biology and life sciences forum

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Proceeding Paper

Fungicide-Free Management of Papaya Anthracnose (*Colletotrichum gloeosporioides* Penz.) Disease Using Combined Bio-Rationales and Bee Wax in Organic Agriculture †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2021; Available online: https://iecps2020.sciforum.net/.

Abstract: Papaya (*Carica papaya* L.) is an economically important orchard crop, mainly cultivated in tropical and sub-tropical countries. Due its excellent medicinal value, papaw is recommended for daily consumption by medical professionals as fresh fruit. Papaya production is being hampered by papaya Anthracnose disease, caused by *Colletotrichum gloeosporioides*, which is inflicting major economic losses of around 40–100% during field cultivation, 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 racemose*, *Azadiracta indica*, *Ocimum tenuiflorum*, *Lantana camara* and *Ocimum cinnamon* combined with bee wax coating against papaya anthracnose disease. Fifty-percent concentrations of botanical were extracted from dried leaves using a 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 a power sprayer, along with a 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, and 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 $p < 0.05$. The results show that disease incidence and severity in *O. basilicum* + bee wax treated fruits was 0% and 5%, respectively, and significant at $p < 0.05$ until the 10th day post-inoculation; thereafter, disease incidence and severity were slowly increased to 15% on the 14th day post-inoculation, but in other treatments and the control, disease incidence and severity varied from 60–80% and 100%, respectively, from the fifth day post-inoculation. Moreover, bee wax-coated papaya fruits showed significantly higher preserved days, to a maximum of 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 *O. basilicum* + bee wax is a promising alternative to nasty fungicides.

Keywords: papaya; bio-rationales; wax; *Ocimum basilicum*; *Ocimum tenuiflorum*

Citation: Srikantharajah, N.; Pakeerathan, K.; Mikunthan, G. Fungicide-Free Management of Papaya Anthracnose (*Colletotrichum gloeosporioides* Penz.) Disease Using Combined Bio-Rationales and Bee Wax in Organic Agriculture. *Biol. Life Sci. Forum* **2021**, *4*, 16. https://doi.org/10.3390/10.3390/IECPS2020-08906

Academic Editor: Yoselin Benitez-Alfonso

Published: 7 December 2020

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 the third ranking world famous largest orchard crop [1]. 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 antioxidants [2].

Commercial as well as small-scale papaya growers in Sri Lanka are frequently being threatened by a globally recognized major post-harvest biotic stress, called "Papaya Anthracnose Disease" (PAD). PAD is caused by *C. gloeosporioides* (Penz.) Penz. & Sacc, which belongs to a large genus, *Colletotrichum* [3,4]. Papaya anthracnose is a catastrophic disease causing pre- and post-harvest losses of up to 40–100%. Partially and fully ripened papaya fruits are vulnerable to Anthracnose disease [5]. 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 [6].

The control of anthracnose papaya diseases is a crucial problem, especially 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 in cold storage conditions. Earlier, fungicides were only useful when *C. gloeosporioides* attacks 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 [7,8], and fungicides are hazards to human and wild animal health. The recent agriculture policy of Sri Lanka emphasizes finding alternatives to fungicides for the management of various post-harvest 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 has made the development of eco-friendly technologies to safeguard the organic papaya industry necessary.

The isolation and application of novel plant extracts, and the 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 [8]. Siqueira et al., in 2012 [9], reported that the application of castor oil on papaya fruits can effectively control the Anthracnose incidence.

Reported research pieces of evidence opened up the idea to investigate the crude extract of different locally available medicinal herbs, previously reported to have antimicrobial compounds, bee-wax coating, and the combined effects 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 cinnamon*) were randomly selected, based on antifungal properties present in [2,10]. Selected plant leaves were surface sterilized by 1% NaOCl and washed with running distilled water to completely remove the impurities. Surface sterilized leaves were allowed for shade dry and finely ground using a mortar and pestle under aseptic conditions. Then, 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 min. 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) [11].

