

Potential of Pre-harvest Application of *Burkholderia spinosa* for Biological Control of Epiphytic and Pathogenic Microorganisms on the Phyllosphere of Banana (*Musa* spp.)

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ABSTRACT: A study was conducted to determine the effects of pre-harvest application of a bacterial antagonist, *Burkholderia spinosa* on the population dynamics of epiphytic and pathogenic microorganisms dwelling on banana (*Musa* spp.) leaves together with the population build-up of the applied bacterial antagonist. Banana cultivar Kolikuttu was established as an outdoor plot experiment and the antagonist was applied by two methods (i.e. a foliar spray and a soil drench) at nine times (i.e. 300 ml per plant per time) repeatedly at weekly intervals. Effects of leaf washings of plants treated with different application methods on germination of *C. musae*, the causal organism of several postharvest diseases of banana, spores were investigated *in vitro*. There was a significant ($p < 0.0001$) interaction effect between time and method of *B. spinosa* application on the density of the bacterial antagonist on banana leaves. A gradual build-up of *B. spinosa* was observed on leaves with a peak density at 228 days after planting when bacterial antagonist was applied as a foliar spray. *Burkholderia spinosa* was not found on leaves of banana when the plants were treated as a soil drench or left untreated with the antagonist. Densities of *Aspergillus* spp. and unidentified fungal species on leaves of banana were significantly influenced by the method of application of *B. spinosa*. In contrast, density of *Fusarium* spp. on the banana phyllosphere varied significantly with the time of application ($p < 0.001$). The density of yeast and unidentified bacterial species on banana leaves was not significantly influenced by the time and method of application or their interaction effect. Application of *B. spinosa* as a soil drench could reduce phyllosphere pathogens, *Aspergillus* spp. and *Fusarium* spp. Percentage germination of *C. musae* spores continued to increase 24 h after incubation in the leaf washing of control banana plants. In contrast, leaf washings of *B. spinosa* treated plants showed a decline in the percentage spore germination. The findings of the present study revealed the ability of *B. spinosa* for building up considerable cell densities on the treated leaf surface and its ability for suppressing a range of microbes. These are desirable features of *B. spinosa* to be used in field application for the control of foliar pathogens of banana.

Keywords: Antagonistic effect, banana, *Burkholderia spinosa*, foliar application, leaf washing, soil drench

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INTRODUCTION

Banana (*Musa* spp.) is an economically-significant fruit crop in many developing countries as a source of energy and nutrients and a valued source of income generation through local and international trade. Potential yield of banana is reduced by a number of pre- and postharvest diseases. Banana fruits grow and develop until harvest in an aerial environment which influences biological events such as disease initiation and development on the host plant (Alvindia *et al.*, 2006). A wide range of fungi and bacteria, both pathogenic and beneficial have been detected on developing banana fruits (De Costa & Subasinghe, 1998; Alvindia *et al.*, 2006). Incidence of some of the postharvest diseases, namely anthracnose and crown rot caused by *Colletotrichum musae* has a significant positive relationship with the population density of the pathogen on flower parts at the pre harvest stage of the crop. Moreover, a positive correlation between the number of dead leaves per plant and the incidence of anthracnose was observed in Queensland (Simmonds & Mitchell, 1940) suggesting that trashed leaves could increase the quantity of primary inoculums. Conidia on plant parts can act as inoculum sources through rain splashing over 2-3 m distance (Waller, 1972). Hence, reducing the inoculum density at preharvest stage is a strategy to reduce disease incidences at the postharvest stage.

In addition to the harbouring of primary inoculum of several postharvest diseases, leaves of banana are affected by foliage diseases such as black sigatoka, yellow sigatoka, cordana and several other leaf spot diseases caused by fungal pathogens *Mycosphaerella fijiensis*, *Mycosphaerella musicola*, *Cordana musae*, *Cercospora* and *Phyllosticta* respectively. Anthracnose and crown rot of banana were primarily controlled by postharvest application of thiabendazole (Rippon & Glennies-Holmes, 1973). However, the treatment has been recently hampered by the emergence of strains resistant to this fungicide (Johanson & Blasquez, 1992). Sterol inhibitor fungicides of the DMI (demethylation inhibitor) group were reported to be very effective but the authorized fungicide residue levels in the fruits for these compounds are very low, thus limiting their use (de Lapeyre de Bellaire and Nolin, 1994). Considering the environmental and health hazards associated with the application of fungicides, there is a strong public and scientific desire to seek for and eco-friendly alternatives for the management of such diseases (Mari *et al.*, 2007).

