Strategic eco-friendly management of post-harvest fruit rot in papaya caused by Colletotrichum gloeosporioides

K. DARSHAN*, S. VANITHA, K. M. VENUGOPALA and S. PARTHASARATHY

Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore - 641003, Tamil Nadu, India

*Corresponding author E-mail: darshuuas@gmail.com

ABSTRACT: Papaya (Carica papaya L.) is one of the important fruits cultivated in the tropical and subtropical regions are widely prone to the post-harvest anthracnose disease. A sum of ten isolates of Colletotrichum gloeosporioides were collected and identified through morphological and molecular method. Morphological characterization of the isolates revealed a wide variation among the isolates with respect to colony colour, topography, margin, pigmentation and zonation. The ITS gene region and the specific primer, MKCgF coupled with ITS-4, which generated amplicons of size 560 bp and 380 bp respectively for C. gloeosporioides. The amplicon (560 bp) of virulent strain Cg1 was partially sequenced [MF062699]. In order to formulate eco-friendly management practices, the in vitro screening of different biocontrol agents viz., Bacillus spp., Pseudomonas spp., plant extracts and essential oils were tested against the C. gloeosporioides. Based on the in vitro efficacy, Bacillus sp. (BSP1) and cinnamon oil were selected and further tested under field conditions as pre harvest spray and after harvest as fruit dipping. The experimental results revealed that pre-harvest spray with Bacillus sp. (BSP1) (5%) + post-harvest dipping with cinnamon oil (0.1%) recorded the lowest PDI of 3.25 when compared to control (70.36) and also increased the shelf life of papaya fruits up to 14 days. Our results show that this novel methodology of use a combination of biocontrol agent as pre-harvest spray and essential oils as post-harvest fruit dipping will protect against post-harvest anthracnose of papaya and use of chemical fungicides can be avoided.

KEYWORDS: Anthracnose, Bacillus spp, Colletotrichum gloeosporioides, cinnamon oil, formulation, thyme oil

INTRODUCTION

Papaya (Carica papaya L.) is one of the important fruits cultivated in the tropical and subtropical regions are widely prone to the post-harvest anthracnose disease. It is native of tropical America (Hofmeyr, 1938). AnKroyrd (1951) ranked it second only to mango as a source of Vitamin-A having 2020 IU/100g of fruit and a fair source of Vitamin-C (Verma and Sharma, 1999; Kelebek et al., 2015). It is a well-accepted fact that consumption of the full fruit is capable of imparting the ample amounts of ascorbic acid; essential minerals such as potassium, iron, calcium and phosphorous; carotenoids; vitamins such as A, B1, B2, and E; and soluble and insoluble dietary fiber (Rufino et al., 2010; Obon et al., 2011; Udomkun et al., 2014). Over the years, papaya has become an important commercial fruit crop and the area under cultivation is increasing worldwide because of its nutritional and pharmaceutical values (Rufino et al., 2010).

In India, papaya is grown in an area of 136 lakh ha, with production of 6107 lakh MT (Anon, 2017-18). Unfortunately, papaya fruits are perishable and have a short shelf-life, due to frequent attack by post harvest pathogens causing heavy loss to the grower (Alvarez and Nishijima, 1987). Among many fungal diseases, anthracnose, caused by Colletotrichum gloeosporioides is the one of the devastating post-harvest diseases in papaya fruit and also one of the major constraints in papaya cultivation (Dickman and Alvarez, 1983; Macedo, 2004). Major portion of harvested produce is lost during handling and transportation which has been estimated to be around 30-35 per cent (Ravindran et al., 2007). It deteriorates the quality and nutritive value of the fruits and renders them unfit for marketing and consumption thereby inflicting severe financial loss to farmers and traders (Jeffries et al., 1990;
Management of post harvest fruit rot in papaya caused by *Colletotrichum gloeosporioides*

Ravindran *et al.*, 2007; Muthulkshmi *et al.*, 2017). Papaya anthracnose can cause crop losses varying from 1 to 93 per cent (Paull *et al.*, 1997).

Though several management practices have been developed to control the pathogen. Currently, synthetic fungicides like benzimidazole and Sterol Biosynthesis Inhibitors (SBIs) were used to reduce losses from post-harvest diseases (Parthasarathy *et al.*, 2017). The extensive use of synthetic fungicides is increasingly being restricted in order to reduce their risks to human health, environmental pollution and also to reduce the aggravating problem of fungicide resistance (Spadaro and Gullino, 2004, Siddiqui and Ali, 2014). Alternative management methods are required to avoid the hazard of excessive toxic residues, considering the fact that papaya fruits are consumed in comparatively short time after harvest as a raw. Although several management practices have been developed to control the pathogen, the present investigation is focused on eco-friendly management strategies that facilitate to reduce the chemical usage and additionally increased consumer demands for agricultural commodities without pesticide residues (Siddiqui and Ali, 2014). Among the various alternatives, use of bio-control agents and natural plant products, including essential oils that are environmentally safe and biodegradable, are catching the attention of scientists worldwide and it has been considered as a novel approach, as it requires low amount of chemical, by reducing the cost of control as well as pollution hazardous (Narayan et al., 2016; Parthasarathy et al., 2016a). Keeping above view, the present work was undertaken to evaluate the efficacies of different biocontrol agents, plant extracts and essential oils for the management of papaya anthracnose causing *C. gloeosporioides*.

