Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Effect of pre-treatments with natural compounds for controlling anthracnose in papaya variety Red Lady

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ARTICLE INFO

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ABSTRACT

Purpose: Papaya (*Carica papaya* L.) is an economically important fruit crop affected by anthracnose caused by *Colletotrichum gloeosporioides*. The study was carried out to test two essential oils; Citronella oil and Cinnamon oil and two leaf extracts; *Lantana camara* and *Ocimum tenuiflorum* on four occasions of fruit development as pre-treatment assay in the field condition. **Research Method:** Essential oils were prepared as an emulsifier and leaf saps were extracted from dried leaves and both were set to 10% concentration. The experiment was conducted in a two-factor factorial experiment with Randomized Complete Block Design. Five treatments including the control were applied for four blocks representing stages of fruit development. Disease severity (0–5 scale) and disease severity index were calculated and statistically analyzed using ANOVA, MINITAB and Tukey's pairwise analysis. **Findings:** According to the obtained results, four occasions of application of the selected treatment were highly significant with a minimum level of DSI (34.67 ± 4.62). *L. camera* leaf extract was highly effective as a pre-treatment with the least values for disease severity percentages (5.78 ± 0.43), disease severity score (0.3 ± 0.17) and disease severity index (26.67 ± 6.36). **Research limitations:** Flower bud initiation was delayed than the date expected due to the unpredicted heavy rainy condition. **Originality/Value:** The most effective block treatment interaction was shown on three occasions of application of *L. camera* leaf extract. This study facilitated the development of the most promising pre-harvest management strategy to control anthracnose disease which causes by the fungal pathogen *C. gloeosporioides*.

INTRODUCTION

Papaya (*Carica papaya*) is a very common home garden fruit crop and popular cultivating fruit in most areas. The cultivation area is about 6000 hectares (Hamangoda et al., 2018) in Sri Lanka. According to the records of the agriculture department, papaya harvest is about 30-40 fruits per plant per year and started fruit setting within 8-10 months after planting. Also, it is possible to continuously obtain an economical harvest for 2-3 years. A study by Buddhinie et al. (2017) recorded that variety Red lady is the most cultivated variety, considering the peel characters and flesh quality, farmers prefer this variety. The green papaya fruits produce latex rich in endopeptidases, which is important for the defense mechanism of plants against pathogens (de Oliveira & Vitória, 2011). Papin, produce by papaya fruits is used as a meat tenderizer in cooking and in applications in the food industry (Su et al., 2009). The papaya is a rich source of Ca^{2+} and an excellent source of many vitamins including A, B1, B2 and C (de Oliveira & Vitória, 2011).

Even though papaya annual fruit production is around 70-85 thousand tons (Hamangoda et al., 2018) about 46% harvest loss has been recorded due to post-harvest diseases (Vidanapathirana, 2019). Sri Lankan farmers pay minimum attention to controlling latent infection (quiescent infection) of postharvest diseases at the pre-harvest stages of fruit development. Improper postharvest handling and mechanical damages are also other considerable factors affecting postharvest losses.

Anthracnose caused by *Colletotrichum gloesporioides* is one of the most common postharvest diseases and recorded severe disease incidence and disease severity of papaya fruit in worldwide. Rahaman et al. (2008) identified that 90-98% of disease incidence and 25-38% of severity was recorded for anthracnose on both wounded and non-wounded fruits inoculated with a conidial suspension of *C. gloeosporioides*.

The most common practice to control postharvest infections is spraying fungicides. Mancozeb and chlorothalonil are the recommended fungicides to prevent fungal infections in papaya (Siddiqui & Ali, 2014). However, Siddiqui and Ali (2014) recorded, the adverse effects of fungicides such as toxicity to human health, adverse environmental impact, and development of resistance to the fungicide could be observed against inorganic fungicides. Lack of information on environmental impacts and residual effects is also a considerable constraint for proper management of pesticide usage among horticultural crops (Wightwick et al., 2010).