To this end, microbial antagonists which occur naturally on plant surfaces can be promoted and managed or microbial antagonists can be artificially introduced (Sharma *et al.*, 2009). Several modes of action have been suggested to explain the biocontrol activity of these microbial antagonists, namely competition for nutrients and space between pathogen and the antagonist, direct parasitism, antibiosis and induced resistance of the host plant (Sharma *et al.*, 2009). Thangavelu & Mustaffa (2012) have highlighted the efficiency of controlling Fusarium wilt of banana, by application of microbial antagonists namely, *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp. as soil treatments, due to synthesis of defense-related enzymes as a result of host plant resistance induced by exogenous application of the microbial antagonists. Therefore, biological control by antagonistic microbes can be used as a potential strategy of reducing the density of pathogen population and a measure of inducing host plant resistance.

Burkholderia spinosa, a bacterium isolated from the peel tissues of banana has been proved for its antagonism against *C. musae*, under *in vitro* conditions and ability to reduce the rate of anthracnose development on different banana varieties when applied as a postharvest dip treatment (De Costa & Erabadupitiya, 2005; De Costa *et al.*, 2008)

The present study was conducted to determine the population dynamics of epiphytic and pathogenic microbes inhabiting on the phyllosphere when the banana plants were treated with *B. spinosa* as a preharvest foliar spray and a soil drench. Moreover, it assessed the retention/colonising efficiency of *B. spinosa* on leaf tissues of banana when the plants were treated by the two methods. Effects of field application of the biocontrol agent on survival and germination of *C. musae* spores were also investigated.

MATERIALS AND METHODS

Crop establishment

A plot experiment was conducted at the experimental field of Faculty of Agriculture, University of Peradeniya, during the period from July 2012 to June 2013. Average minimum and maximum day temperature, mean rainfall per day and mean sunshine hours during the experiment period were 20.9°C, 29.4°C, 9.1 mm per day and 6.3 h respectively. Banana plants (cultivar Kolikuttu) were purchased from Tissue culture laboratory, Mahaweli Authority of Sri Lanka, Embilipitiya, planted and maintained according to recommendations of Department of Agriculture. Each plant was covered with a polythene guard by inserting a 15 cm section below the ground level. This was done to prevent the attack from wild animals and to prevent the lateral mixing of different treatments.

Microbial cultures

Collectotrichium musae was isolated from banana fruit (cultivar Kolikuttu) showing typical anthracnose symptoms and pure cultures were maintained on PDA medium supplemented with the Streptomycin sulphate (10 µg/ml). *B. spinosa* was retrieved from stock cultures in sterile distilled water and maintained at the Laboratory of Plant Pathology and Microbiology, Department of Agricultural Biology, Faculty of Agriculture, and confirmed the purity and antagonism. Antagonism was confirmed by dual culture plate method (De Costa *et al.*, 2008). *B. spinosa* cell suspensions were prepared in nutrient broth to have a final cell concentration of 10⁸ CFU/ml.

Application of treatments

Cell suspensions of *B. spinosa* at a concentration of 10⁸ CFU/ml were applied to banana plants as a Foliage Spray (FS) and a Soil Drench (SD). Foliage spray (300 ml per plant) was applied to both sides of the leaves using a hand sprayer until the leaves were thoroughly wet. A volume of 300 ml of the cell suspension was added to the base of the plants as a Soil Drench per plant at a time. Water was used for the control treatment in both application types. Applications were repeated at weekly intervals, from 130 days after planting till 208 days after planting (i.e. 9 times). Each treatment was replicated three times and the treatments were arranged in the field according to a completely randomized design.

Quantifications of epiphytic microorganisms

Leaf samples were collected one week after treatment with *B. spinosa* either as a FS or a SD (i.e. 137 days after planting) and continued 9 times at weekly intervals (i.e. until 215 days after planting). Ten leaf discs having a 2 cm diameter were cut from randomly selected areas of fully grown leaves from each plant. At a time, ten discs were taken per plant. Leaf discs from each plant were vortexed with 1 ml sterile distilled water and plated on PDA medium

and Nutrient agar (NA) medium separately by serial dilution plate technique. Fungal including yeasts and bacterial colonies which were appeared on PDA and NA media respectively were quantified as colony forming units per ml till five days after incubation at 28 ± 2 °C. Quantification was repeated twice with three replicates per treatment at each experiment.