**MATERIALS AND METHODS**

**Isolation of *Colletotrichum gloeosporioides* pathogen**

Initially, disease infected samples showing typical symptoms of anthracnose were collected from different markets and from farmer's field around Coimbatore district (Fig. 1a and Fig. b). The suspected pathogen was isolated from the infected fruits showing typical symptoms of anthracnose. The pathogen was isolated from different samples using standard tissue isolation procedure of Rangaswami (1958). The infected fruit samples were cut into small pieces along with some healthy portion and rinsed in sterile water, dried on filter paper and placed in Petri dishes containing Potato Dextrose Agar (PDA) medium under aseptic conditions. The inoculated Petri plates were incubated (28±2°C) for fungal growth. The growth of the fungus was observed morphologically as well as under microscope for mycelium and spores. A total of ten isolates of *C. gloeosporioides* were isolated, purified by single spore isolation technique and designed as Cg1-Cg10. The pathogenicity of the isolates was tested by pin prick method (Parthasarathy et al. 2016b) and used for further studies.

**Morphological and molecular characterization of *Colletotrichum gloeosporioides* isolates**

In order to study the morphological characters of *C. gloeosporioides* isolates (10 isolates), the cultures were grown on PDA medium and incubated at 28±2°C. Colony colour, substrate colour, margin of colony and other phenotypic appearance were assessed. Observations of the conidia were taken. Measurement of the spores were also calculated using an image analyzer at 40x magnification (Medline Lab Instruments). The mean values and the range were determined.

The mycelium of *C. gloeosporioides* isolates grown on PD broth was macerated to form a dry powder using liquid nitrogen. The DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The quantity and quality of extracted DNA were examined by using nanodrop100 spectrophotometer. The rDNA region of ITS1 and ITS4 were amplified in ten isolates of *C. gloeosporioides* by polymerase chain reaction (PCR) (White et al. 1990; Stracieri et al. 2016). The PCR amplifications of DNA were performed with a program consisted of following condition for *C. gloeosporioides*: an initial step of 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 60 sec, annealing at 58°C for 2 min, extension at 72°C for 60 sec and a final extension at 72°C for 5 minutes (Kamle et al., 2013). The *C. gloeosporioides* species specificity was confirmed using species specific primer MKCgF 5’TTGCTTTCGCGGATTGTC3’ and MKCgR 3’ACGCAAAAGGAGGCTCCGGA5’ (Kamle et al., 2013). The amplification was performed in an Eppendorf thermal cycler using the following conditions an initial step of 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 60s, annealing at 63°C for 2 min, extension at 72°C for 60 sec and a final extension at 72°C for 5 minutes. The PCR products were resolved in electrophoresis (1.2% Agarose gel) and visualized under UV tech image analyzer. Later the amplicon of virulent strain Cg1 obtained with the primer pair ITS1 and ITS 4 were partially sequenced. The sequence identity of the isolate Cg1 was compared with the available resources using NCBI database. Further, these sequences were submitted to NCBI GenBank.

**Isolation and molecular identification of biocontrol agents**

Fourteen *Bacillus* spp. and seven *Pseudomonas* spp. were isolated from phyllosphere and fructosphere regions of papaya fruits using serial dilution method. Swab technique was followed and then streaked onto nutrient veg agar for *Bacillus* spp and King's B media for *Pseudomonas* spp.
and incubated at 25°C for 5 days. The genomic DNA from selected biocontrol agents was isolated by CTAB method with slight modifications. The previous study, the same author (Darshan et al., 2018) was performed the PCR and confirmed the strains of *Pseudomonas* spp., and *Bacillus* spp., by using gene specific primers ITSIF (5′-AAGTCGTAACAGGTAG-3′) and ITS2R (5′GACCATATATAACCCCAAG-3′) (Rameshkumar et al. 2002) and for *Bacillus* spp. 16S rDNA intervening sequence (Cano et al., 1994).

**In vitro screening of biocontrol agents against Colletotrichum gloeosporioides**

A dual culture technique (Dennis and Webster, 1971) was followed to determine mycelium inhibition against sixteen isolates of *Bacillus* spp. and eight isolates of *Pseudomonas* spp. Evaluation of biocontrol agent, the test bacterial isolates were streaked 1.0 cm away from the margin of a petri dish containing PDA. An agar plug cut from actively growing mycelium of *C. gloeosporioides* using a cork borer (8 mm diameter) was placed 1 cm away from the margin of the opposite site of the PDA. The plates were incubated 28 ± 2°C for further ten days. Radial growth of *C. gloeosporioides* was measured and per centage of inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

**Collection and extraction of botanicals**

The fresh leaves of different plant material were collected from botanical garden, Tamil Nadu Agricultural University, Coimbatore and washed with tap water to remove dust particle. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water at the ratio of 1:1 w/v. Then slurry was filtered through two layer of clean muslin cloth and then centrifuged at 5,000 rpm for 10 min (Kulshrestha et al., 2015). Then supernatant was collected and the final volume was made to 100 ml, thus finally filtrate obtained was used as standard stock solution as 100%. The standard solution was stored at 4°C for further use.

