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Studies on the Eco-friendly Management of Papaya anrthracnose caused by *Colletotrichum papayi* (Penz.) Penz & Sacc. and *Lasiodioplodia theobromae* (Pat.) Griffon & Maubl

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ABSTRACT
Papaya (Carica papaya)_is a tropical fruit having commercial importance because of its high nutritive and medicinal value. Papaya cultivation had its origin in South Mexico and Costa Rica. Papaya is highly susceptible to disease caused by many micro organisms especially fungi. Among these, anthracnose of papaya is caused by complex pathogen viz., Colletotrichum papayi (Penz.) Penz & Sacc. and Lasiodioplodia theobromae. (Pat.) Griffon & Maubl. This disease causes great economic losses because it affects the production and marketing of papaya. Post-harvest disease of fruits are traditionally managed by using chemical fungicides, however residues play a major role in the application of fungicides during harvest. Modern agriculture is highly dependent on chemical fungicides. However, the repeated use of such chemicals may result in the environmental pollution and may lead to development of resistance in target organism. Management of crop diseases through bio-control agents has been realized as an alternate to chemical fungicides. In vitro studies were carried out to test the efficacy of bacterial bio-agents and plant oils against papaya pathogen. Among the bacterial isolate tested isolate PfP_1 recorded the maximum inhibition zone of 11.00 mm with a minimum of 28.33 mm mycelial growth of L. theobromae, accounting 67.68 per cent reduction of the mycelial growth over control and maximum inhibition zone of 11.67 mm with a minimum of 21.33 mm mycelial growth of C. papayi, accounting 75.94 per cent reduction of the mycelilal growth over control. This was followed by BsP_2 and BsP_1. With regard to the plant oils tested, Eucalyptus oil showed minimum inhibitory concentration at 0.05% and minimum lethal concentration of 0.1 % against C. papayi and L. theobromae followed by lemon grass oil. Further, the effect of selected plant oils and bio control agents with fungicide check (Thiabendazole @ 1000 ppm) were tested against the anthracnose disease of papaya after inoculating the spore suspension. The treatment T_4-dipping the fruits in Eucalyptus oil (0.05 %) was found to completely reduces the disease development at 8DAI (days after inoculation) by recording 91.27 per cent disease reduction over control. It was followed by T_5-lemongrass oil consortium and consortium of P. fluorescens (PfP_1) + B. subtilis (BsP_1).
Key words: *Carica papaya, Pseudomonas fluorescens, Bacillus subtilis, Plant oils*

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INTRODUCTION

Papaya (*Carica papaya L.*) is an important fruit crop belonging to a family Caricaceae. It is native to Southern Mexico and neighboring Central America. It is an important fruit crop cultivated in tropical and subtropical areas. It is known as "Common mans fruit " and it is one of the most important fruit crop in home gardens. The major papaya producing regions

are Asia, South America, North Central America and Africa. About 65 % of the world's production is from South America. Another 35% is from North Central America and Africa. India ranks first in papaya production. In India Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Gujarat and Assam are major papaya producing states. India is the largest producer with an area of 136 ha with production of 6018 MT [3]. It is a rich source of vitamin A, C and calcium. The fruit has an important antioxidant activity and its consumption provides health benefits, particularly by its medicinal properties and improved digestion [9].

The shelf life of papaya is often limited as it is highly perishable and susceptible to various post-harvest diseases due to its drastic changes in physiochemical properties [47]. Papaya is highly susceptible to disease caused by many micro organisms especially fungi. Diseases like *Alternaria* fruit rot, Black spot, Black rot, Anthracnose, Brown spot, Chocolate spot and Collar rot. Among these, papaya anthracnose cause serious problem both in field and storage level. The maximum post-harvest losses are mainly due to pathogenic micro organisms which can invade fruits through injury or even latent infection during the pre-harvest [5].

Papaya anthracnose incidence can reach around 70-80 per cent with a resulting reduction in the commercial value of the fruit [16, 34]. Anthracnose has been reported to cause substantial yield loss in the major papaya producing regions of the world including Hawaii, Brazil, Africa and Asia [15]. The two commonly grown papaya cultivars 'Red lady' and 'Tainung no 2 F_1 Hybrid' are highly susceptible to anthracnose disease with disease incidence in mature ripe fruits approaching 80 per cent because the disease is only apparent on mature ripe fruits. The green fruit remains largely unaffected by this disease.