Quantification of *C. musae* on banana leaves

Spore suspension of *C. musae* having a concentration of 10^8 spores per ml was sprayed on to the leaves of banana using a hand held sprayer. The spraying of *C. musae* spores was done two days after application of *B. spinosa* either as a FS or a SD. Only a spore suspension *C. musae* was applied to the leaves of banana plants maintained as control treatments. Ten leaf discs, each having a 2 cm diameter were collected two days after the spraying of *C. musae* from banana leaves sprayed with *C. musae*. Leaf discs were separately vortexed in sterile distilled water. *C. musae* remaining on the leaf surface was quantified as colony forming units per ml by plating the vortexed sample on PDA and NA plates according to dilution plate technique. Quantification was repeated twice with three replicates per treatment at each experiment.

Effects of leaf washing on germination of *C. musae*

Similar area of banana leaves taken from the three treatments (i.e. FS, SD and control) was washed separately with sterile distilled water. The washings were filter sterilized using disposable syringe filters (having a pore diameter of 0.2 μ m). Similar aliquots taken from the filter sterilized washing were placed in sterile glass Petri dishes. An aliquot of 0.5 ml of *C. musae* spore suspension (10^3 spores/ml) was added to the sterilized washing and incubated at room temperature. Percentage spore germination was quantified at 18 h, 24 h, 48 h and 72 h after incubation. All the Petri dishes were introduced with 1 ml of 4% filter sterilized glucose solution, as germinated spores were not found in the absence of a nutrient source.

Data collection and analysis

When quantification of the microbial colonies, number of deep yellow colonies appearing on NA medium was quantified as *B. spinosa* colonies as they produce characteristic circular shiny deep yellow coloured colonies on NA. The identity of these colonies was confirmed by Gram staining, 3% KOH test and antagonism against *C. musae* by dual culture plate method (De Costa *et al.*, 2008). Total bacterial, fungal and yeast colonies appearing on PDA plates were separately recorded based on colony and spore morphology. Density of each microorganism dwelling on banana leaves was calculated as cfu/ml. Significance of the variation of colony densities with different methods of application was tested using analysis of variance and means were separated using Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Population dynamics of *B. spinosa* on banana leaves

B. spinosa density on banana leaves of the plants treated with different treatment methods over the experimental period is given by Table 1. Density of the bacterial antagonist varied significantly by the method of treatment, time of application of the treatment and the interaction effect of the method of treatment x time of application of the treatment

($p < 0.0001$). Viable cells of *B. spinosa* were not present on banana leaves of the plants which were treated with the SD and the plants which were not treated with *B. spinosa* by any application method (control) (Table 1). A gradual increase of *B. spinosa* was observed till 228 days after planting (DAP) and the highest density was reported at 228 DAP and a reduction thereafter. At 242 DAP, the colony density was significantly lower than that of 228 DAP but higher to the density reported during period from 130 – 214 DAP. *B. spinosa* colony density on phyllosphere from 130 DAP to 214 DAP had no significant difference among each other.

Table 1. Fluctuation of colony density of *B. spinosa* on banana leaves treated with three different treatments

DAP	<i>B. spinosa</i> –foliar spray (cfu/ml) x 10 ³	<i>B. spinosa</i> - soil drench (cfu/ml) x 10 ³	Control - (cfu/ml) x 10 ³
130	2.94 ^c	0.0	0.0
144	2.42 ^c	0.0	0.0
158	2.82 ^c	0.0	0.0
172	4.18 ^c	0.0	0.0
186	2.92 ^c	0.0	0.0
200	8.60 ^{bc}	0.0	0.0
214	25.00 ^{bc}	0.0	0.0
228	66.00 ^a	0.0	0.0
242	34.00 ^b	0.0	0.0

Note: Means values having the same letter along a column are not significantly different at $p=0.05$.

Population dynamics of other naturally-dwelling fungi on banana leaves

Colony density of *Aspergillus* spp. varied significantly among plants treated with different treatments ($p < 0.0059$). A significantly higher colony density of *Aspergillus* spp. was recorded on the leaves of the control treatment in comparison to the other two treatments (i.e. BS-FS and BS-SD) (Fig. 1). In contrast to colony density of *B. spinosa* on banana leaves of control plants, *Aspergillus* spp. showed the highest colony density at 130 DAP and then a gradual reduction throughout the experimental period with the exception at 186 and 242 DAP. The lowest colony density throughout the experimental period with the exception at 186 DAP, was reported on the plants treated with *B. spinosa* as a foliar spray. Fig. 1 indicates the ability of *B. spinosa* in reducing the phyllosphere inhabiting *Aspergillus* spp. when applied either as a foliar spray or a soil drench. Despite the absence of *B. spinosa* colonies on leaves of the plants treated with a soil drench (Fig. 1), reduction of the colony density of *Aspergillus* spp. could be due to a possible influence by induced host plant resistance caused by the microbial antagonist. Similar findings have been highlighted by Thangavelu & Mustaffa (2012) and Kumar *et al.* (2011) in controlling foliar and systemic diseases when plant growth promoting rhizobacteria are applied as a soil treatment.

