

Proceedings

Fungicide free management of papaya Anthracnose (*Colletotrichum gloeosporioides* Penz.) disease using combined bio-rationales and bee wax in Organic Agriculture †

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Abstract: Papaya (*Carica papaya* L.) is an economical important orchard crop mainly cultivated in tropical and sub-tropical countries. Due its excellent medicinal value, papaw is recommended to consume daily by medical professionals as fresh fruit. Papaya production is highly being hampered by Papaya Anthracnose disease caused by *Colletotrichum gloeosporioides* which is inflicting major economic loss of around 40-100% while cultivation in field, transportation and storage in organic agriculture. An investigation was planned to assess the antifungal capacity of the medicinal plants *Spinacia oleracea*, *Limonia acidissima*, *Allium sativum*, *Achyranthes aspera*, *Calotropis gigantea*, *Ocimum basilicum*, *Mukia scabrella*, *Ficus racemosa*, *Azadiracta indica*, *Ocimum tenuiflorum*, *Lantana camara* and *Ocimum cinnamomth*, combined with bee wax coating against papaya anthracnose disease. Fifty percent concentrations of botanical were extracted from dried leaves using methanol based solvent extraction method. Two sets of partially ripened non-infected marketable papaya fruits were collected and treated with 50% concentration of botanical extracts and allowed to dry. One set was coated with melted wax by spraying under cool conditions using power sprayer along with non-treated control. These experimental setups were arranged in a complete randomized design with five replicates. Four hours after wax coating, both sets were inoculated with spores of *C. gloeosporioides*. Data on disease incidence, disease severity (0-5 scale), number of days for disease free period, pH, TSS were measured in both sets and ANOVA was performed using SAS software. Duncan's Multiple Ranges Test (DMRT) was used to determine the least significant differences among the treatments at $P < 0.05$. Results show that disease incidence and severity in *Ocimum basilicum* + bee wax treated fruits was 0% and 5%, respectively and significant at $P < 0.05$ until 10th day of post-inoculation, thereafter, disease incidence and severity were increased slowly to 15% at 14th day of post-inoculation, but in other treatments and control, disease incidence and severity were varied from 60-80% and 100%, respectively, from 5th day of post-inoculation. Moreover, bee wax-coated papaya fruits showed significantly higher preserved days of maximum 17.047 ± 3.86 . Weight loss percentage, pH and TSS were not significantly on par among wax-coated treatments but were significant when compared with wax-free treatments. This study concludes that the combined application *Ocimum basilicum* + bee wax is a promising alternative to nasty fungicides.

Keywords: Papaya, Bio-rationals, wax, *Ocimum basilicum*, *Ocimum tenuiflorum*

1. Introduction

Tropical fruit crop papaya (*Carica papaya*) belongs to the family Caricaceae and native to the Northwest of South America. It is cultivated as a 3rd rank world famous largest orchard crop [8]. Papaya is commonly known as a common man's fruit because of its enriched source for calcium, vitamins (A, B1, B2, and C), fibers, and plenty of anti-oxidants [1].

Commercial as well as small scale papaya growers in Sri Lanka are being threatened frequently by a globally recognized major post-harvest biotic stress called “Papaya Anthracnose Disease” (PAD). PAD is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc which belongs to a large genus *Colletotrichum* [3,11]. Papaya anthracnose is a catastrophic disease causing pre and post-harvest losses up to 40-100%. Partially and fully ripened papaya fruits are vulnerable to Anthracnose disease [15]. Therefore, proper diagnosis and management of anthracnose disease are important to decline the papaya yield loss in the field as well as in the market pipelines [17].

The control of anthracnose diseases of papaya is a crucial problem specially since the latent onset of anthracnose disease symptoms are often devastating and lead to significant postharvest losses and restricts its export to other countries, even, cold storage conditions. Earlier, fungicides were only useful when *C. gloeosporioides* attacks on young trees, leaves and immature fruits. Application of fungicides to mature and ripened fruits are not advisable because of the emergence of fungicide-resistant strains [13,6], and fungicides are hazards to human and wild animal’s health. The recent agriculture policy of Sri Lanka emphasizes to finding alternatives to fungicides for the management of various postharvest diseases to build up the “Toxin-Free Nation”. The boom and bust cycle of Anthracnose incidence on the highly perishable nature of the papaya fruits alarmed to invent quick eco-friendly technologies to safeguard the organic papaya industry.

