: Soil application with strain Bs @ 50 g plant^{-1}, T5: Rhizome bacterization with both strains Pf51 and Bs @ 50 g plant^{-1}, T6: Soil application with both strains Pf 51 and Bs @ 50 g plant^{-1}, T7: Rhizome bacterization and soil application with both strains Pf51 and Bs @ 50 g plant^{-1}, T8: Copper oxy chloride (0.25%): T9: Control. Sterile mixture consisting of river sand, soil and farm yard manure in the ratio of 1:1:1 was filled in the pits and cardamom clones were planted after the treatment @ 3 clones per pit. The fresh peat formulations were prepared separately for two antagonists. In the case of rhizome treatment, 50 g of formulation (25 g from each antagonist) was mixed with required quantity of water and uniform thick paste was made which was coated uniformly in all sides of rhizome and they were kept under shade for 1 h. The bacterized rhizomes were planted in the pits. In case of soil application the formulation of both the antagonists were incorporated uniformly in the potting mixture as individually and in combination basis (25g from each) at the rate of 50 g

plant^{-1} at the time of planting. The cardamom clones were then planted in the mixture which contained the antagonist or antagonists. The fungicide copper oxy chloride were used as check. Copper oxy chloride was drenched in the pits containing sterile potting mixture at the time of planting. Control plots with out any treatment were also maintained. The pathogen *P. meadii* was multiplied in carrot agar medium (Rajan *et al*., 2002). The freshly multiplied pathogen was applied at the base of the plants in the soil two week after the application of antagonistic bacteria. Observations on growth parameters and capsule infection was recorded. The capsule infection was calculated by counting the total number of capsules and infected capsules.

RESULTS AND DISCUSSION

Among 90 rhizobacterial strains screened *in vitro* against the pathogen *P. meadii* only 19 of them were found inhibitory to the pathogen. The short listed strains include *P. fluorescens* strains (7,10,15,47,51,62,75,84 and 90) and *B. subtilis* strains (Ba, Br, Bc, Bd, Bs, Be, Bq, Bf, Bg and Bh). Among 19 promising bacterial strains, *P. fluorescens* strain Pf51 and *B. subtilis* strain Bs were highly inhibitory. Strain Pf 51 exhibited highest inhibition (40.2%) against *P. meadii* followed by *B.subtilis* strain Bs (39.7%) (Table 1). High degree of *in vitro* antagonism against different kinds of pathogens by fluorescent pseudomonads (Rangeswaran and Prasad 2000; Sivakumar and Sharma, 2007) and *Bacillus* spp.(Sivakumar *et al*., 2011) was already reported. The compatibility study revealed that there was no inhibition between *P. fluorescens* and *B. subtilis* and it clearly indicated the compatible nature of both the antagonists. Earlier studies also reported that the both bacteria are compatible and the combination was highly successful in controlling crop diseases (Salaheddin *et al*., 2010).

The population of both strains in different formulation was assessed at different intervals up to 270 days of storage. Among the carriers peat supported the survival of both strains Pf51 and Bs for 270 days with a viable population of 4.3 x10^7 cfu g^{-1} and 6.2 x10^7 cfu g^{-1} respectively (Table 2 and Table 3).

Peat based formulations of *P. fluorescens* strain Pf 51 and *B subtilis* strain Bs were evaluated individually and in combination under microplots in different methods for suppressing the capsule rot disease of cardamom. Both the strains performed better and found on par in growth promotion and disease suppression activities as compared to control (Table 4). Regarding the methods of application, rhizome treatment was found to be better than soil application. The most effective management was achieved when the peat based formulations of both strains Pf51 and Bs were applied in combination of rhizome bacterization and soil application. Application of antagonists in combination through rhizome bacterization and soil application resulted in 60% reduction of capsule infection over control as compared to individual treatments as rhizome bacterization (53%) and soil application (46%). Application of copper oxychloride resulted in 73% reduction of capsule infection. Better growth of the cardamom plants was noticed following application of the bacterial antagonists as compared to chemical. Maximum height (169.7cm) and number of tillers (36.3) were recorded due to the application of mixture of both the strains through rhizome and soil (Table 4) as compared to the single strain and individual application method. The increase in plant growth might be due to