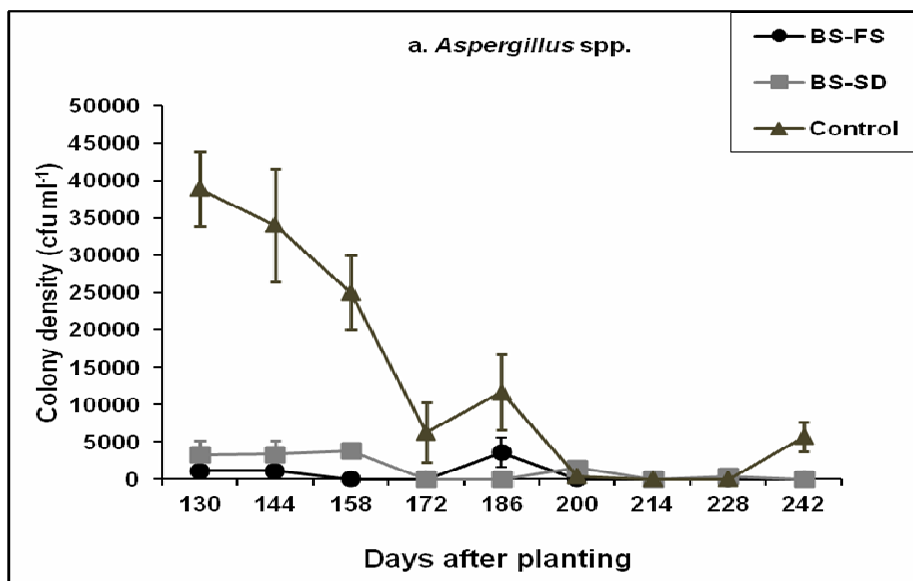


Fig. 1. Fluctuation of colony density of *Aspergillus* spp. on banana leaves treated with three different treatments with time (BS-FS: *B. spinosa* as a foliar spray, BS-SD: *B. spinosa* as a soil drench, control: no application of *B. spinosa* either as a foliar spray or a soil drench)

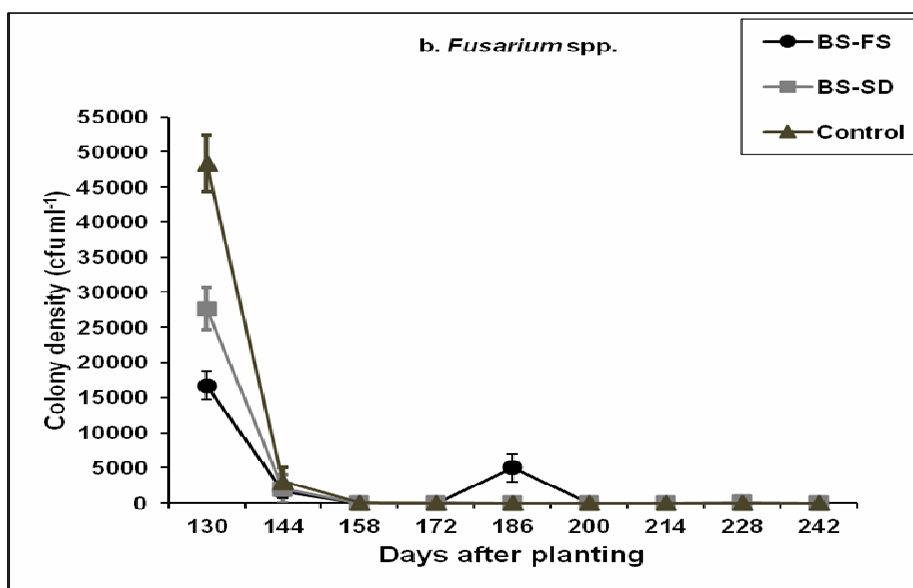


Fig. 2. Fluctuation of colony density of *Fusarium* spp. on banana leaves treated with three different treatments (BS-FS: *B. spinosa* as a foliar spray, BS-SD: *B. spinosa* as a soil drench, control: no application of *B. spinosa* either as a foliar spray or a soil drench)