Isolation and application of novel plant extracts, application of Sodium bicarbonate associated with the yeast *Candida oleophila*, protein and polysaccharides were found to exhibit the antifungal activity against *C. gloeosporioides* in the stored and shipped papaya fruits [13]. Siqueira et al., 2012 reported that the application of castor oil on papaya fruits can control the Anthracnose incidence effectively.

Reported research pieces of evidences opened an idea to investigate the crude extract of different locally available medicinal herbs previously reported to have antimicrobial compounds, bee-wax coating, and combined effect of both against papaya anthracnose disease especially in organic cultivation.

2. Experiments

2.1 Crude extraction of botanicals

Naturally growing abundantly available medicinal plants (*Spinacia oleracea*, *Limonia acidissima*, *Allium sativum*, *Achyranthes aspera*, *Calotropis gigantea*, *Ocimum basilicum*, *Mukia scabrella*, *Ficus racemosa*, *Azadiracta indica*, *Ocimum tenuiflorum*, *Lantana camara* and *Ocimum cinnamomth*) were randomly selected based on antifungal properties present in [1,2]. Selected plant leaves were surface sterilized by 1 % NaOCl and washed with running distilled water to remove the impurities completely. Surface sterilized leaves were allowed for shade dry and finely ground using motor and pestle under aseptic conditions. 14 g of each finely ground leaf powder was mixed with 250 mL analytical grade ethanol (95%) and volatile components were extracted using Soxhlet apparatus. The extract was centrifuged at 4000 rpm for 10 minutes. The supernatant was diluted to 20% concentrations by adding double distill water and sterilized in an autoclave at standard conditions (121 °C, 15 min, 15 psi) [20].

2.2 In-Vivo assay of bio-rationales against *Colletotrichum gloeosporioides*

As per the Bron et. al 2006 [7] partially ripened (stage 3: 26-50% of skin yellowing) disease-free ‘red leady’ papaya fruits were collected from a certified organic farm and surface sterilized using NaOCl (1%). Following treatments of plant leaves extracts (**Table 1**) were prepared with three replicates based on the preliminary experiments. Plant extracts were applied using a hand sprayer on the cleaned fruits topically under aseptic conditions at the rate of 5-7 mL/fruit (Both set-1&2)

allowed for three hours drying. For the wax coating, plant extract applied fruits (set-2) were sprayed uniformly with the melted wax under a cool atmosphere at the same rate as plant extracts applied. Each treatment was replicated three times and arranged in Complete Randomized Design.

Four hours after wax coating, both sets were inoculated with spores of *C. gloeosporioides* ($\times 10^8$ spores/mL). Data on disease incidence, disease severity (0-5 scale) [22], number of days for disease-free period, pH, TSS were measured in both sets.

Table 1: list of treatments

Treatment Number	Treatment-Set 1	Treatment-Set 2
T1	<i>Ocimum basilicum</i>	<i>Ocimum basilicum</i> +wax
T2	<i>Ocimum tenuiflorum</i>	<i>Ocimum tenuiflorum</i> +wax
T3	<i>Allium sativum</i>	<i>Allium sativum</i> +wax
T4	<i>Azadiracta indica</i>	<i>Azadiracta indica</i> +wax
T5	<i>Lantana camara</i>	<i>Lantana camara</i> +wax
T6	<i>Ocimum cinnamon</i>	<i>Ocimum cinnamon</i> +wax
T7	Control (surface sterilized organic fruit)	Control + wax

2.2 Data analysis

Data collected in the whole study was analyzed by Microsoft Excel 2013 and SAS software (9.1 version). Duncan's Multiple Ranges Test (DMRT) was used to determine the least significant differences among the treatments at $P > 0.05$.

