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Research Article

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

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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;

Ravindran *et al.,* 2007; Muthulakshmi *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 (Narayanan *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. gloeosporoides* 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`TTGCTTCGGCGGGTAGGGTC3` and MKCgR 3`ACGCAAAGGAGGCTCCGGGA5` (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 60s extension at 72°C for 60s; and a final extension at 72°C for 5 minutes.The PCR products was 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'-AAGTCGTAACAAGGTAG-3') and ITS2R (5'GACCATATATAACCCCAAG-3') (Rameshkumar *et al.* 2002) and for *Bacillus* spp. 16S rDNA intervening sequence specific BCF1 (CGGGAGGCAGCAGTAGGGAAT); BCR2 (CTCCCCAGGCGGAGTGCTTAAT) primers (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 gleosporoides* 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. gleosporoides 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 farmer's 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

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 gleosporioides*

Isolates	Conidial length (µm)	Conidial width (µm)	Conidial characters	
			Colour	Shape
Cg1	15.50^{ab}	5.32^{ab}	Hyaline	Cylindrical
Cg2	11.48^{ab}	3.79^{d}	Hyaline	Cylindrical
Cg3	16.15^{ab}	5.29^{ab}	Hyaline	Oblong to cylindrical
Cg4	14.67^{d}	4.89^{bc}	Hyaline	Cylindrical
Cg5	14.59^{f}	4.32^{cd}	Hyaline	Cylindrical
Cg6	16.23^{bcd}	6.26^{a}	Hyaline	Cylindrical
Cg7	14.01^{cde}	4.11^{cd}	Hyaline	Cylindrical
Cg8	13.33^{g}	4.88^{bc}	Hyaline	Cylindrical
Cg9	17.50^{g}	5.29^{b}	Hyaline	Cylindrical
Cg10	16.78^{a}	5.90^{a}	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 gleosporioides* isolates on PDA medium

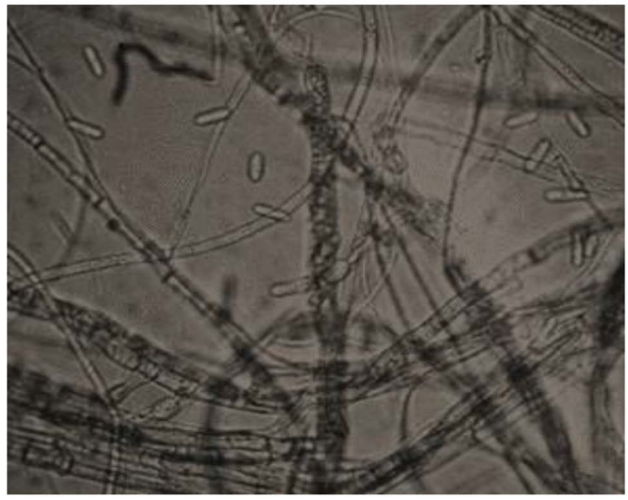
Isolates	Colony color	Pigmentation	Margin	Zonation	Growth rate	Acervuli production
Cg1	Light pinkish white / creamy mycelia	Dark orange / salmon color	Smooth-thin, sharp edge	With concentric zonation (4 to 5)	Very fast Growth	Dense and more in number
Cg2	Milky whitish / powdery	Light orange / orange yellow	Very smooth and sharp edge	With concentric zonation (2 to 3) with V shaped sporulation	Moderate Growth	Sparsely
Cg3	Light white	Light salmon orange	Smooth to serrated	With concentric zonation (4 to 5) and with V shaped sporulation	Moderate Growth	Less scattered
Cg4	Dull white with powdery	Light brownish white	Smooth	With concentric zonation (2 to 3) with V shaped sporulation	Moderate Growth	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

Cg9	Grey white / cement color	Dark brownish to charcoal color	Smooth with wavy margin	Without concentric zonation	Moderate growth	Sparsely
Cg10	Whitish grey	Creamy to charcoal	Smooth	With concentric zonation (2 to 3) with V shaped sporulation	Fast growth	Scattered

a. Initial infection in the field concentric manner

b. Brown to black lesions with acervuli in a

c. Pure culture of pathogen after re-isolation

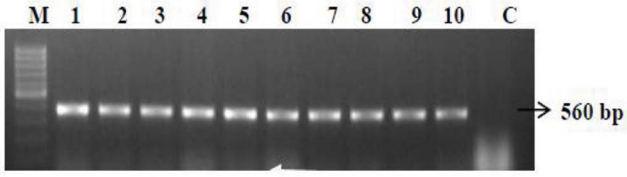


d. Mycelium and Conidia

Fig. 1. Sympotamatic and morphological characterization of *Colletotrichum gloeosporioides*

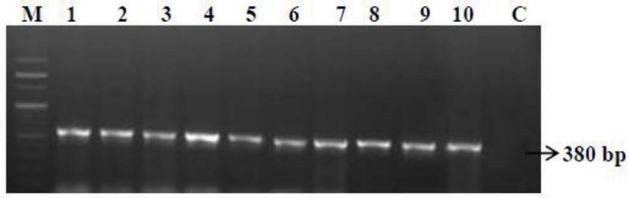
media. The results revealed that the appearance of the fungal mycelium of different *C. gloeosporioides* isolates varied from cottony white to greyish brown, superficial, septate and branched (Fig. 1c). The topography of the mycelium was flat in certain isolates, while in the others, the mycelium was 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).