Colony density of *Fusarium* spp. was significantly varied with time of application ($p < 0.0001$). At the initial stage of treatment of the plants (i.e. 130 DAP) colony density of *Fusarium* spp. was highest on leaves of control plants. The density of colonies varied significantly at 130 DAP, however the density showed no difference among each other at the rest of the time intervals (from 144 to 242 DAP). In general, density of *Fusarium* spp. dwelling on banana leaves was higher in comparison to epiphytic *Aspergillus* spp. on banana leaves. Plants treated with *B. spinosa* either as a FS or a SD showed a rapid decline to zero level, from the second application of the bacterial antagonist, indicating an antagonistic effect on survival of *Fusarium* spp. (Fig. 2). However, reasons for rapid decline reported for *Fusarium* spp. on leaves of control-treated plant from 144 DAP onwards are not clear.

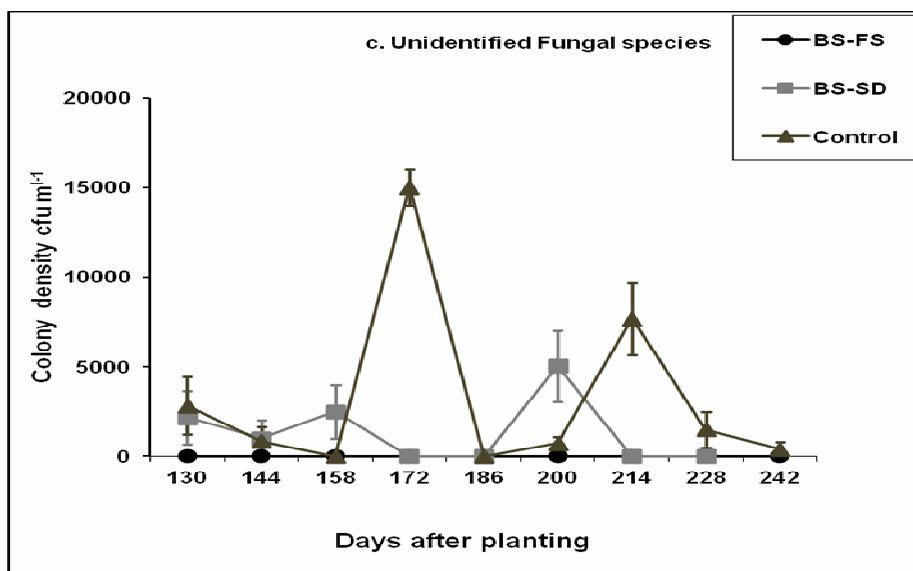


Fig. 3. Fluctuation of colony density of unidentified fungi on banana leaves treated with three different treatments with time of application (BS-FS: *B. spinosa* as a foliar spray, BS-SD: *B. spinosa* as a soil drench, control: no application of *B. spinosa* either as a foliar spray or a soil drench)

Density of unidentified fungal species differed among the three treatment methods significantly ($p < 0.0409$) (Fig. 3). Plants maintained as control treatment reported significantly higher density of fungi in comparison to plants treated with *B. spinosa* as a FS. Plants treated with *B. spinosa* as a FS did not allow the development of fungi classified as 'unidentified fungi' on leaves of banana. Leaves of control-treated plants and the plants treated with *B. spinosa* as a SD showed several peaks of fungal densities approximately in bi weekly intervals.

Population dynamics of yeast and unidentified bacterial species on banana leaves

Figure 4 presents the fluctuation of colony density of yeast and unidentified bacteria (b) on banana leaves treated with three different treatments.