Table 1. *In vitro* screening of rhizobacteria against *Phytophthora meadii*

Isolates of bacteria	*In vitro* inhibition (%) of *Phytophthora meadii*
Pseudomonas fluorescens	
Pf 7	12.6
Pf 10	24.3
Pf 15	18.2
Pf 47	14.3
Pf 51	40.2
Pf 62	13.1
Pf 75	11.4
Pf 84	9.2
Pf 90	14.7
Bacillus subtilis	
Ba	15.5
Br	20.1
Bc	22.8
Bd	13.5
Bs	39.7
Be	11.3
Bq	14.7
Bf	12.3
Bg	19.1
Bh	14.5
Control	0.0
CD (P = 0.05)	0.72

Table 2. Shelf life of *Pseudomonas fluorescens* strain Pf 51 in four different formulations

Formulations	Population (× 10^7 cfu/g)								
	Days after storage								
	0	30	60	90	120	150	180	240	270
Peat	66.0	56.0	49.2	38.0	26.0	18.1	14.2	9.2	4.3
Talc	59.5	43.1	40.3	31.6	19.3	4.1	1.0	0.1	0.1
Vermiculite	52.0	41.0	37.4	20.2	10.2	1.0	0.1	0.1	0.1
Lignite	52.2	40.0	35.2	17.2	5.3	1.0	0.1	0.1	0.1
CD (P = 0.05)	1.01	0.99	1.15	0.88	1.10	0.74	1.31	0.04	0.01

Table 3. Shelf life of *Bacillus subtilis* strain Bs in four different formulations

Formulations	Population (× 10^7 cfu/g)								
	Days after storage								
	0	30	60	90	120	150	180	240	270
Peat	76.0	63.0	52.2	42.0	28.0	20.0	15.4	11.2	6.2
Talc	68.5	58.0	43.0	33.6	20.3	6.3	2.0	1.0	0.1
Vermiculite	62.0	44.0	38.5	22.2	16.3	1.1	0.1	0.1	0.1
Lignite	64.0	42.0	36.1	19.2	10.2	1.0	0.1	0.1	0.1
CD (P = 0.05)	1.1	1.02	1.12	1.25	2.21	0.31	1.21	0.03	0.02

Table 4. Bioefficacy of bacterial antagonists on growth and capsule infection of cardamom under microplots

Treatment	No. of tillers	Plant height (cm)	Capsule infection (%)	Percent reduction over control
Rhizome bacterization with *P. fluorescens* strain Pf 51 @ 50 g plant^{-1}	27.2	146.4	40.3	41.0
Rhizome bacterization with *B. subtilis* strain Bs @ 50 g plant^{-1}	26.3	145.7	41.6	40.0
Soil application with *P. fluorescens* strain Pf51 @ 50 g plant^{-1}	22.2	142.5	46.5	32.0
Soil application with *B. subtilis* strain Bs @ 50 g plant^{-1}	21.4	143.6	45.4	34.0
Rhizome bacterization *P. fluorescens* strain Pf 51 and *B. subtilis* strain Bs @ 50 g plant^{-1}	33.5	159.3	32.14	53.0
Soil application with *P. fluorescens* strain Pf 51 and *B. subtilis* strain Bs @ 50 g plant^{-1}	30.3	153.2	37.2	46.0
Rhizome bacterization and soil application with *P.fluorescens* strain Pf51 and *B. subtilis* strain Bs	36.3	169.7	27.2	60.0
Copper oxy chloride (0.25%)	21.1	138.3	18.2	73.0
Control	9.2	89.43	69.4	0.0
CD (P = 0.05)	2.01	3.31	3.21	