**Testing the fungitoxicity of plant extracts against Colletotrichum gloeosporioides in vitro**

The antifungal activity of sixteen different plant extracts was tested against *C. gloeosporioides* by poisoned food technique (Nene and Thapliyal, 1982). Plant extracts prepared as above were filtered through sterilized microbial filters of pore size 0.4 μm (Millipore filters), then 10 ml of stock solution aseptically mixed with 90 ml of sterilized PDA medium in order to get 10 per cent concentration of each extracts separately and the medium was thoroughly shaken for uniform mixing of extracts. Twenty ml of each of the poisoned medium was poured into sterilized petri plates separately and allowed to solidify. Each plate was seeded with an actively growing culture disc (8 mm diameter) of pathogen was placed at the center of petri dish and also maintained control without treatments. The plates were incubated at 28 ± 2°C for ten days. The radial mycelial growth (mm) of the pathogen was recorded. Per cent inhibition of the mycelia growth was then calculated by using the formula (Pandey et al., 1982).

**In vitro efficacy of essential oils against Colletotrichum gloeosporioides at different concentrations**

The efficacy of five essential oils was assayed at 0.05, 0.1, 0.2 and 0.25 per cent concentrations separately against the mycelial growth of *C. gloeosporioides* in vitro (Table 2). The required quantity of each essential oil was added separately in to sterilized PDA flask. Later 20 ml of poisoned medium was poured into sterilized Petri plates separately and allowed to solidify. Mycelial disc of actively growing culture was placed at the center of each agar plate. Control was maintained without adding any essential oil to the medium. Each treatment was replicated thrice and the plates were incubated at room temperature (28 ± 2°C) for ten days and the radial mycelial growth (mm) of the pathogen was recorded. The efficacy of an essential oil was expressed as inhibition of mycelial growth over control that was calculated by using the formula (Vincent, 1947).

**Field experiment**

Field trial was conducted in farmers' field at Thondamuthur, Coimbatore district, Tamil Nadu during March 2017 to May 2017 in papaya cv. red lady. For this *Bacillus* sp. bioformulation was developed as per the protocol (Manikandan et al., 2010) and for cinnamon oil 10 % EC formulation was prepared by mixing recommended quantities of 10 ml plant oils, 70 ml emulsifying agent (Unitox), 1 ml stabilizing agent (Epichlorohydrin) and remaining part was filled with organic solvent (Cyclohexanone) (Vanitha, 2010). The field experiment was laid out with ten treatments by following Randomized Block Design (RBD). The treatments were imposed as pre-harvest spray 15 days before harvesting of papaya. The same treatments were also followed as post-harvest dipping. Fruits were dipped in different treatments for 10 min. Periodical observations were taken.

**Statistical analysis**

The experimental data of the present study were analyzed using Analysis of Variance (ANOVA) by Agres
Management of post harvest fruit rot in papaya caused by *Colletotrichum gloeosporioides*

Statistical Software Package Version 3.01 (Agres, 1994). The Least Significant Difference (LSD) analysis was performed to separate the group mean when ANOVAs were significant at $p = 0.05$ and treatment means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Phenotypic characterization

The morphological characteristics of different isolates of *C. gloeosporioides* were studied on PDA

Table 1a. Conidial characters of different isolates of *Colletotrichum gloeosporioides*

| Isolates | Conidial length (µm) | Conidial width (µm) | Conidial characters |
|----------|----------------------|---------------------|---------------------|
|          |                      |                     | Colour             | Shape             |
| Cg1      | 15.50ab              | 5.32ab              | Hyaline            | Cylindrical       |
| Cg2      | 11.48ab              | 3.79ab              | Hyaline            | Cylindrical       |
| Cg3      | 16.15ab              | 5.29ab              | Hyaline            | Oblong to cylindrical |
| Cg4      | 14.67c               | 4.89bc              | Hyaline Cylindrical |
| Cg5      | 14.59f               | 4.32cd              | Hyaline Cylindrical |
| Cg6      | 16.23bcd             | 6.26a               | Hyaline Cylindrical |
| Cg7      | 14.01e               | 4.11d               | Hyaline Cylindrical |
| Cg8      | 13.33f               | 4.88bc              | Hyaline Cylindrical |
| Cg9      | 17.50g               | 5.29b               | Hyaline Cylindrical |
| Cg10     | 16.78a               | 5.90a               | Hyaline Cylindrical |
| SEd      | 0.193                | 0.212               |                     |                   |
| CD (p=0.05) | 0.391            | 0.365               |                     |                   |

In a column, means followed a common letter differ significantly at the 5% level by DMRT