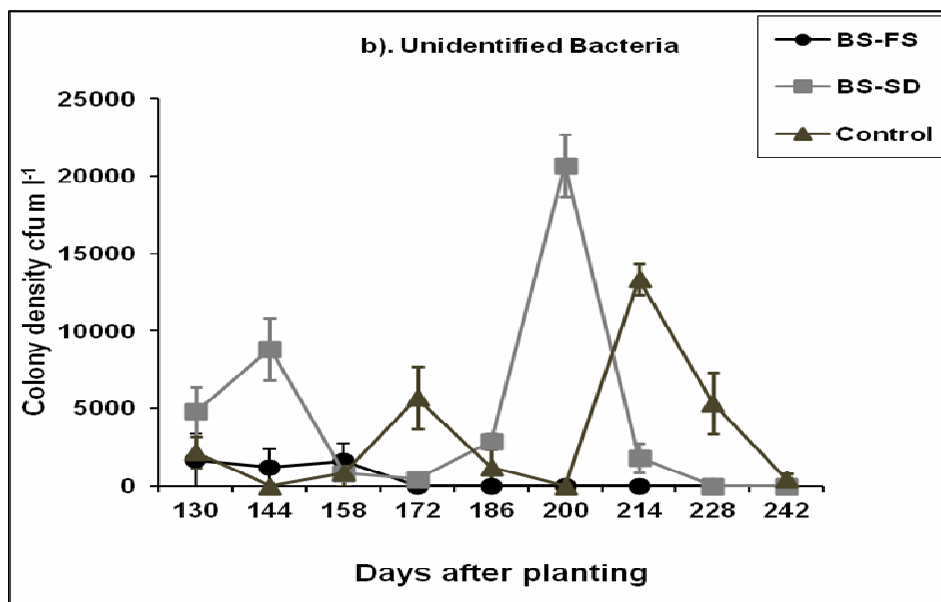


Fig. 4. Fluctuation of colony density of yeast (a) and unidentified bacteria (b) on banana leaves treated with three different treatments (BS-FS: *B. spinosa* as a foliar spray, BS-SD: *B. spinosa* as a soil drench, control: no application of *B. spinosa* either as a foliar spray or a soil drench)

Method of treatment, time after treatment or their interaction effect did not influence significantly on the colony densities of yeast and unidentified bacteria (other than *B. spinosa*) present on banana leaves ($p=0.05$). At the initial period of treatment, higher yeast density was reported by the plants treated as control. By 144 DAP the yeast density on control plants showed a reduction. In contrast, plants treated with *B. spinosa* as a foliar spray did not report yeast species throughout the experimental period, indicating an unfavourable microenvironmental condition for the growth of yeast due to the presence of *B. spinosa* on leaf surface. However, colony density of unidentified bacteria became zero from the 172 DAP and till then a higher colony density than that of control plants were reported from plants treated with *B. spinosa* as a FS. Leaves treated with *B. spinosa* as a soil drench reported a slightly higher colony density of yeast and unidentified bacteria than the plants treated with *B. spinosa* as a foliar spray with peak densities at 172 and 200 DAP. Higher colony density of unidentified bacterial species could be the endophytes or epiphytes present at times on plant leaves which had no detrimental effects by the microbial antagonist when applied as a soil drench. If these yeast and bacterial species are inhibitory to pathogens dwell on the leaves of the plant, they will provide an additional advantage on disease management.

Quantification of *C. musae* on banana leaves

C. musae colonies were not produced on PDA medium from the vortexed sample obtained from banana leaves which were artificially inoculated with a spore suspension of *C. musae*. This indicates the absence of viable spores on inoculated-leaves even after two days of inoculation. This could be due to the harmful effects of radiation and desiccation on spores. Environmental factors such as moisture, temperature and radiation have a large influence on the survival of fungal spores (Caesar & Pearson, 1983; Stevenson & Pennypacker, 1988).

Although short wavelength UV radiation (250-270 nm) is a very small component of solar radiation, it is the main fungicidal element of solar radiation (Boyd-Wilson *et al.*, 1998).