To control post-harvest diseases, the application of essential oil and plant sap could be a promising practice in fruit and it also is a great approach to reduce the risk of fungicide usage in fruit preservation (Abd-alla et al., 2013; Abeywickrama et al., 2009; Gurjar et al., 2012; Hewajulige et al., 2010). The secondary metabolites from plants such as flavonoids, iso-flavonoids, saponins, steroids, tannins, phenols, phenolic acids, coumarins, and pyrones are significantly used to control different fungal pathogens (Gurjar et al., 2012).

Pre-harvest procedures, including as field sanitation, adequate fertilizer and pesticide application, and post-harvest treatments like anthracnose disease infection, are far more successful than post-harvest measures in preventing severe post-harvest loss. Practicing of effective fungicide spray program at the beginning of the fruit set and continuing at appropriate intervals while the plants are producing fruit is an effective approach to control such kind of disease incidence (Pernezny et al., 1999). Fungicide treatment against papaya anthracnose is practiced by applying before rainfall and at the stage of the first appearance of flowers and vastly recommended application of fungicides at two- to four-week intervals to prevent disease incidence under the field condition (Maeda & Nelson, 2014).

The overall aim of this research is the development of an environmental friendly, effective management strategy to control anthracnose disease in the papaya Red Lady variety. Hence this research helps identify the most promising natural extractions to control anthracnose disease in papaya fruit with combining of scheduled pre and postharvest treatment strategies. These research findings directly facilitated to development of the most promising alternative management to control anthracnose disease which causes by the fungal pathogen *C. gloeosporioides*. This will be beneficial for the farmers and high-level companies who are involved in the industry and the government will be benefited especially because nowadays much focus is on promoting the national level business based on organic products.

MATERIALS AND METHODS

Site selection and establishment of papaya cultivation

Papaya cultivation was established at the Sri Lanka School of Agriculture, Kuruwita, premises belonging to the Department of Agriculture. The location WL1 (low country wet zone-1) agro-climatic zone in Sri Lanka. Papaya (*C. papaya*) variety Red Lady seeds were established in the nursery on March 2021. Required seeds were obtained from the "Onach" Seed Company and each seed was established in polybags according to the nursery establishment procedure referenced by the Department of Agriculture. The field was prepared three weeks before planting. Plant spacing among rows and in between rows was 2.5 m. Planting hole parameters were 45 cm×45 cm× 45 cm. Holes were filled with 5 kg of compost mixed up with topsoil. The basal dressing was added into the planting holes and mixed well as the recommendation, one week before planting. Blocks were separated by a 1 ½ feet path. One-month-old healthy papaya seedlings were selected from the nursery and established in prepared planting holes. All the cultural practices such as irrigation, application of top dressings (Table 1), and weeding were carried out according to the recommendations of the Agriculture Department.

Preparation of treatments

Citronella oil and cinnamon oil were purchased from commercial merchandisers. Solutions with 1000 μL L^{-1} of cinnamon oil and citronella oil concentration were prepared as an emulsion in sterilized water containing the surfactant Tween 80 (0.05%) (Samithri et al., 2020; Sarkhosh et al., 2018).

Lantana camara leaf samples were collected from Udawalawa National Park and *Ocimum tenuiflorum* leaf samples were collected from the School of Agriculture. Leaf samples were washed and disinfected with sodium hypochlorite at 1%. Samples were air-dried at room temperature (25-28 $^\circ$C) and ground into a fine powder. Ten grams of air-dried fine powder was weighed separately and the stock solution was prepared following Gurjar et al. (2012). The extraction was adjusted to the concentration of 10% by diluting.

Table 1. Fertilizer recommendation for papaya (per plant).

Fertilizer	Basal dressing	Top dressing I	Top dressing II
Urea	60 g	60 g	65 g
TSP	40 g	40 g	35 g
MOP	130 g	130 g	135 g

Table 2. Treatment schedule for each block for each treatment (factor 02).

Block/Stage	A	B	C	D
Flower bud initiation	✓	✓	✓	✓
Fruit setting	✓	✓	✓	x
Fruit maturation	✓	✓	x	x
Harvesting (color breaking stage)	✓	x	x	x

✓ - indicated that the treatment was applied for the respective plot.
X - Indicated that the treatment was not applied for the respective plot.

Design of the experiment

All the treatments were used at 10% concentration expecting a similar effect from each combination to withstand of time schedule for pre-treatments.