Table 1b. Cultural characteristics of *Colletotrichum gloeosporioides* isolates on PDA medium

| Isolates | Colony color                           | Pigmentation                          | Margin                  | Zonation                               | Growth rate   | Acervuli production |
|----------|----------------------------------------|---------------------------------------|-------------------------|----------------------------------------|---------------|---------------------|
| Cg1      | Light pinkish white / creamy mycelia   | Dark orange / salmon color             | Smooth-thin, sharp edge | With concentric zonation (4 to 5)       | Very fast     | Dense and more in number |
| Cg2      | Milky whitish / powdery                | Light orange / orange yellow           | Very smooth and sharp edge | With concentric zonation (2 to 3) with V shaped sporulation | Moderate     | Sparsely            |
| Cg3      | Light white                            | Light salmon orange                   | Smooth to serrated      | With concentric zonation (4 to 5) and with V shaped sporulation | Moderate     | Less scattered       |
| Cg4      | Dull white with powdery                | Light brownish white                  | Smooth                  | With concentric zonation (2 to 3) with V shaped sporulation | Moderate     | Randomly scattered   |
| Cg5      | Yellowish white                        | Whitish orange                        | Smooth                  | Without concentric zonation            | Fast growth   | Sparsely            |
| Cg6      | Creamy white                           | Clear dark orange                     | Smooth to wavy          | With concentric zonation (2)           | Slow growth   | Very less number     |
| Cg7      | Milky white                            | Whitish with light orange             | Smooth with undulation  | Without concentric zonation and V shaped sporulation | Slow growth   | Less segregates      |
| Cg8      | Olive grayish - white                  | Greenish brown                        | Smooth                  | With concentric zonation (2)           | Fast growth   | Randomly distributed |
Fig. 1. Symptomatic and morphological characterization of *Colletotrichum gloeosporioides*

The results revealed that the appearance of the fungal mycelium of different *C. gloeosporioides* isolates varied from cottony white to greyish brown, superficial, septate and branched (Fig. 1c). The topography of the mycelium was flat in certain isolates, while in the others, the mycelium was slightly raised and fluffy in appearance. In majority of the isolates concentric zonation was seen on the underside of the culture plates except in three of the ten isolates, viz., Cg 5, Cg 7 and Cg 9. All of them produced pigments in culture that varied from shiny dark oranger to light orange in color.

Microscopic examination of infected tissue revealed that *C. gloeosporioides* produced hyaline, single celled, smooth walled, oblong or cylindrical conidia with 1-2 centrally placed oil globule. Significant variations were observed among the isolates with respect to conidial dimension. The conidial length and width of ranged from 11.48 - 17.50 × 3.79-6.26 µm. Majority of the isolates produced acervuli which were sparsely/randomly scattered on culture. Acervuli were circular, covered with a mucilaginous mass containing numerous conidia. Setae were arising through this mass, dark brown to black and they were erected in habit (Table 1a and 1b, Fig. 1c).
Management of post harvest fruit rot in papaya caused by \textit{Colletotrichum gloeosporioides}

\section*{Genotypic characterization}
To address the genetic position of the pathogen, the genomic DNA of \textit{C. gloeosporioides} isolates were extracted and Polymerase Chain Reaction (PCR) amplification was done by using universal primer pair ITS-1 and ITS-4. Agarose gel electrophoresis revealed the amplicons of size approximately 560 bp for all the ten isolates of \textit{C. gloeosporioides} (Fig. 2). All the ten isolates were subjected to PCR amplification with species specific primer for \textit{C. gloeosporioides} (MKCgF/R) and the results revealed that all ten isolates gave uniformly amplicon size of 380 bp on agarose gel, thereby confirming their identity as \textit{C. gloeosporioides} (Fig. 3). The amplicon (560 bp) obtained from the PCR amplification of virulent strain Cg1 isolate obtained with the primer pair ITS1 and ITS 4 was partially sequenced. The sequence identity of the isolate Cg1 was compared with the available sequences in Genbank. Further, this sequence was submitted to NCBI and obtained accession number as MF062699.

\section*{In vitro screening of biocontrol agents}
Sixteen isolates of \textit{Bacillus} spp. and eight isolates of \textit{Pseudomonas} spp. were screened against mycelial growth of \textit{C. gloeosporioides}. The results revealed that \textit{Bacillus} spp. significantly inhibited the growth of the pathogen by producing clear inhibition zone and was superior to \textit{Pseudomonas} spp. Among the sixteen \textit{Bacillus} spp. isolates, the highest percent inhibition over control was recorded in the BSP1 (47.83\%) and followed by BSP2 which recorded 46.83 per cent as compare to control. The least mycelial inhibition of 20.00 per cent was recorded in BSP13 with the mycelial growth of 7.0 cm (Fig. 8 & 9).

\section*{In vitro assay of plant extracts}
Sixteen different plant extracts at 10 per cent concentration was tested against the mycelial growth of \textit{C. gloeosporioides}. The results revealed that \textit{Melia azedarach} (10\%) leaf extract recorded the highest per cent inhibition of mycelial growth (65.20\%) and the next effective was the extract from \textit{Phyllanthus niruri} which recorded 54.07 per cent inhibition. The least effective was \textit{Withania somnifera} leaf extract (4.44\%). (Table 2).