Lane M: 100 bp marker; Lane 1-10: *C. gleosporoides* isolates *Cg* 1-10; Lane C: Control

Fig. 2. PCR amplification of ITS region of *Colletotrichum gleosporoides* isolates.



Lane M: 1000 bp marker; Lane 1-10: C. gleosporoides isolates Cg 1-10; Lane C:Control

Fig. 3. Amplification of *Colletotrichum gleosporoides* isolates with species specific primers.

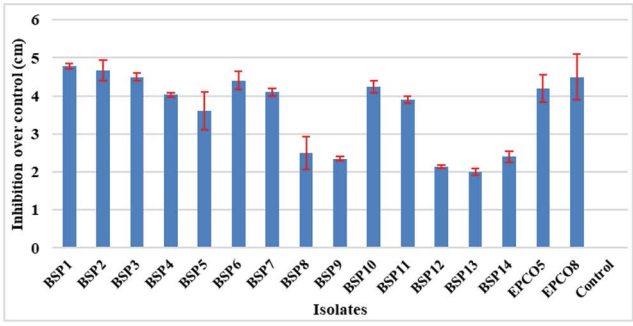


Fig. 4. In vitro efficacy of biocontrol agents *Bacillus spp.* against *Colletort. gleosporioides*

Genotypic characterization

To address the genetic position of the pathogen, the genomic DNA of *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 *C. gloeosporioides* (Fig. 2). All the ten isolates were subjected to PCR amplification with species specific primer for *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 *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.

In vitro screening of biocontrol agents

Sixteen isolates of *Bacillus* spp. and eight isolates of *Pseudomonas* spp. were screened against mycelial growth of *C. gloeosporioides*. The results revealed that *Bacillus* spp. significantly inhibited the growth of the pathogen by producing clear inhibition zone and was superior to *Pseudomonas* spp. Among the sixteen *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).

In vitro assay of plant extracts

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

In vitro evaluation of essential oils

Five essential oils were assayed at four different concentrations *viz*., 0.05, 0.10, 0.20 and 0.25 per cent oils separately against the mycelial growth of *C. gleosporoides* under *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 *C. gloeosporoides* (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).

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 *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 *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 *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 (%)
	Comman Name	Scientific Name			
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 sepulae*	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 sinctum*	2.63 d (1.62)	1.86	41.48
9	Nandiyavattai	*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	*Pongamia 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
SEd			0.0341		
CD (0.05)			0.0931		

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

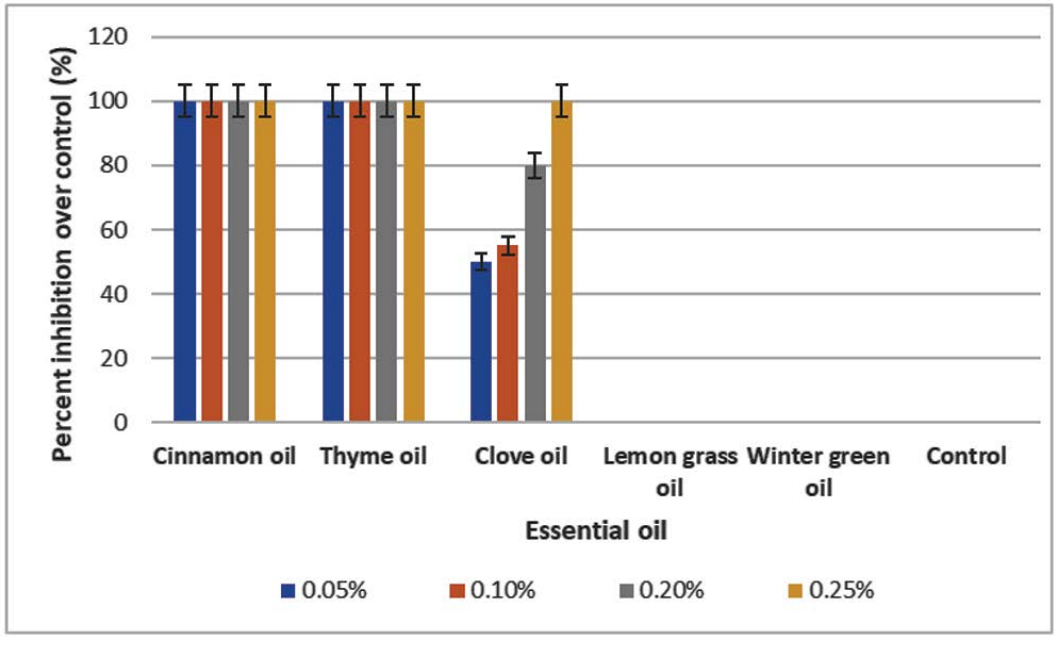


Fig. 5. Testing the efficacy of different of essential oils against anthracnose of papaya.

Table 3. Pre and post-harvest testing of biocontrol agents and other treatments against *Colletotrichum gleosporioides* 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

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)

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 to *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.

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