3. Results

3.1. In-Vivo evaluation of disease incidence and disease severity.

Plant extracts were prepared and tested on papaya fruits with and without edible bee wax coating. Disease incidence and severity were observed every day (Figure 1 and 2). The highest incidence and severity were recorded in the absence of a wax coat. Among the wax-free treatments, *Ocimum cinnamon*, control, *Allium sativum* showed 100% incidence within 5 days but others showed an incidence of 60 % except *Ocimum basilicum* which was free from disease (Incidence 0 %). At 10th days after inoculation, all the botanicals treated fruits showed the disease incidence of 100 % except for *Ocimum basilicum* and *Ocimum tenuiflorum* which showed 33.34 % and 77.78 % of incidence, respectively.

Among the wax-coated treatments, Anthracnose disease incidence was comparably very low. Disease incidence was 0 % at 5th day of inoculation in all the treatments whereas at 10th day of inoculation disease incidence was varied from 0-15 % (Figure 1). Control set exhibited a papaya anthracnose disease severity score of 5 (80-100 %) in 12th days of inoculation whereas the *Ocimum basilicum* showed a lower value of disease severity score of 0 (0 %) (Figure 2). Other treatments of wax coated fruits were exhibited a level of disease severity score from 1-4 but the level of severity less than without wax applied fruits.

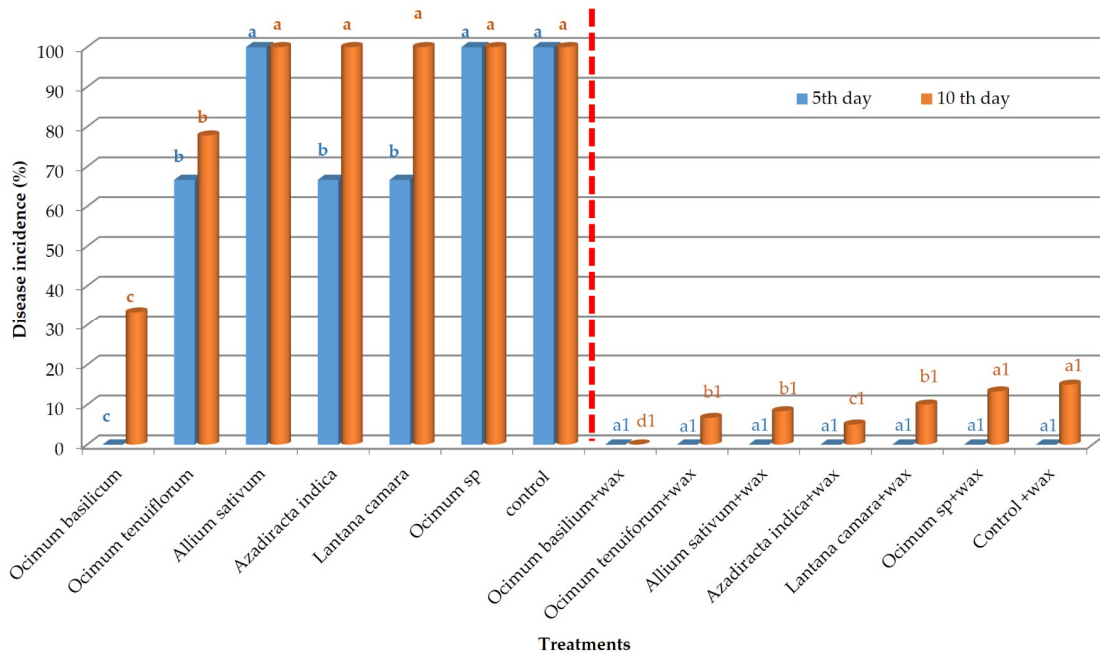


Figure 1. Comparison of percentage of disease incidence in papaya fruits after preservation using bio rationales alone and in combination of wax.