\section*{In vitro evaluation of essential oils}
Five essential oils were assayed at four different concentrations \textit{viz.}, 0.05, 0.10, 0.20 and 0.25 per cent oils separately against the mycelial growth of \textit{C. gloeosporioides} under \textit{in vitro}. Among the five different essential oils tested, cinnamon oil and thyme oil completely inhibited the mycelial growth and recorded the highest per cent inhibition of mycelial growth of \textit{C. gloeosporioides} (100.00\%) which was followed by clove oil. Lemon grass oil and wintergreen oil had no effect on the pathogen at any of the concentrations tested (Fig. 5).

\section*{Field efficacy tests}
Emulsifiable Concentration (10EC) formulations of thyme and cinnamon oils were prepared for the management of papaya anthracnose under field and after harvest as fruit dipping (Fig. 11). The field experimental results revealed that pre-harvest spray with \textit{Bacillus} (BSP1) (5\%) + post-harvest dipping with cinnamon oil (0.1\%) recorded the lowest disease severity of 3.25 and also increased the shelf life of treated papaya up to 14 days when compared to untreated control which had a shelf life of only 5 days. The next effective treatment was pre-harvest spray with \textit{Bacillus} (BSP1) (5\%) + post-harvest dipping with EC formulated thyme oil (0.1\%) which recorded a disease severity of 5.50 PDI when compared to control (70.36 PDI). Least effective was pre-harvest spray of \textit{Melia azedarach} (PDI of 46.26) (Table 3).

Papaya fruits are consumed in moderately brief time after harvest. The use of chemicals in post-harvest disease management is not advisable because of its residual problem and can pose direct danger to the consumer who are mainly human. So, the alternative is biological control which is
Table 2.  *In vitro* efficacy of different botanicals against *Colletotrichum gleosporoides*

| S.No. | Botanicals                              | Mean Mycelial growth (cm) | Inhibition over control (cm) | Percentage inhibition over control (%) |
|-------|-----------------------------------------|----------------------------|-----------------------------|---------------------------------------|
| 1     | Madagiri vambu, *Melia azedarach*       | 1.56 a (1.24)             | 2.93                        | 65.20                                 |
| 2     | Phyllanthus, *Phyllanthus niruri*       | 2.06 b (1.43)             | 2.43                        | 54.07                                 |
| 3     | Terminelia seed, *Terminelia sepulcans* | 2.13 b (1.45)             | 2.36                        | 52.60                                 |
| 4     | Lantana, *Lantana camera*               | 2.43 cd (1.55)            | 2.06                        | 45.93                                 |
| 5     | Solanum, *Solanum nigrum*               | 2.40 c (1.54)             | 2.10                        | 46.66                                 |
| 6     | Anona, *Anona reticulata*               | 2.63 d (1.62)             | 1.86                        | 41.48                                 |
| 7     | Neelavambu, *Andrographis paniculata*   | 2.60 cd (1.61)            | 1.90                        | 42.22                                 |
| 8     | Ocimum, *Ocimum sanctum*                | 2.63 d (1.62)             | 1.86                        | 41.48                                 |
| 9     | Nandiavattai, *Tubernaemontana divaricata* | 2.90 e (1.70)             | 1.60                        | 35.55                                 |
| 10    | Neerium, *Neerium indicum*              | 3.06 e (1.74)             | 1.43                        | 31.85                                 |
| 11    | Pongum, *Pongania pinnata*              | 3.30 fg (1.81)            | 1.20                        | 26.66                                 |
| 12    | Periwinkle, *Vinca rosea*               | 3.33 g (1.82)             | 1.16                        | 25.92                                 |
| 13    | Vilvum, *Aegel marmelos*                | 3.50 g (1.87)             | 1.00                        | 22.22                                 |
| 14    | Gundumuthu, *Abrus precatorium*         | 3.76 h (1.93)             | 0.73                        | 16.29                                 |
| 15    | Coleus, *Plectranthus scutellaroides*   | 4.03 hi (2.00)            | 0.46                        | 10.37                                 |
| 16    | Aswagandha, *Withania somnifera*        | 4.30 ij (2.07)            | 0.20                        | 4.44                                  |
| 17    | Control                                 | 4.50 j (2.12)             | 0.00                        | 0.00                                  |

In a column, means followed a common letter differ significantly at the 5% level by DMRT.