the growth-promoting compounds such as gibberellins, cytokinins, auxin from tryptophan produced by biocontrol agents (Pal *et al*., 2000). Several approaches have been used to control crop diseases which include the combined application of two or more biocontrol strains to enhance the level and consistency in disease control (Raupach and Kloepper, 1998). Biological control with multi mechanisms may be achieved by using one biocontrol agent exhibiting several mechanisms or by applying more than one biocontrol agent in a mixture. *P. fluorescens* was found compatible with *B. subtilis* (Salaheddin *et al*., 2010) and the compatible combination was effective in controlling various diseases. Studies on the mode of action of *B. subtilis* have shown that the increase in crop growth is due to the release of bacterial metabolites having precursors of auxin (indole-3-pyruvic acid) or inducers (G3 fraction) for auxin synthesis (Bochow and Dolej, 1999). Biosynthesis of antibiotics, production of lytic enzymes, production of siderophores, production of hydrogen cyanide, competition for substrates and induced systemic resistance are the major mechanisms responsible for the bicontrol activity of various fluorescent Pseudomonads (Bloemberg and Lugtenberg, 2001). The present study confirmed the compatibility between these two bacteria and application of this mixture through rhizome bacterization and soil application could effectively control the capsule infection of cardamom.

ACKNOWLEDGEMENT

The authors are grateful to Kerala State Council for Science, Technology, Trivandrum and Environment for the financial assistance to carry out the research work. The authors are also thankful to National Bureau of Agriculturally Important Insects, Bangalore for the identification service.

REFERENCES

Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R. 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. *Crop Prot.* **23**: 835–843.

Bloemberg GV, Lugtenberg BJJ. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Current *Opinion in Plant Biol.* **4**: 343–350.

Bochow H, Dolej S.1999. Mechanisms of tolerance induction in plants by root colonizing *Bacillus subtilis* isolates, pp. 411–416 In: Lyr H (Eds.). Proceedings 12th International Reinhardsbrunn Symposium on Modern Fungicides and Antifungal Compounds, Thuringia, Germany, Intercept Publications, UK.

Pal KK, Dey R, Bhat DM, Chauhan, SM. 2000. Enhancement of groundnut growth yield by plant growth promoting rhizobacteria. *Int Arachis News Lett.* **19**: 51–53.

Rajan PP, Sarma YR, Anandaraj M. 2002. Management of foot rot disease of black pepper with *Trichoderma* spp. *Indian Phytopathol.* **55**: 34–38.

Rangeshwaran R, Prasad RD. 2000. Isolation and screening of rhizobacteria for control of chickpea diseases. *J Biol Control,* **14**: 9–15.

Raupach GS, Kloepper JW.1998. Mixtures of plant growth promoting rhizobacteria enhanced biological control of multiple cucumber pathogens. *Phytopathol.* **88**: 1158–1164.

Salaheddin K, Valluvaparidasan V, Ladhalakshmi D, Velazhahan R. 2010. Management of bacterial blight of cotton using a mixture of *Pseudomonas fluorescens* and *Bacillus subtilis*. *Plant Prot Sci.* **46**: 41–50.

Sivakumar G, Sharma, RC. 2007. Management of banded leaf and sheath blight of maize using *Pseudomonas fluorescens*, *SAARC J Agri.* **5**: 79–85.

Sivakumar G, Rangeshwaran R, Sriram S. 2011. Screening and identification of potential *Bacillus* spp. for management of bacterial wilt of brinjal. *J Biol Control* **25**: 229–235.

Thomas J, Vijayan AK. 2003. PGPR induced growth promotion biocontrol activity in small cardamom. In: Reddy MS, Anandaraj M, Santhosh J Eapen, Sarma YR, Kumar A. (Eds.). In 6th International Workshop on Plant Growth Promoting Bacteria. Abstracts and Short Papers, 5–10 October 2003, Calicut (p. 44). Indian Society for spices, Calicut.

Tsao PH, Guy SO.1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora* isolation medium containing hymexazole, *Phytopathol.* **67**: 796–801.