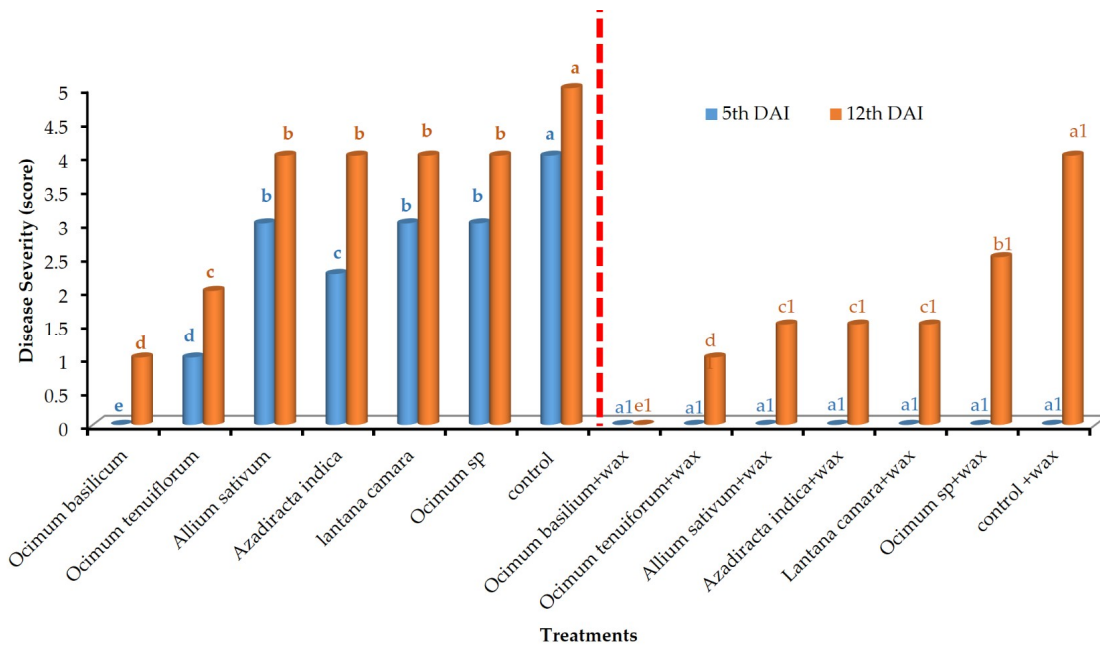


Figure 2. Comparison of disease severity in papaya fruits after preservation using bio rationales alone and in combination of wax.

3.2. In-Vivo evaluation of physiochemical (pH, TSS, and weight loss) changes of papaya fruits with different coatings.

Among the wax free treatments, pH was varied from 4.83 ± 0.01 - 5.32 ± 0.35 on 5th day after treatment to 5.51 ± 0.11 - 5.92 ± 0.02 on 12th day after application and significant among the treatments at $P < 0.05$. But pH change was not significantly on par between *O. basilicum* (5.32 ± 0.35), (5.42 ± 0.059), and *O. tenuiflorum* (5.28 ± 0.04), (5.50 ± 0.17) at 5th and 12th day of application, respectively (A1). In wax-coated experiment, huge pH variations was observed between the 5th and 12th day of application. In wax-free treatments TSS variation was 9.10 ± 0.57 - 11.80 ± 0.15 and significant at $P < 0.05$ on 5th day.

Whereas in wax coated experiments, the TSS variation was mild on 5th day. In both wax-free and wax-coated experiments TSS revealed increasing trend from 5th day to 12th day. But the increasing trend was not significant in wax coated *O. basilicum* treated fruits on 5th day and 12th day.

A highly significant percentage of weight loss was observed among the wax-free treatments between the 5th and 12th days of application. but this variation was less and non-significant among the wax-coated treatments at 5th day, 12th day of application. The wax-coated fruits showed a lower weight loss percentage (WLP) (0.8334 ± 0.5460 %), (2.749 ± 1.26 %) on the 5th day, 12th day, respectively.

4. Discussion

Papaya anthracnose can manage through different means such as physical, biological, chemical and botanicals but fungicides work better than others [17,23]. Development of natural fungicides from plant extracts would possibly decrease the negative impact of synthetic agents, such as high cost, residues in the food, resistance development in fungal pathogens and environment pollution.