Anthracnose of papaya is caused by complex pathogen *viz., Colletotrichum papayi* (Penz.) Penz & Sacc. and *Lasiodioplodia theobromae*. (Pat.) Griffon & Maubl. This disease causes great economic losses because it affects the production and marketing of papaya [42]. Anthracnose can infect papaya leaves, but the fruit is much more severely affected [12]. The symptoms of anthracnose in papaya are characterized by round brownish depressed lesions and in some cases, salmon-colour areas formed by the conidial masses that cover the lesion. *Colletotrichum* species can directly penetrate the fruit skin [18].

Post-harvest disease of fruits are traditionally managed by using chemical fungicides, however residues play a major role in the application of fungicides during harvest. Modern agriculture is highly dependent on chemical fungicides. However, the repeated use of such chemicals may result in the environmental pollution and may lead to development of resistance in target organism. Management of crop diseases through bio-control agents has been realized as an alternate to chemical fungicides. It is an important component of integrated disease management system in order to reduce the risk of pesticide hazard as well as to prevent resistance development in pathogens to fungicides. Out of the diverse microbial species of bio-agents, a few genera like *Pseudomonas* and *Bacillus* have been successfully exploited in different crops. *Pseudomonas* and *Bacillus* are two mostly used bio-control agents as they have antifungal, plant growth promoting and plant defense inducing activities [14]. The potential of essential oils as an alternative disease control for several fruit crops [29] has been well-studied. To date, a wide range of essential oils such as thyme, *Eucalyptus*, Mexican lime, lemongrass, cinnamon and castor oil applied on fruits have proven to be effective against *C. gloeosporioides* and *L. theobromae* [30, 1].

With due to the concentration of the above fact, the present study is formulated with the following objectives i) to isolate and identify the pathogen ii) laboratory evaluation of bio-control agents and plant oils against pathogen iii) to develop a management strategy by using plant oils and antagonists against anthracnose disease of papaya.

MATERIAL AND METHODS

Sampling and isolation of anthracnose pathogens

The pathogen causing anthracnose disease in papaya was isolated from diseased fruit samples. The variety namely, CO-1, CO-2, CO-3 and Red lady were collected from retail markets (Chidambaram, Peelamedu, Karur, Tiruchengodu and Salem) of Tamil Nadu during 2016-2017 for isolation of pathogen(s). The symptomatic tissues (1 cm^2) from the skin of papaya were surface-sterilized by using 2 per cent (v/v) sodium hypochlorite for 5 min, followed by three washes with purified water and drying on sterile paper. The edges of the

surface-sterilized tissues were cut off and the tissues were cultured on Petri dishes containing potato dextrose agar (PDA) and incubated at room temperature (28 ± 2 °C) for 5-7 days. The emerging colonies were sub cultured onto PDA plates. Single spore isolation of the fungus or single hyphal tip method was followed for making the pure cultures and maintained on PDA slants [3].

Among the 12 isolations made, 9 isolates were identified as *L. theobromae* and named as LT_1 to LT_9. Likewise, three isolates were identified as *C. papayi* and named as CP_1 to CP_3 accordingly. In all isolation studies, the isolates of both *L. theobromae* and *C. papayi* frequently obtained from var. Red lady. The identity of all isolates of *L. theobromae* and *C. papayi* was confirmed by microscopical observation. All isolates of *L. theobromae* examined during this study agreed with the description given by Goos *et al.* [20] and Punithalingam [35]. All isolates of *C. papayi* examined were agreed with the descriptions given by Sutton and Waterson [43]. Based on the virulence studies, the isolates LT_1 and CP_1 were used for further studies.

Isolation of bacterial antagonists

Antagonistic bacteria were isolated from fruit skin of papaya following the method described by Zahavi *et al.* [46]. Papaya fruit peel was taken and mixed with 5 ml of sterile distilled water in a flask which was shaken on a shaker for 30 min. Then 1 ml of suspension was added to a Petri plate containing nutrient agar medium and incubated at room temperature (28±2°C) for 48 h. The growing colony was sub cultured on nutrient agar (NA) using single colony isolation. The slant was kept at 10°C in refrigerator and used as stock culture.

Totally 9 isolates of bacteria were isolated and they were identified based on Bergey's Manual of Systematic Bacteriology. Of these, 7 isolates were identified as *Pseudomonas fluorescens* and named as PfP_1, PfP_2, PfP_3, PfP_4, PfP_5, PfP_6 and PfP_7. Likewise, 2 isolates were identified as *Bacillus subtilis* and named as BsP_1 and BsP_2 accordingly.