2.2. In Vivo Assay of Bio-Rationales against Colletotrichum Gloeosporioides

As per Bron et al. 2006 [12], 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 the treatments of plant leaves, extracts (Table 1) were prepared with three replicates, based on the preliminary experiments. Plant extracts were topically applied using a hand sprayer on the cleaned fruits under aseptic conditions at a rate of 5–7 mL/fruit (Both sets 1 and 2) with three hours for drying. For wax coating, plant extract-applied fruits (set 2) were uniformly sprayed with the melted wax under a cool atmosphere at the same rate as the applied plant extracts. Each treatment was replicated three times and arranged in complete randomized design.

Table 1. List of treatments.

Treatment Number	Treatment-Set 1	Treatment-Set 2
T1	*Ocimum basilicum*	*Ocimum basilicum* + wax
T2	*Ocimum tenuiforum*	*Ocimum tenuiforum* + wax
T3	*Allium sativum*	*Allium sativum* + wax
T4	*Azadiracta indica*	*Azadiracta indica* + wax
T5	*Lantana camara*	*Lantana camara* + wax
T6	*Ocimum cinnamon*	*Ocimum cinnamon* + wax
T7	Control	Control + wax

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) [8], number of days for disease-free period, pH, and TSS were measured in both sets.

2.3. Data Analysis

Data collected during the 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 (Figures 1 and 2). The highest incidence and severity were recorded in the absence of a wax coat. Among the wax-free treatments, *O. cinnamon*, control, *A. sativum* showed 100% incidence within 5 days, but others showed an incidence of 60%, excluding *O. basilicum* which was free from disease (Incidence 0%). At 10 days after inoculation, all the botanically treated fruits had a disease incidence of 100%, excluding *O. basilicum* and *O. tenuiforum*, which had 33.34% and 77.78% incidences, respectively.

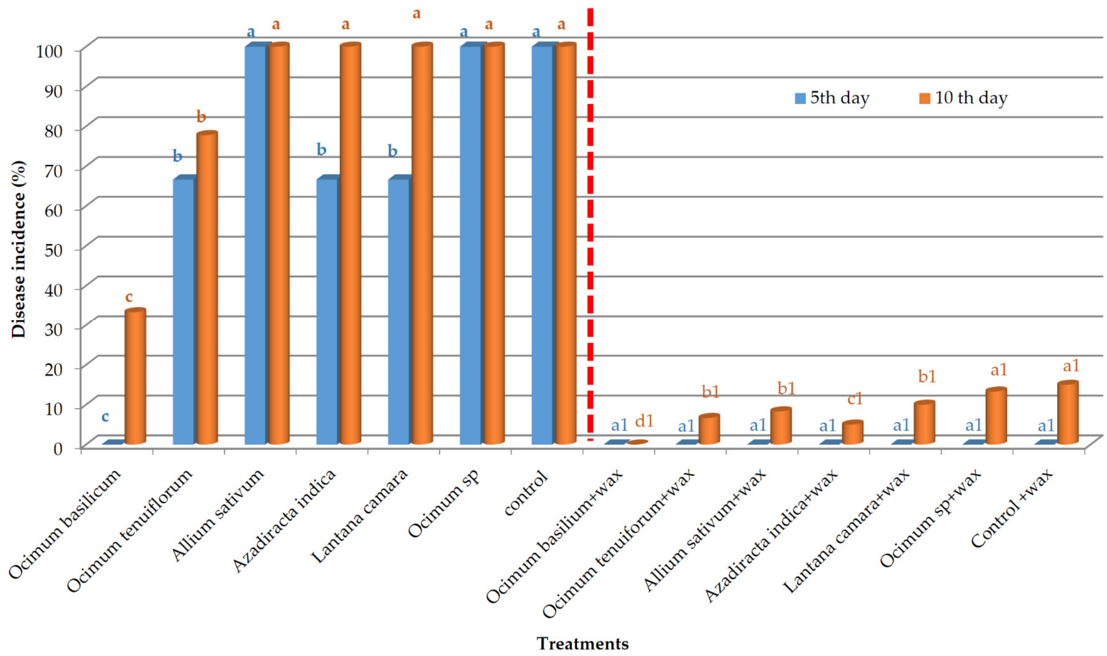

Figure 1. Comparison of percentage of disease incidence in papaya fruits after preservation using bio rationales alone and in combination of wax.