In the present investigation, nine plant extracts were evaluated under *In-vitro* condition against *C. gloeosporioides* to know the fungi toxic nature of their extracts. A significant decrease in disease incidence and severity was observed when treating leaf extracts of *O. basilicum* and *O. tenuiflorum*. *Ocimum basilicum* with wax coatings minimize the disease incidence and severity- 0 percentage level at 12th day after preservation.

The *Ocimum* species are consisting of highly volatile metabolites that are toxic to microbes especially bacteria and fungi [12]. Application of essential oils or their volatile compounds at the postharvest stage has been shown to control postharvest diseases in different fruits. Basil contains 57.42% aromatic oxygenated monoterpenes that could be responsible for higher antifungal activity [12]. Antifungal activity of thyme oil is well documented and proven to inhibit the fungal growth of *C. gloeosporioides* in-vitro or in-vivo in avocado cultivars [22]. Early phases of growth of *C. gloeosporioides* is very minimal in the presence of natural antimicrobial compounds produced on the surface of many unripe tropical fruits. As soon as the tropical fruit reaches its ripe stage, the natural antimicrobial compounds concentration tends to decrease, therefore, *C. gloeosporioides* attack easily. Bee wax act as a coating to prevent the attachment of and penetration of fungal spores and germ tube. Moreover, edible wax covers the natural opening like lenticels and stoma on the fruit coat, therefore, no way to fungal penetration and prevent the loss of natural antimicrobial compounds. Moreover, natural wax is present in the papaya fruit coat and hot treatment melt the wax and block the natural opening, improve the physical appearance and keeping quality of the fruits. Hazarika *et al.* (2017) also reported that parafilm wax coating prevents microbial contact, loss of weight, increases the firmness of the treated fruits. Wax, plays a role in blocking moisture and oxygen that can accelerate food spoilage but if treated properly it is the cheapest source to preventing postharvest disease incidence [5].

The results obtained from these experiments revealed that the wax coating prolonged the fruit's shelf life by delaying the ripening and preventing infection of *C. gloeosporioides* and other microbes. Moreover, the total soluble solids, pH, weight loss percentage in wax coated fruits were lesser than the uncoated fruits. Wax-coatings slowed the changes in pH, effectively by delaying fruit senescence [20]. Delaying senescence means the slow process of conversion of carbohydrates into free sugars (TSS). Wax coated fruits retarded TSS development because the gel decreases the respiration and eventually catabolism of sugars [15]. Slow down the rate of weight loss by preventing water losses through evaporation. Mukherjee *et al.*, 2011 reported that the rate of weight loss is depending on the water gradient between the surrounding atmosphere. The paraffin wax emulsion served as a physical barrier around the fruit which partially closes the stomatal openings and lenticels thereby reduces the rates of transpiration and respiration. Different organic compounds like wax, milk protein, celluloses, lipids, starch, zein, and aliquate [9], Chitosan, Sodium carboxymethyl cellulose [4] mainly been used as edible coatings to prevent weight loss in fruits. These coatings not only minimize the water loss but also maintain the standard colour, taste, aroma, and flavours and retained acid [18].

5. Conclusions

Present investigation identified *O. basilicum* and *O. tenuiflorum* as are best bio-rationals to minimize the Anthracnose disease incidence in field and storage. Combined application of these bio-rationales with bee-wax further minimizes the disease incidence and disease severity by 40-60 %, respectively while maintaining the physiochemical properties of papaya fruits.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix

Table A1: Comparison of pH, TSS and WLP of papaya fruits after preservation using bio rationales alone and in combination of wax