Biochemical tests for identification of e bacterial isolates

For the identification of bacterial isolates certain biochemical tests were conducted Starch hydrolysis, nitrate reduction, fluorescent pigment, gelatin liquefication, gram staining, KOH test and motility) according to Bergey's Manual of Determinative Bacteriology [21].

***In vitro* testing of bacterial antagonists**

The antagonistic activity of nine bacterial isolates against *L. theobromae* (LT_1) and *C. papayi* (CP_1) was tested by dual culture technique separately [7] using PDA medium. At one end of the sterile Petri plate containing fifteen ml of sterilized and solidified PDA medium, a eight mm culture disc of pathogen obtained from seven days old culture of *L. theobromae* and *Colletotrichum* was placed at 1.5 cm away from the margin of the Petri plate. Similarly, one cm long streak was gently made on to the medium using 48h old culture of bacterial isolates just opposite to the pathogenic culture at equidistance under aseptic condition. A control was maintained by inoculating *L. theobromae and C. papayi* alone at one end of the Petri plate. The plates were incubated at room temperature (28±2°C) for 48 h. The radial growth (in mm) of the pathogen was measured after incubation. The effective antagonists were selected based on the inhibition of the growth of pathogen. The per cent inhibition of mycelial growth as calculated according to Vincent [45].

$$\text{Per cent inhibition (I)} = C\text{-}T \,/\, C \times 100$$

Where, C- Radial growth in control

T- Radial growth in treatment

I- Inhibition per cent

Based on the dual culture technique the effective bacterial isolates of PfP_1 and BsP_1 were used for further studies.

Mechanism of action of bacterial antagonists *in vitro*

Preparation of the culture filtrate

The antagonistic bacterial isolates of *P. fluorescens* (PfP_1) and *B. subtilis* (BsP_1) were inoculated into Erlenmeyer flasks containing 50 ml of sterile King's B broth and Nutrient broth, respectively. Then the flasks were kept on a rotary shaker at 100 rpm for 48 h. After, incubation the cultures were filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Effect of culture filtrates of bacterial antagonists on the growth of *L. theobromae* (LT_1) and *C. papayi* (CP_1)

The culture filtrates of the bacterial antagonists were separately incorporated into sterilized PDA medium at 5, 10 and 15 per cent by adding the calculated quantity of the culture filtrate to the medium by means of a sterile pipette. The amended media were transferred to sterile Petri plates separately @ 15 ml and allowed to solidify. Each plate was inoculated at the centre with seven day's old (eight mm) culture disc of *L. theobromae* and *C. papayi* grown on PDA. Three replications were maintained for each treatment. Sterile water served as control. The diameter of the mycelial growth (in mm) of *L. theobromae* and *C. papayi* was measured when the mycelial growth fully covered the control plates.

Effect of plant oils on the growth of *L. theobromae* (LT_1) and *C. papayi* (CP_1)

Solid bioassay

Fungistatic and fungicidal activities of different plant oils were assessed against *L. theobromae* and *C. papayi* by radial growth assay following poisoned food technique. Plant oils were tested in the concentration range of 0.02-1.0 per cent. To 50 ml of sterilized PDA, different concentrations of plant oils were mixed separately and dispensed into sterile Petri plates. All the plates were gently rotated for even dispersal of oil. A plate with the PDA medium without oil was served as control. Eight mm mycelia disc of the test fungi taken from advancing edge of a seven day-old culture placed on oil-containing PDA and incubated at 28 ±2°C for seven days. Three replications were maintained for each treatment. All the operations were carried out under aseptic conditions.

Liquid bioassay

The oils, which showed effectiveness in solid assay at the lowest concentrations, were further tested in liquid bioassay for confirmation. Conical flasks (100 ml) containing 50 ml of PDB were autoclaved separately for 20 min. at 21°C. The test oils at 0.02-1.0 per cent (v/v) were prepared and added to the flasks separately. Tween 80 at 0.01 per cent was used as a surfactant to disperse the oil. The contents were thoroughly mixed by placing the flasks on a shaker at 28 ±2°C. Anthracnose pathogen(s) inoculum (8 mm mycelia disc) was aseptically introduced into flasks. After seven days, the mycelium was recovered on pre-weighed filter paper (What man No.1), washed three times with sterile distilled water, and placed in a hot air oven at 60°C for 36 h. and weighed. The per cent inhibition was determined using the method described by Baratta *et al.* [7].