Effects of leaf washing on germination of *C. musae*

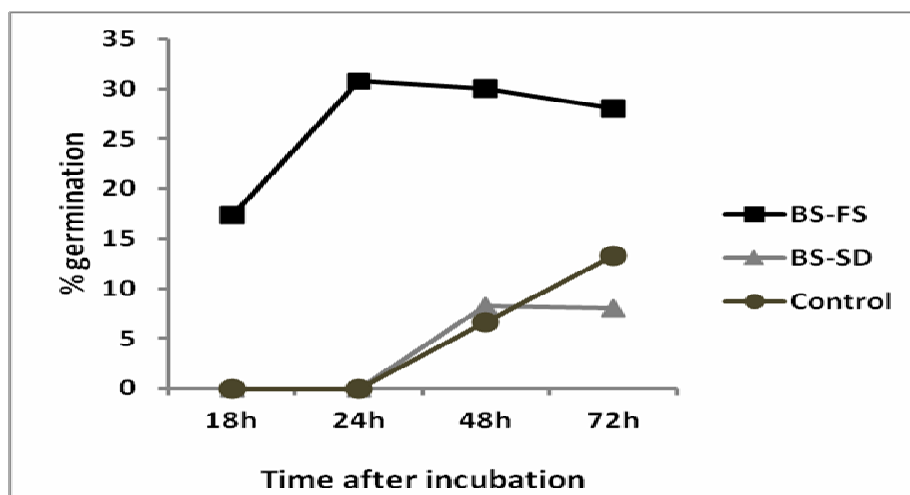


Fig. 5. Percentage germination of spores of *C. musae* on leaf washings obtained from banana treated with three treatments (BS-FS: *B. spinosa* as a foliar spray, BS-SD: *B. spinosa* as a soil drench, control: no application of *B. spinosa* either as a foliar spray or a soil drench)

Percentage germination of conidia continued to increase 24 h after incubation in the leaf washings obtained from control banana leaves. However, the leaf washings of the other two treatments showed a decline of spore germination. The higher percentage spore germination observed in leaf washings obtained from foliar sprayed-leaves could be due to the nutrients of the culture medium remained on the leaves when the microbial antagonist was sprayed as a foliar spray.

The microbial communities of leaves are diverse and include many different genera of bacteria, actinomycetes, filamentous fungi, yeast and less frequently protozoa and nematodes (Brighna *et al.*, 1997). Findings of the present study agree with the rich diverse nature of microbial community on the phyllosphere of banana having a range of fungal, yeast and bacteria. *Aspergillus* spp. such as *A. nidulans*, *A. flavus* and *Fusarium* spp. such as *F. semitectum*, *F. moniliforme* have been reported as pathogens of banana (Rodage & Suryawanshi, 2013; Jones & Stover, 2000; Murihead & Jones, 2000). Raaijmakers *et al.* (1995) reported that effective colonization and high population size of the introduced bacterial biological control agents on plant surfaces have been considered to be an important factor in the successful control of plant diseases. Results of the present study are also in agreement with Raaijmakers *et al.* (1995) showing the potential of effective colonization and high population size of the introduced biological control agent, *B. spinosa* on phyllosphere. Ji & Wilson (2003) also studied the enhancement of population size of a biological control agent and their bio-efficacy in the control of bacterial speck of tomato through salicylate and ammonium surface amendments. Use of phyllosphere inhabiting actinomycete for the control

of foliar pathogen of cucumber (*Corynespora cassicola*) has been reported (Wang & Ma, 2011).

When considered the population build-up on banana leaves and the ability to suppress other microbial colonies on banana leaves, use of *B. spinosa* as a foliar spray can be considered as an effective method to control pathogenic microbial populations growing on the phyllosphere. The potential ability of *B. spinosa* to be used as a field spray was further supported by the simulation *in vitro* study of spore germination of *C. musae* in different types of leaf washings.

CONCLUSIONS

Application of *B. spinosa* as a foliar spray in weekly intervals for nine repetitive times suppresses the abundance of *Aspergillus* spp., *Fusarium* spp. and several other unidentified bacteria, fungi and yeast spp. dwell on banana leaves. *B. spinosa* when applied as a soil drench could reduce phyllosphere pathogens, *Aspergillus* spp. and *Fusarium* spp. Foliar spraying of *B. spinosa* built up the colony density on banana leaves at a concentration of 10^5 cfu/ml by the end of 228 DAP.

REFERENCES

- Alvindia, D.G., Kobayashi, T. and Natsuaki, K.T. (2006). The aerial and fruit surface populations of fungi in nonchemical banana production in the Philippines. *J. Gen. Plant Pathol.* 72, 257 - 260.
- Brighna, L.M., Ravenelli, A., Monelli, A. and Ercoli, R. (1997). The use of epiphyte as bioindicator of air pollution in Costa Rica. *Science and Environment*, 198, 175 - 180.
- Boyd-Wilson, K.S.H., Perry, J.H. and Walter, M. (1998). Persistence and survival of saprophytic fungi antagonistic to *Botrytis cinerea* on kiwifruit leaves. *Proceedings of the 51st New Zealand Plant Protection Conference*, 51, 96 - 101.
- Caesar, A.J. and Pearson, R.C. (1983). Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. *Phytopath.* 73, 1024 - 1030.
- De Costa, D. M. and Subasinghe, S.S.N.S. (1998). Antagonistic bacteria associated with the fruit skin of banana in controlling its postharvest diseases, *Tropical Science*. 38, 206 - 212.
- De Costa, D.M., Erabadupitiya, H.R.U.T. (2005). An integrated method to control postharvest diseases of banana using a member of the *Burkholderia cepacia* complex. *Postharvest Biol Tech.* 36, 31 - 39.
- De Costa, D.M., Zahra, A.R.F., Kalpage, M.D. and Rajapakshe, E.M.G. (2008). Effectiveness and molecular characterization of *Burkholderia spinosa*, a prospective biocontrol agent for controlling postharvest diseases of banana. *Biol. Control*, 47, 257 - 267.
- De Lapeyer de Bellaire, L. and Nolin, J. (1994). Amélioration du contrôle du chancre sur les bananes d'exportation et traitements post-récolte. *Fruits*. 49, 179 - 185.