![Graph](image.png)

Fig. 5.  Testing the efficacy of different of essential oils against anthracnose of papaya.
considered as one of the ecofriendly and safest methods for lessening the damage caused by post-harvest plant pathogens. In the present study biological control combined with IPM strategy was evaluated against identified virulent *Colletotrichum gloeosporioides*. Joshi *et al.* (2015) reported that the isolate of *C. gloeosporioides* produced fluffy mycelial growth on potato dextrose agar. *C. truncatum* isolated from green gram produced pinkish white mycelial growth with smooth margin in PDA (Roopadevi and Jamadar, 2016). Identification of *C. gloeosporioides* based on colony and spore morphology, which showed that acervuli were circular to elliptical, setae were erect in habit, conidia were hyaline, single celled and smooth walled. The conidial length and width of *C. gloeosporioides* ranged from 11.48-17.50 µm × 3.79-6.26 µm. These characters are in agreement with those of Bose *et al.* (1973) where the size of conidia varied from 11-16 × 4-6 µm. Sutton (1992) reported that the conidia of *C. gloeosporioides* were hyaline, smooth and thin walled, cylindrical or oval, straight and size of the conidia varied from 9-24 × 4-12 µm. In the present study, the ITS regions of *C. gloeosporioides* was amplified using the universal primers ITS-1 and ITS-4 by PCR. The primers amplified a fragment corresponding approximately to 560 bp for the *C. gloeosporioides* isolates. These results were in line with the earlier findings of Xiao *et al.* (2004) and Kamle *et al.* (2013). Pandey *et al.* (2012) amplified the genomic DNA from 12 isolates of *C. gloeosporioides* belonging to different regions by PCR with *C. gloeosporioides* species-specific primers. All the isolates amplified a uniform DNA fragment of size 450 bp.

The indiscriminate use of chemicals is not only hazardous to microbial population but also cause serious environmental pollution with toxic residual effects. Over the decade biological control has been considered as one of the most effective, ecofriendly and alternative approach for any disease management practice. *In vitro* screening of biocontrol agents will aid in the identification of potential isolates, the biocontrol agents *Bacillus* spp. significantly inhibited the growth of the pathogen by producing clear inhibition zone (Rahman *et al.*, 2007). The antimicrobial activity of plant extracts was studied by several authors (Mahesh and Satish, 2008; Kagale *et al.*, 2004). The plant derived essential oils possess antimicrobial properties and they were used against plant diseases and some of the essential plant oils are considered as resistance inducers (Kessmann *et al.*, 1994).

Currently, biological control is considered as a very promising alternative to synthetic fungicide in the control of postharvest decay of fruits and vegetables (Parthasarathy *et al.*, 2017). In the present study, the liquid formulation of *Bacillus* sp. (BSP1) was developed and Emulsifiable Concentrate (EC)

### Table 3. Pre and post-harvest testing of biocontrol agents and other treatments against *Colletotrichum gloeosporioides* on papaya

| S. No. | Treatments | Per cent Disease Index (PDI) | Total shelf life (Days) |
|--------|------------|-----------------------------|-------------------------|
| 1.     | T1 - Pre-harvest spray with *Bacillus* sp. (BSP1) (5%) | 24.36f | 10 |
| 2.     | T2 - Pre-harvest spray with 10EC formulated Thyme oil (0.1%) | 35.32h | 8 |
| 3.     | T3 - Pre-harvest spray with 10EC formulated Cinnamon oil (0.1%) | 29.85g | 9 |
| 4.     | T4 - Pre-harvest spray extracts of *Melia azedarach* (10%) | 46.26i | 7 |
| 5.     | T5 - Pre-harvest spray with *Bacillus* sp. (BSP1) (5%) + Post harvest dipping with 10EC formulated Thyme oil (0.1%) | 5.50b | 13 |
| 6.     | T6 - Pre-harvest spray with *Bacillus* sp. (BSP1) (5%) + Post-harvest dipping with 10EC formulated Cinnamon oil (0.1%) | 3.25a | 14 |
| 7.     | T7 - Pre-harvest spray with *Bacillus* sp. (BSP1) (5%) + Post-harvest dipping with extracts of *Melia azedarach* (10%) | 10.90c | 13 |
| 8.     | T8 - Post harvesting dipping with 10EC formulated Thyme oil (0.1%) | 14.90d | 12 |
| 9.     | T9 - Post harvesting dipping with 10EC formulated Cinnamon oil (0.1%) | 20.23e | 12 |
| 10.    | T10 - Control | 70.36j | 5 |

*Critical value of t @ 0.05* 2.08596

*Least significant difference* 0.0547

*Mean of three replications and means with the same letter are not significantly different*
formulations of cinnamon oil and thyme oil were prepared at 10 per cent EC concentration were used for foliar spray for field evaluation. Vanitha (2010) developed 10 per cent EC formulation of lemongrass oil and wintergreen oil and effectively managed the Alternaria chlamydospora causing leaf blight of Solanum nigrum at 0.1 per cent. Similarly, Brankica et al. (2013) developed EC formulations of thyme oil and tested against the growth of Monilinia fructigena. From the above results, it was clearly demonstrated that the Bacillus sp. (BSP1) and cinnamon oil (0.1%) highly inhibited C. gloeosporioides in vitro. Pre-harvest application of Bacillus sp. (BSP1) 5 per cent bioformulation and post-harvest dipping of cinnamon oil (0.1%) 10 per cent EC effectively reduced the disease incidence, latent symptom activity and also increased shelf life of papaya in field conditions. Hence it can be adopted as suitable management strategies in organic disease management system.