$$\text{Per cent inhibition (MIC)} = \frac{(C\text{-}I) - (T\text{-}I) \times 100}{C\text{-}I}$$

Where,

T = mean weight of mycelium in the treatments

C = mean weight of mycelium in control

I = mean weight of initial inoculums

MIC values of oils for each treatment were recorded. Where the growth was completely inhibited by oils, fungal discs were transferred to fresh PDA plates, incubated for seven days and later soaked in one per cent solution of TTC for 30 min.

Evaluation of selected plant oils and bacterial antagonists against papaya anthracnose (with pathogen inoculation)

To evaluate the efficacy of plant oils and bacterial antagonists against anthracnose disease of papaya under *in vitro* condition. Papaya fruits (var. Red lady) were collected from the farmer's field and the experiment was conducted. Papaya fruits were washed thoroughly with tap water, air dried and surface sterilized with sodium hypochlorite (2%). Then fruits were injured (pin-prick method) with a sterile needle and conidia scrapped out from the seven days old culture of *L. theobromae* (LT_1) and *C. papayi* (CP_1) with a glass rod and suspended in sterile distilled water. Then the concentration was adjusted to 10^5 conidia/ml by using Haemocytometer and this suspension was sprayed over each fruit and the fruits were left a side for 12 h. Pin pricked fruits inoculated with only pure sterile distilled water were used as control. Subsequently, fruits were dipped with different treatments *viz.*, *P. fluorescens* (PfP_1) @0.2 per cent, *B. subtilis* (BsP1) @0.2 per cent, consortium of *P. fluorescens* (PfP_1) plus *B. subtilis* (BsP_1) each @ 0.2 per cent, *Eucalyptus* oil @0.05 per cent, lemongrass oil @ 0.06 per cent and Thiabendazole @ 1000 ppm was served as chemical control. This experiment was conducted at room temperature and the average temperature reported at the time of experimentation was around 28°C and incubated for 10 days.

Treatment schedule

T_1	:	*P. fluorescens* (Pfp_1) @0.2 %
T_2	:	*B. subtilis* (BsP_1) @0.2 %
T_3	:	$T_1 + T_2$
T_4	:	*Eucalyptus* oil @0.05%
T_5	:	Lemongrass oil @0.06 %
T_6	:	Thiabendazole @ 1000 ppm
T_7	:	Inoculated control
T_8	:	Healthy control

Statistical analysis

The data on the effect of the treatments on the growth of pathogen and disease incidence were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT). The data on disease incidence was arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1eveloped by the Biometrics Unit of the International Rice Research Institute, The Philippines.

RESULTS AND DISCUSSION

Isolation of pathogen associated with papaya anthracnose in the market survey

Isolation of pathogen from infected papaya fruits in different varieties revealed that the pathogen *L. theobromae* and *C. papayi* were isolated in variety Red lady with respect to the fruits collected from Chidambaram and Peelamedu market. The pathogen *L. theobromae* was alone isolated in Co-1, Co-2 and Co-3 varieties. Hence, the variety Red lady was taken for subsequent experiments. The pathogen *L. theobromae* was designated as LT and *C. papayi* was designated as CP.

Biochemical tests for the identification of antagonistic bacterial isolates

The results of the gram reaction and biochemical tests performed for the identification of native bacterial isolates showed that all the isolates produced similar results with regard to gram staining, KOH test, nitrate reduction and starch hydrolysis growth test (PfP_1, PfP_2, PfP_3, PfP_4, PfP_5, PfP_6, PfP_7) showed negative whereas fluorescent pigment test showed positive results. Hence they are identified as *P. fluorescens*. The isolates Bsp_1 and Bsp_2 showed positive in gram staining and KOH test, growth test, whereas fluorescent pigment showed negative results. Hence, they are identified as *B. subtilis*. Generally all the isolates showed positive results in motility test and gelatin liquefaction test. This is in agreement with the findings of Joseph *et al.* [23] in their studies for characterization of plant growth promoting rhizobacteria associated with chickpea reported that out of 150 isolates, 40 belonged to genus *Bacillus,* 35 to *Pseudomonas,* 40 to *Azotobacter* and 35 to *Rhizobium.*. Of these, 25 were representatives of genus *Bacillus* and 25 of *Pseudomonas*. The above results lend support to the present findings