Four treatments with control; T1 (Citronella oil treatment), T2 (Cinnamon oil treatment), T3 (*L. camara* leaf extract), T4 (*O. tenuiflorum* leaf extract) and T5 (control-distilled water) were assigned as factor one. Four stages of fruit development (Block A, B, C and D) were assigned as factor two representing the time of application as per the development stage of the fruit (Table 2). Each treatment was applied in four stages of fruit development, separately covering selected development stage combinations. Complete Randomized Block Design was applied according to the two-factor factorial experiment with three replicates.

The research site consisted of 60 plants belonging to three replicates and each replicate consisted of 20 plants that were treated with five (including control) selected treatments (factor 1) according to the four pretreatment time schedule (factor 2).

During the experiment, the treatments were applied according to the schedule presented in Table 02. Block A was sprayed at all four scheduled occasions within fruit development, block B was sprayed at three scheduled occasions within fruit development, block C was sprayed at two scheduled occasions within fruit development, and block D was sprayed at one scheduled occasion within fruit development. Papaya fruits were harvested at the time of color breaking stage and were ripened for 14 days which consider an expected post-harvest life span at the farm level. Three fruits from the middle whorl of the fruiting area of each plant were harvested from a total number of 60 plants. A total of 180 papaya fruits were selected and subjected to the treatments to collect data at the end of 14-day ripening period. Observations were recorded according to the disease severity scale (Table 3).

Table 3. Disease severity scale.

Severity score	Scale
0	0-5% fruit surface showing symptoms
1	6-10% fruit surface showing symptoms
2	11-25% fruit surface showing symptoms
3	26-50% of fruit surface show symptoms
4	More than 50% of fruit surface shows symptoms

Development of disease severity index to monitor anthracnose
Three fruits from each treated plant were randomly harvested at the correct harvesting stage from the middle whorl of the crown. The fruits were washed and cleaned fruits were allowed to drip dry for 30 minutes on a laboratory bench, and kept until ripening at room temperature wrapped with papers on cardboard layers following the storing method of farmers. Selected fruits showing the gradual development of anthracnose lesions were photographed daily and the anthracnose disease severity of the fruits was manually examined and recorded using a severity scale (Table 3). Disease severity was determined as the percentage of anthracnose lesions concerning the total area of the fruit. A disease severity index was prepared using the photographs along with their percentage anthracnose values.

The disease severity Index (DSI) of postharvest disease was calculated by using the formula (1) stated by Dissanayake et al. (2019).

$$\text{DSI} = \frac{\text{Sum of individual disease rating}}{\text{Number of samples}} \times \frac{100}{\text{Maximum disease grade}} \quad (1)$$

Statistical analysis
Statistical analysis of the results was carried out using the MINITAB 17 statistical software. Data obtained for the disease severity index were analyzed using a two-way analysis of variance with replicates (ANOVA) and mean separation was done using Tukey's multiple comparison test and regression analysis.

RESULTS AND DISCUSSION

The experiment was carried out to develop a suitable pre-treatment strategy to control papaya anthracnose using natural compounds which can use as pre-treatment organic fungicides. The botanicals used in this study, citronella oil, cinnamon oil, and leaf extracts of *L. camera* and *O. tenuiflorum* were selected depending on available scientific information on their antifungal effect (Abeywickrama et al., 2009; Ademe et al., 2015; Dissanayake et al., 2019; Maqbool et al., 2011) on *C. gloeosporioides,* the post-harvest fungal pathogen course to the disease anthracnose in many topical fruits.

Most of the previous research conducted *in-vivo* reported not enough evidence for pre-treatment applications for identified plant extracts under field conditions for papaya anthracnose disease (Abeywickrama et al., 2009; Srikantharajah et al., 2020). Therefore, this research was conducted *in vitro* to find out the most effective natural compound among these four treatments to control anthracnose disease in papaya and secondly, to determine the effective schedule for the application of the natural compounds for anthracnose disease on papaya fruits in field level.