- Ji, P. and Wilson, M. (2003). Enhancement of population size of a biological control agent and efficacy in control of bacterial speck of tomato through salicylate and ammonium sulfate amendments, *App. Environ. Microbiol.* 69(2), 1290 - 1294.
- Johanson, A. and Blasquez, B. (1992). Fungi associated with banana crown-rot on field-packed fruit from the Wind-ward islands and assessment of their sensitivity to the fungicides thiabendazole, prochloraz and imazalil. *Crop Prot.* 11, 79 - 83.
- Jones D.R. and Stover, R.H. (2000). Fungal diseases of banana fruit, preharvest diseases. In: Jones, D.R. (Ed.), pp. 173-190. *Diseases of Banana, Abacá and Enset*. CABI Publishing, Wallingford, UK.
- Kumar, A., Prakash, A. and Johri, B.N. (2011). *Bacillus* as PGPR in crop ecosystem. Pp 37-59. In: Maheshwari, D.K. (Ed.). *Bacteria in Agrobiolgy: Crop Ecosystems*. Springer-Verlag, Berlin, Heidelberg.
- Mari, M., Torres, R., Casalini, L., Lamarca, N., Mandrin, J.F., Lichou, J., Larena, I., de Cal, M.A., Melgarejo, P. and Usall, J. (2007). Control of postharvest brown rot on nectarine by *Epicoccum nigrum* and physico-chemical treatments. *J. Sci. Food Agriculture.* 87(7), 1271 - 1277.
- Muirhead, I.F. and Jones, D.R. (2000). Fungal diseases of banana fruit, postharvest diseases. In: Jones, D.R. (Ed.). *Diseases of Banana, Abacá and Enset*, pp. 190-206. Wallingford, UK: CABI Publishing.
- Raaijmakers, J. M., Leeman, M., van Oorschot, M. M. P., van der Sluis, I., Schippers, B. and Bakker, P. A. H. M. (1995). Dose-response relationship in biological control of *Fusarium* wilt of radish by *Pseudomonas* spp. *Phytopathol.* 85, 1075 - 1081.
- Rippon, L.E. and Glennies-Holmes, L. (1973). Post-harvest dipping of bananas. *Agricultural Gazette of New South Wales.* 84, 229 - 231.
- Rodage, T.S. and Suryawanshi, N.S. (2013). Isolation of fungal pathogens from infected banana fruits and its control using *Trichoderma*, *Bionano Frontier*, 6(2), 1 - 2.
- Simmonds, J.H. and Mitchell, R.S. (1940). Black end and anthracnose of the banana with special reference to *Gleosporium musarum* and *Colletotrichum*. *Bulletin of Country Science and Industrial Research of Australia.* No. 131.
- Sharma, R.R., Singh, D. and Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biol. Control.* 50, 205-221.
- Stevenson, R.E. and Pennypacker, S.P. (1988). Effect of radiation, temperature and moisture on conidial germination of *Alternaria solani*. *Phytopathol.* 78, 926 - 930.
- Thangavelu, R. and Mustafa, M.M. (2012). Current advances in *Fusarium* wilt disease management in banana with emphasis on biological control. In: Cumagun, C.J. (Ed.) *Plant Pathology*, InTech, Available from: <http://www.intechopen.com/books/plant-pathology/current-advancesin-the-fusarium-wilt-disease-management-in-banana-with-emphasis-on-biological-control>

Waller, J.M. (1972). Water-borne dispersal in coffee berry disease and its relation to control. *Ann. Appl. Biol.* 71, 1 - 18.

Wang, M. and Ma, Q. (2011). Antagonistic actinomycete XN-1 from phyllosphere microorganisms of cucumber to control *Corynespora cassicola*, *Cucurbit Genetics Cooperative Report*, 33-34, 17-21.