***In vitro* inhibition of mycelial growth of *L. theobromae* (LT_1) and *C. papayi* (CP_1) by selected bacterial isolate.**

L. theobromae

All the six bacterial isolates showed varying degree of antagonism to *L. theobromae.* and *C. papayi.* Among these, isolate PfP_1 recorded the maximum inhibition zone of 11.00 mm with a minimum of 28.33 mm mycelial growth of *L. theobromae*, accounting 67.68 per cent reduction of the mycelial growth over control (Table 1). This was followed by BsP_2 (10.33 mm; 29.66 mm; 66.16%).

Among the bacterial isolate tested, isolate PfP_1 recorded the maximum inhibition zone of 11.67 mm with a minimum of 21.33 mm mycelial growth of *C. papayi*, accounting 75.94 per cent reduction of the mycelilal growth over control (Table 2). This was followed by isolate BsP_1. Similarly, Kedar *et al.* [25] reported that five known bioagents tested by dual culture technique showed that *P. fluorescens* and *B. subtilis* were strong antagonism to *L. theobromae* (banana fruit rot) by inhibiting the mycelial growth up to 75.83 and 70.50 per cent, respectively. Zaitoun *et al.* [48] stated that *B. theobromae* was significantly inhibited by using *P. fluorescens* and *B. subtilis*. *B. subtilis* caused the highest suppression in case *B. theobromae* with an inhibition zone of 0.71cm. On the other hand, testing of *P. fluorescens*

significantly inhibited the growth of *B. theobromae* of 0.42 cm. *In vitro* studies revealed that *P. fluorescens* and *B. cereus* showed 47 to 52 per cent mycelial growth inhibition of *C. gloeosporioides* [11]. Recently, Udhayakumar *et al.* [44] reported that among the bacterial isolates tested, isolate BIB2 recorded the maximum inhibition zone of 12.67 mm with a minimum of 21.33 mm mycelia growth of *C. gloeosporioides,* accounting for 76.30 per cent reduction of the mycelial growth over control.

Studies on the effect of culture filtrate of selected bacterial isolates on the mycelial growth of *L. theobromae* and *C. papayi* revealed that the culture filtrates of the isolate PfP_1 completely inhibited the mycelial growth of both the pathogen at 15 per cent concentration followed by the isolate BsP_1 and PfP_7 (Table 1 and2). Similarly, El-Banna *et al.* [13] reported that cell free culture filtrate of *P. putida* is most effective in reducing the mycelial growth of *L. theobromae* causing die-back of grapevine. Recently, Udhayakumar *et al.* [44] reported that among the isolates, the culture filtrate of the isolate BIB2 completely inhibited the mycelial growth of *C. gloeosporioides* at 15 per cent concentration under *in vitro* conditions followed by the isolate BIB4, which recorded 94.82 per cent inhibition.

Production of siderophores and chitinases are two factors that may be involved in biological control activity. Indeed, it is known that chitinolytic activity and siderophore production are correlated with antifungal activity [24, 36]. In addition, *P. fluorescens* is capable of solubilizing phosphate and producing IAA, characteristics that may enhance its potential use as an effective biological control agent to contribute to the control of *L. theobromae*. Mahesh [28] suggested that fungal growth is mainly inhibited by HCN production and siderophore production. All these earlier results lend support to the present findings.

In addition to this, *Pseudomonas* spp. are well known for production of broad spectrum antibiotics such as phenazine by *Pseudomonas* sp. B-109 in tomato [8]; 2, 4-diacetylphloroglucinol (2,4-DAPG) by *Pseudomonas* sp. 28r/-96 in wheat [37]; Pyoluteorin by *P. fluorescens* CHAO in tobacco [26]; Pyrrolnitrin by *P. fluorescens* BL 915 in cotton [27]; Viscosinamide by *P. fluorescens* D1254 in sugarbeet [32] and Zwittermycin A by *B. cereus* UW in alfalfa [41] which proved to be a major mechanism involved in their biocontrol activity. Moreover, Baker *et al.* [6] reported that ability of some *Pseudomonas* spp. in producing siderophores, antibiotics and lipopolysaccharides as important factors in improving the effectiveness of the antagonist. All the above reports were in line with the present observations.