ACKNOWLEDGEMENTS

The authors would like to thank Professor and Head, Department of Plant Pathology and The Dean, School of Post Graduate Studies, Tamil Nadu Agricultural University, Coimbatore for providing facilities to successfully carry out the research program.

REFERENCES

Agres. 1994. Statistical Software Version 3.01. Pascal International Software Solutions, USA.

Alvarez AM, Nishijima WT. 1987. Postharvest diseases of papaya. Plant Dis. 71: 681-686. https://doi.org/10.1094/PD-71-0681

Anonymous. 2017. National Horticulture database. National Horticulture Board, Govt. of India, Gurgaon, India. pp. 182. www.nhb.gov.in

Bose SK, Sindhar GS, Pande BN. 1973. Studies on the die-back disease of mango in the Tarai region of Kumaon. Prog Hort. 5: 41-53.

Brankica T, Slavica G, Jovana H, Milica M, Mila G, Goran D, Marija S. 2013. Development of a thyme essential oil formulation and its effect on Monilinia fructigena. Pestic Phytochem. 28: 273-280. https://doi.org/10.2298/PIF1304273T

Dickman MB, Alvarez AM. 1983. Latent infection of papaya caused by Colletotrichum gloeosporioides. Plant Dis. 67: 748-750. https://doi.org/10.1094/PD-67-748

Dennis C, Webster J. 1971. Antagonistic properties of species groups of Trichoderma: production of non-volatile antibiotics. Trans Br Mycol Soc. 57: 25-39. https://doi.org/10.1016/S0007-1536(71)80077-3

Hofmeyr JDJ. 1938. Genetical studies of Carica papaya L. South Afr J Sci. 35: 300-304.

Jeffries P, Dodd JC, Jeger MJ, Plumbley RA. 1990. The biology and control of Colletotrichum species on tropical fruit crops. J Plant Pathol. 39: 343-366. https://doi.org/10.1111/j.1365-3059.1990.tb02512.x

Joshi PV, Kadam JJ, Joshi MS, Pawar SV. 2015. Symptomatology, pathogenicity, host range and cultural study of Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. causing tip blight of jackfruit (Artocarpus heterophyllus L.). Periodic Res. 4: 1-5.

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. 2004. Antimicrobial activity and induction of systemic acquired resistance in rice by leaf extract of Datura metel against Rhizoctonia solani and Xanthomonas oryzae pv. oryzae. Physiol Mol Plant Pathol. 65: 91. https://doi.org/10.1016/j.pmpp.2004.11.008

Kamle M, Pandey BK, Kumar P, Muthu Kumar M. 2013. A species-specific PCR based assay for rapid detection of mango anthracnose pathogen Colletotrichum gloeosporioides (Penz. and Sacc.) Penz. and Sacc. causing tip blight of mango pulp. Pmc: 2004.11.008

Kelebek H, Selli S, Gubbuk H, Gunes E. 2015. Comparative evaluation of volatiles, phenolics, sugars, organic acids and antioxidant properties of Sel-42 and Tainung papaya varieties. Food Chem. 173: 912-919. https://doi.org/10.1016/j.foodchem.2014.10.116 PMid:25466106

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzo J. 1994. Induction of systemic acquired resistance in rice plants by chemicals. Ann Rev Phytopathol. 32: 439-459. https://doi.org/10.1146/annurev.py.32.090194.002255 PMid:18479201

Kulshrestha S, Chaturvedi S, Jangir R, Agrawal K. 2015. In vitro Evaluation of antibacterial activity of some important medicinal plants against plant and human pathogens. World J Agrl Sci. 4: 839-843.
Manikandan R, Saravanakumar D, Rajendran L, Raguchander T, Samiyappan R. 2010. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. *Biol Control.* 54: 83-89. https://doi.org/10.1016/j.bico.2010.04.004

Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8(19): 4321-4325. doi: 10.1093/nar/8.19.4321 https://doi.org/10.1093/nar/8.19.4321 PMid:7433111 PMcid:PMC324241

Muthulakshmi P, Ragavi B, Parthasarathy S. 2017. Influence of abiotic factors on the growth of *Colletotricum musae* causing post-harvest anthracnose disease in banana. *Int J Agric Sci Res.* 7:121-128.

Narayanan P, Parthasarathy S, Rajalakshmi J, Arunkumar P, Vanitha S. 2016. Systemic elicitation of defense related enzymes suppressing Fusarium wilt in mulberry (*Morus spp.*). *Afr J Microbiol Res.* 10: 813-819. https://doi.org/10.5897/AJMR2015.7900

Nene YL, Thapliyal PN. 1982. *Fungicides in Plant Diseases Control.* Oxford and IBH Publishing Co Pvt. Ltd., New Delhi. pp.163.