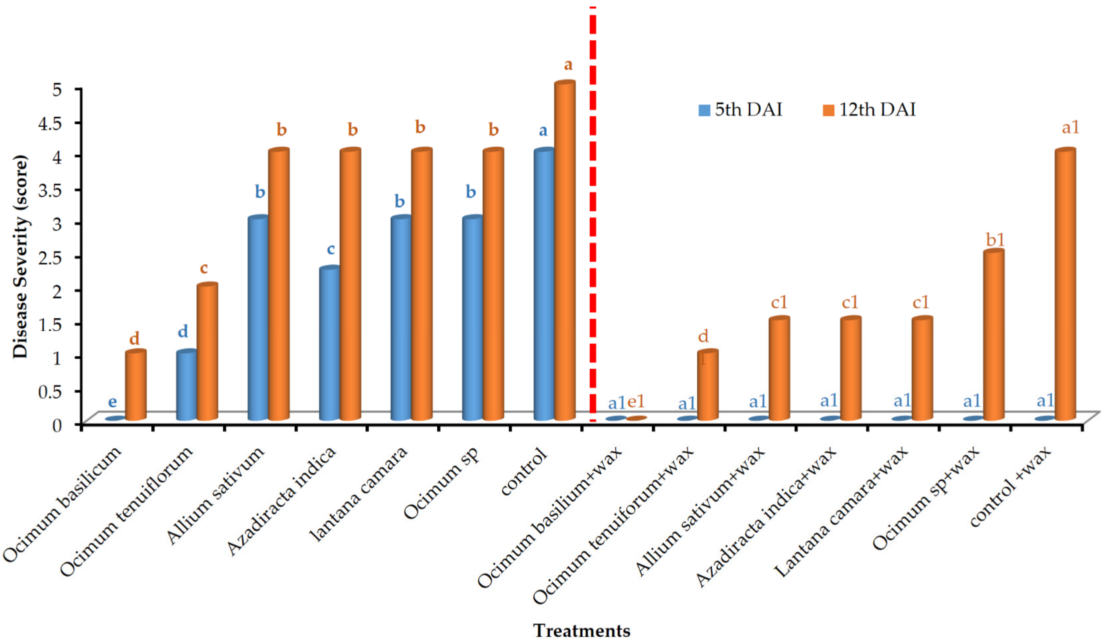

Figure 2. Comparison of disease severity in papaya fruits after preservation using bio rationales alone and in combination of wax.

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

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 varied from 4.83 ± 0.01–5.32 ± 0.35 on the fifth day after treatment to 5.51 ± 0.11–5.92 ± 0.02 on 12th day after application and was significant among the treatments at $p < 0.05$. However, pH change was not significantly on par between *O. basilicum* (5.32 ± 0.35) (5.42 ± 0.059), and *O. tenuiforum* (5.28 ± 0.04) (5.50 ± 0.17) at 5 and 12 days of application, respectively (Table A1). In the wax-coated experiment, huge pH variations were observed between the 5th and 12th days of application. In wax-free treatments, TSS variation was 9.10 ± 0.57–11.80 ± 0.15 and significant at $p < 0.05$ on the fifth day. In wax coated experiments, the TSS variation was mild on the fifth day. In both wax-free and wax-coated experiments, TSS revealed an increasing trend from the 5th day to the 12th day. However, the increasing trend was not significant in wax-coated *O. basilicum* treated fruits on 5th and 12th days.