In vitro inhibition of plant oils against *L. theobromae* (LT_1) and *C. papayi* (CP_1)

L. theobromae

Among eight different plant oils tested *viz.,* lemon grass oil, citronella oil, palmarosa, *Eucalyptus* oil, geranium oil, pungam oil, gingelly oil and castor oil were tested against the mycelial growth of *L. theobromae in vitro.* Among these, *Eucalyptus* oil showed minimum inhibitory concentration of 0.05 % and minimum lethal concentration of 0.1% followed by lemon grass oil (Table 3).

C.papayi

Among eight different plant oils tested, *Eucalyptus oil* showed minimum inhibitory concentration at 0.05% and minimum lethal concentration of 0.1 % followed by lemon grass oil (Table 4). Activity of oil was found to be conc. dependant while microorganisms showed differential sensitivity to the different oils.

Sangeetha [38] reported that palmarosa oil, tulsi oil, geranium oil and lemongrass oil showed antifungal activities (*L. theobromae* and *C. musae*) even at the least concentration of 0.04 per cent under *in vitro* condition. Muthukumar and Sanjeevkumar [31] reported that lemongrass oil was found to be more effective and caused complete growth inhibition of *L. theobromae* even at 0.005 per cent concentration. *C. musae*. Sarala (2016) reported that cinnamon oil (0.05 % and 0.1 %) was found to be superior in reducing the mycelia growth of *Gloeosporium papayi* over control [33, 39].

The most abundant antifungal compounds identified in the *Eucalyptus* oil were 1, 8-cineole (16.2%), α-pinene (15.6%), α-phellandrene (10.0%), and p-cymene (8.1%) (Gakuubi *et al.,* 2017). Lemongrass oil is characterized for monoterpenes compounds, and citral is the major component followed by geranial (trans-citral, citral A) and neral (cis-citral, citral B). In addition to citral, the lemongrass oil consists of small quantities of geraniol, geranyl acetate and monoterpene olefins, such as myrcene [40]. Their mechanism of action appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage

and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration [4]. Because of high volatility and lipophilicity of the essential oils, they are readily attached to penetrate into the cell membrane to exert their biological effect [22].

Effect of selected plant oils and bacterial antagonist against anthracnose of papaya *in vitro*

The effect of selected plant oils and bio control agents with fungicide check (Thiabendazole @ 1000 ppm) were tested against the anthracnose disease of papaya after inoculating the spore suspension (each 10^5 conidia / ml) of *L. theobromae* and *C. papayi.* The maximum disease incidence was observed in the control (76.33 per cent disease index) treatment on the seventh day after incubation. Among these treatments, the treatment T_4-dipping the fruits in *Eucalyptus* oil (0.05 %) was found to completely reduces the disease development at 8DAI (days after inoculation) by recording 91.27 per cent disease reduction over control (Table 5). It was followed by T_5-lemongrass oil (0.06%) by recording 89.51 per cent of disease reduction over control at eight days after inoculation and this was followed by consortium of *P. fluorescens* (PfP_1) + *B. subtilis* (BsP_1) was found to be superior by recording 86.03 per cent of disease reduction over control.

Similarly, the treatment of banana fruit var. Robusta (Cavendish-AAA) with oils of *O. sanctum*, *C. citrateu*, *C. nardus*, and *C. martini* not only reduced the crown rot severity (*C. musae* and *L. theobromae*) significantly, but also increased the shelf life of banana fruits [38]. Ali *et al.* [1] stated that 'Sekaki' papaya were exposed to lemongrass oil fumigation (0, 7, 14, 28 µl 1^{-1}) for 18 h and at room temperature for 9 days. Lemongrass oil vapour at a concentration of 28 µl1^{-1} was most effective against anthracnose of artificially inoculated papaya fruit, while quality parameters of papaya were not significantly altered. Coatings formed by synergistic chitoson (5 mg/ml) and *Cymbopogan citrates* oil (0.15, 0.3 or 0.6 µl/ ml) combination decreased anthracnose lesion development in guava, mango and papaya inoculated with *Colletotrichum* species during storage [33]. Contrarily, Sarala [39] reported that dipping the fruits in cinnamon oil (0.1 %) was found to completely inhibit the disease development art 8 DAI by recording 95.44 per cent disease reduction over control in papaya. These earlier reports are in line and lend support to the results observed in the present study.