Botanicals	pH after preservation		TSS after preservation		Weight loss percentage after preservation (WLP)	
	5th day	12th day	5th day	12th day	5th day	12th day
<i>Ocimum basilicum</i>	5.32±0.35 ^{Aa}	5.42±0.059 ^{Adc}	10.10±0.57 ^{Bbc}	12.44±0.57 ^{Acb}	9.128±1.9 ^{Bba}	23.055±5.87 ^{Abac}
<i>Ocimum tenuiflorum</i>	5.28±0.04 ^{Aa}	5.50±0.17 ^{Ac}	9.77 ^{Bbc}	11.48±0.170 ^{Afed}	7.283±1.62 ^{Bb}	17.047±3.86 ^{Ac}
<i>Allium sativum</i>	5.11±0.18 ^{Bcd}	5.339±0.29 ^{Abc}	9.10±0.57 ^{Bc}	11.63±0.25 ^{Aced}	8.299±2.99 ^{Bba}	18.797±6.4 ^{Abc}
<i>Azadiracta indica</i>	5.01±0.086 ^{Bcd}	5.55±0.036 ^{Abc}	10.77 ^{Bbac}	12.58±0.15 ^{Ab}	11.538±797 ^{Ba}	27.14±8.64 ^{Aa}
<i>lantana camara</i>	5.26±0.078 ^{Ba}	5.92±0.022 ^{Aa}	9.73±1.75 ^{Bbc}	12.14±0.63 ^{Acbd}	10.215±4.15 ^{Bba}	20.349±5.02 ^{Abac}
<i>Ocimum cinnamon</i>	4.83±0.01 ^{Bfed}	5.23±0.173 ^{Ad}	11.33±2.21 ^{Bba}	13.37±1.22 ^{Aa}	11.472±1.67 ^{Ba}	24.739±4.017 ^{Aba}
<i>control</i>	4.88±0.088 ^{Bced}	5.51±0.11 ^{Ac}	11.80±0.15 ^{Ba}	13.67±0.1 ^{Aa}	7.004±.492 ^{Bb}	16.764±.322 ^{Ac}
<i>Ocimum basilium +wax</i>	4.822±0.002 ^{Bfed}	5.77±0.133 ^{Aba}	10.40±1.09 ^{Bbac}	10.78±0.005 ^{Afeg}	1.6367±0.825 ^{Bc}	4.777±2.98 ^{Ad}
<i>Ocimum tenuiforum +wax</i>	4.97±0.061 ^{Bced}	5.78±0.026 ^{Aba}	9.59±0.23 ^{Bc}	10.77±0.05 ^{Afeg}	0.6927±0.226 ^{Bc}	1.58±0.52 ^{Ad}
<i>Allium sativum +wax</i>	4.53±0.056 ^{Bg}	5.79±0.26 ^{Aba}	10.103±0.65 ^{Bbc}	11.51±0.22 ^{Afed}	1.1753±0.415 ^{Bc}	2.86±1.6028 ^{Ad}
<i>Azadiracta indica +wax</i>	5.02±0.009 ^{Bcd}	5.84±0.0569 ^{Aa}	9.59±0.24 ^{Bc}	11.85±0.085 ^{Acbd}	0.3507±0.044 ^{Bc}	1.657±0.6411 ^{Ad}
<i>lantana camara +wax</i>	4.71±0.076 ^{Bf}	5.44±0.0152 ^{Adc}	9.63±0.10 ^{Bc}	10.61±0.33 ^{Acbd}	0.6827±0.33 ^{Bc}	2.416±0.974 ^{Ad}
<i>Ocimum cinnamon +wax</i>	4.80±0.072 ^{Bfed}	5.40±0.168 ^{Adc}	9.51±0.42 ^{Bc}	10.53±0.35 ^{Ag}	1.7353±1.20 ^{Bc}	4.118±1.821 ^{Ad}
<i>control +wax</i>	4.36±0.02 ^{Bh}	5.77±0.55 ^{Aba}	10.29±0.25 ^{Bc}	11.55±0.69 ^{Afed}	0.0642±0.270 ^{Bc}	1.618±.0367 ^{Ad}

All the values are from mean of three replicates. Values having same letter in a column are not significantly different according to the significant mean separation at 0.05 α and 95% confidence interval, capital letters are indicating column viz means separation, small letters indicating row viz mean separation

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