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

4. Discussion

Papaya anthracnose can manage through different means, such as physical, biological, chemical and botanicals, but fungicides work better than others [6,13]. The 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 better understand 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% on the 12th day after preservation.

The *Ocimum* species consist of highly volatile metabolites that are toxic to microbes, especially bacteria and fungi [14]. The application of essential oils or their volatile compounds at the post-harvest stage has been shown to control post-harvest diseases in different fruits. Basil contains 57.42% aromatic oxygenated monoterpenes that could be responsible for the greater antifungal activity [14]. The 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 [15]. Early phases of growth of *C. gloeosporioides* are 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 compound concentration tends to decrease; therefore, *C. gloeosporioides* attack easily. Bee wax acts as a coating to prevent the attachment and penetration of fungal spores and germ tubes. Moreover, edible wax covers the natural opening in a similar manner to lenticels and stoma on the fruit coat; therefore, fungal penetration is not possible the loss of natural antimicrobial compounds is avoided. Natural wax is present in the papaya fruit coat and hot treatment to melt the wax and block the natural opening improves the physical appearance and maintains the quality of the fruits. Hazarika et al. (2017) [16] also reported that parafilm wax coating prevents microbial contact, loss of weight, and 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 post-harvest disease incidence [17].

The results obtained from these experiments revealed that the wax coating prolonged the fruit's shelf life by delaying the ripening and preventing the infection of *C. gloeosporioides* and other microbes. Moreover, the total soluble solids, pH, and weight loss percentage in wax coated fruits were lesser than the uncoated fruits. Wax coatings slowed the changes in pH, by effectively delaying fruit senescence [11]. 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 [5]. Slow down the rate of weight loss by preventing water losses through evaporation. Mukherjee et al., 2011 [18] reported that the rate of weight loss is dependent on the water gradient of the surrounding atmosphere. The paraffin wax emulsion served as a physical barrier around the fruit, which partially closes the stomatal openings and lenticels, thereby reducing the rates of transpiration and respiration. Different organic compounds such as wax, milk protein, celluloses, lipids, starch, zein, and aliquate [19], Chitosan, Sodium carboxymethyl cellulose [20] are used as edible coatings to prevent weight loss in fruits. These coatings not only minimize the water loss but also maintain the standard color, taste, aroma, and flavors, and retained acid [21].

5. Conclusions

The present investigation identified *O. basilicum* and *O. tenuiflorum* as the best bio-rationales to minimize 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%, while maintaining the physiochemical properties of papaya fruits.

Supplementary Materials: The supplementary file is available online at https://www.mdpi.com/article/10.3390/10.3390/IECPS2020-08906/s1.

Author Contributions: G.M. and K.P. conceived the research idea; N.S. conducted experiments; N.S. and K.P. wrote the manuscript; G.M. and K.P edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: This research was carried out in JICA granted Laboratory at the Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna. The authors thank the Japanese government for donating to the research and laboratory complex through the JICA grant. The authors wish to thank N. Senthilkumaran and K. Uthayakumar, Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, for their technical assistance throughout this research.

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

Appendix A

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
Ocimum basilicum	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
Ocimum tenuiflorum	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
Allium sativum	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
Azadiracta indica	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
lantana camara	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
Ocimumcinnamon	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
control	4.88±0.088 Bced	5.51 ± 0.11 Ac	11.80 ± 0.15 Ba	13.67 ± 0.1 Aa	7.004 ± 0.492 Bb	16.764 ± 0.322 Ac
Ocimum basilium + wax	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
Ocimum tenuiforum + wax	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
Allium sativum + wax	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
Azadiracta indica + wax	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
lantana camara + wax	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
Ocimum cinnamon + wax	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
control + wax	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 ± 0.0367 Ad

All the values are from mean of three replicates. Values with the same letter in a column are not significantly different according to the mean separation at 95% confidence interval; capital letters indicate columns; viz means separation; small letters indicating row; viz mean separation.

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