Table 1. *In vitro* inhibition of mycelial growth of *L. theobromae* (LT_1) by selected bacterial isolates

Isolates	Dual culture technique			Poisoned food technique					
				Mycelial growth (mm) / conc. of culture filtrate					
	Mycelial growth (mm)	Per cent inhibition over control	Inhibition on zone (mm)	5%	Per cent inhibition over control	10%	inhibition over	15%	inhibition over
PfP_1	28.33[a]	67.68	11.00[a]	20.66[a]	76.25	10.46[a]	88.15	0.00[a]	100
BsP_2	29.66[b]	66.16	10.33[b]	24.32[b]	72.04	11.33[b]	87.17	4.33[b]	95.07
PfP_4	34.33[d]	60.83	8.33[d]	36.60[d]	57.93	29.46[d]	66.64	12.66[d]	85.61
PfP_6	36.66[e]	58.17	8.00[e]	46.66[e]	46.36	36.25[e]	58.96	14.00[e]	84.09
PfP_7	32.00[c]	63.49	9.00[c]	32.36[c]	62.80	18.66[c]	78.87	8.00[c]	90.90
Control	87.66	-	-	87.00	-	88.33	-	88.00	-

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

Table 2. *In vitro* inhibition of mycelial growth of *C. papayi* (CP_1) by selected bacterial isolates

Isolates	Dual culture technique			Poisoned food technique					
				Mycelial growth (mm) / conc. of culture filtrate					
	Mycelial growth (mm)	Per cent inhibition over control	Inhibition on zone (mm)	5%	Per cent inhibition over control	10%	Per cent inhibition over control	15%	Per cent inhibition over control
PfP_1	21.33[a]	75.94	11.67[a]	19.00[a]	78.40	11.33[a]	87.17	0.00[a]	100
BsP_1	25.33[b]	71.43	9.67[b]	28.00[b]	68.18	18.66[b]	78.87	7.66[b]	91.19
PfP_4	33.00[d]	62.77	7.67[d]	38.66[d]	56.06	27.00[d]	69.43	18.66[d]	78.55
PfP_6	55.00[e]	37.96	5.33[e]	48.00[e]	45.45	31.33[e]	64.53	20.00[e]	77.01
PfP_7	28.67[c]	67.66	8.33[c]	33.36[c]	62.09	24.33[c]	72.45	12.00[c]	86.20
Control	88.66	-	-	88.00	-	88.33	-	87.00	-

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p=0.05)

Table 3. *In vitro* inhibition of plant oils against *L. theobromae* (LT1)

S. No	Plant oils	Minimum inhibitory concentration (MLC)	Minimum lethal concentrations (MLC)
1	Lemon grass oil	0.06	0.08
2	Citronella oil	0.1	0.1
3	Palmara oil	0.06	0.06
4	*Eucalyptus* oil	0.05	0.1
5	Geranium oil	0.060	0.08
6	Pungam oil	No control	No control
7	Gingelly oil	No control	No control
8	Castor oil	No control	No control

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p= 0.05)

Table 4. *In vitro* inhibition of plant oils against *C. Papayi* (CP_1)

S. No	Plant oil	Minimum inhibitory concentration (MLC)	Minimum lethal concentrations (MLC)
1	Lemon grass oil	0.06	0.06
2	Citronella oil	0.08	0.1
3	Palmarosa oil	0.06	0.06
4	*Eucalyptus* oil	0.05	1.0
5	Geranium oil	0.06	0.06
6	Pungam oil	No control	No control
7	Gingelly oil	No control	No control
8	Castor oil	No control	No control

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p= 0.05)

Table 5. Effect of selected plant oils and bacterial antagonist against anthracnose of papaya

Treatment No.	Treatments	Per cent disease index (PDI)	Disease reduction over control
T_1	*P. fluorescens* (PfP_1) @ 0.2%	12.00 (20.26)	84.27
T_2	*B. subtilis* (BsP_1) @ 0.2%	14.33 (22.24)	81.22
T_3	*P. fluorescens* (PfP_1) + *B. subtilis* (BsP_1) each @ 0.2%	10.66 (19.05)	86.03
T_4	*Eucalyptus* oil @ 0.05%	6.66 (14.95)	91.27
T_5	Lemon Glass oil @ 0.06%	8.00 (16.42)	89.51
T_6	Thiabendazole (1000 ppm)	0.12 (1.98)	99.84
T_7	Inoculated Control	76.33 (60.88)	-
T_8	Healthy Control	10.66 (19.05)	86.03

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p= 0.05)

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