Obon JM, Diaz-Garcia MC, Castellar MR. 2011. Red fruit juice quality and authenticity control by HPLC. *J Food Compos Anal.* 6: 760-771 https://doi.org/10.1016/j.jfca.2011.03.012

Pandey A, Yadava LP, Manoharan M, Chauhan UG, Pandey BK. 2012. Effectiveness of cultural parameters on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose disease of mango (*Mangifera indica* L.). *J Biol Sci.* 12: 123-133. https://doi.org/10.3844/jbisc.2012.123.133

Pandey DK, Tripathi RN, Tripathi NN. 1982. Antifungal activity in some seed extracts. *Environ India.* 4: 164-167.

Parthasarathy S, Rajalakshmi J, Narayanan P, Arunkumar K, Prabakar K. 2017. Bio-control potential of microbial antagonists against post-harvest diseases of fruit crops: A review. *Res Rev: J Bot Sci.* 6: 17-23.

Parthasarathy S, Rajalakshmi J, Narayanan P, Prabakar K. 2016a. Botanicals in eco-friendly post-harvest disease management. *Innovative Farm.* 1: 67-71.

Parthasarathy S, Thiribhuvanamala G, Subramanium KS, Prabakar K. 2016b. Bacterial antagonists and hexanal-induced systemic resistance of mango fruits against *Lasiodiplodia theobromae* causing stem-end rot. *J Plant Interact.* 11: 158-166. https://doi.org/10.1080/17429145.2016.1252068

Paull RE, Nishijima W, Reyes M, Cavaletto C. 1997. Postharvest handling and losses during marketing of papaya (*Carica papaya*). *Postharvest Biol Technol.* 11: 165-179. https://doi.org/10.1016/S0925-5214(97)00028-8

Rangaswami G. 1958. An agar block technique for isolating soil micro-organisms with special reference to Pythiaceous fungi. *Sci Culture* 24: 85-94.

Ravindran C, Kohli, Anshuman, Murthy and Srinivas. 2007. Fruit production in India. *Chronica Horticulturae* 42: 21-26.

Rahman MA, Kadhur J, Mahmud TMM, Abdul R, Begum S. 2007. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gloeosporioides* in papaya. *Asian J Plant Sci.* 6: 12-20. https://doi.org/10.3923/ajps.2007.12.20

Roopadevi, Jamadar MM. 2016. In-vitro bioassay of different fungicides against anthracnose of green gram caused by *Colletotrichum truncatum* (Schw.) Andrus and Moore. *Environ Ecol.* 34: 132-135.

Rufino MS, Alves RE, de Brito ES, Perez-Jimenez J, Saura-Calixto F, Mancini-Filho J. 2010. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chem.* 121: 996-1002. https://doi.org/10.1016/j.foodchem.2010.01.037

Siddiqui Y, Ali A. 2014. *Colletotrichum gloeosporioides* (Anthracnose). *Post-harvest Decay* 4: 337-364. https://doi.org/10.1016/B978-0-12-411552-1.00011-9

Spadaro D, Gullino ML. 2004. State of the art and future prospects of the biological control of postharvest fruit disease. *Intern J Food Microbiol.* 91:185-194. https://doi.org/10.1016/S0168-1605(03)00380-5

Stracieri J, Pereira FD, da Silveira AL, Magalhães HM, de Goes A. 2016. Morphocultural and molecular characterization of papaya tree *Colletotrichum spp.* *Afr J Agric Res.* 11: 1755-1764. https://doi.org/10.5897/AJAR2016.10868

Sutton BC. 1992. The genus *Glomerella* and its anamorph *Colletotrichum.* pp. 1-26. In: Bailey JA, Jeger MU (Eds.). *Colletotrichum biology, pathology and control.* CAB International, Wallingford.
DARSHAN et al.

Taale E, Savadogo A, Zongo C, Somda MK, Sereme SS, Karou SD, Soulama I, Traore, AS. 2015. Biochemical and molecular characterization of strains isolated bacteria from Soumbala. *Int J Adv Res Biol Sci.* 2: 279-290.

Vanitha S. 2010. Developing new formulation using plant oils and testing their physical stability and antifungal activity against *Alternaria chlamydospora* causing leaf blight in *Solanum nigrum*. *Res J Agric Sci.* 1: 385-390.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 150: 850. https://doi.org/10.1038/159850b0 PMid:20343980

Udomkun P, Nagle M, Mahayothee B, Nohr D, Koza A, Müller J. 2014. Influence of air drying properties on non-enzymatic browning, major bio-active compounds and antioxidant capacity of osmotically pre-treated papaya. *LWT-Food Sci Technol.* 60: 914-922. https://doi.org/10.1016/j.lwt.2014.10.036

White TJ, Bruns TD, Lee SB and Taylor JW. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. pp. 315-322. In: Innis MA, Gelfand DH, Sninsky JJ and White TJ (Eds). *PCR protocols: A guide to methods and applications*. Academic Press, New York.

Xiao CL, Mac Kenzie SJ, Legard DE. 2004. Genetic and pathogenic analyses of *Colletotrichum gloeosporioides* from strawberry and non-cultivated hosts. *Phytopathology* 94: 446-453. https://doi.org/10.1094/PHYTO.2004.94.5.446 PMid:18943762