Assessment of the best treatment to control anthracnose in papaya variety Red Lady
According to the results (T3) *L. camera* leaf extract showed the highest significant effect on papaya postharvest disease of anthracnose caused by *C. gloeosporioides* (Table 4, Fig. 1). It recorded the least severity percentage (5.78 ± 0.43), severity score value (0.3 ± 0.17) and disease severity index (26.6667 ± 6.36) among all other three treatments. The *L. camera* leaf extract was tested many times as an antifungal agent for anthracnose disease in different papaya varieties in other countries (Ademe et al., 2015; Prasad, 2015). Fayaz et al. (2017) reported that the methanol extract of *L. camera* shows a higher antifungal effect against plant pathogens.

The *L. camera* leaf extract was tested for *C. gloeosporioides in-vivo* resulting in a higher significance inhibition value compared to the other leaf extracts (Dissanayake et al., 2019; Mdee et al., 2009). Sousa et al. (2012) recorded that *L. camera* essential oils from leaves extracted by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) contain a high percentage of sesquiterpene hydrocarbons, like bicyclogermacrene (19.4%), isocaryophyllene (16.7%), valencene (12.9%) and germacrene D (12.3%).

Dissanayake et al. (2019) recorded that the fruit quality parameters such as weight loss, total soluble solid and pH not declined significantly by applying *L. camera* as a post-harvest treatment in-vivo level. Kumar et al. (2018) reported that anthracnose disease in papaya arises as a pre-harvested quiescent infection and, is rapidly available in humid areas. The disease is quiescent until ripening started to express visible symptoms on the peel. Due to these reasons, the *L. camera* leaf extract pre-treatment with three times application schedule will be highly effective for the reduction of *C. gloeosporioides* population or inhibiting growth of the pathogen in the post-harvest period of the papaya fruit.

Table 4. Results of Tukey's pairwise comparison showing the interaction of each treatment.

Factor (Treatment)	Number of replicates	DSI
T1 (Citronella oil)	12	46.6667[a]±6.72
T2 (Cinnamon oil)	12	49.4444[a]±3.98
T3 (*L. camera* leaf extract)	12	26.6667[b]±6.36
T4 (*O. tenuiflorum* leaf extract)	12	51.6667[a]±5.23
T5 (Control)	12	51.1111[a]±4.45

Each data point represents the mean of twelve replicates ± standard error.
Means sharing a common letter(s) within the same column is not significantly different by Tukey's pairwise comparison test (p = 0.000).
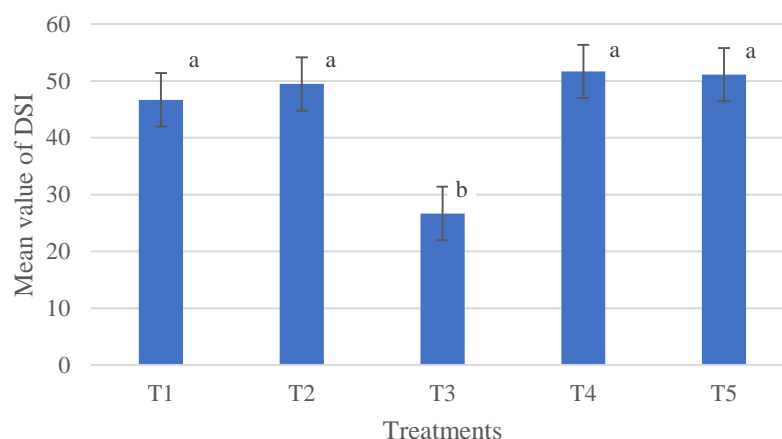
Fig. 1. Significance of treatment effect×T1 (Citronella oil), T2 (Cinnamon oil), T3 (*L. camera* leaf extract), T4 (*O. tenuiflorum* leaf extract), T5 (Control).

Determination of the effective time schedule for the application of pre-treatment

L. camera leaf extract showed the highest significant effect in two blocks (A and B) in this study (Table 5 and Fig. 2). Block A also showed a significant difference in the combination of *L. camera* leaf extract applied at four times of fruit development known as flower bud initiation, fruit setting, fruit maturation and harvesting (at the color breaking stage), reported a 15.56 value for DSI while block B, which applied *L. camera* leaf extract three times during fruit development stages known as flower bud initiation, fruit setting, and fruit maturation reported the lowest DSI of 6.67 compared to other blocks (Fig. 2). Therefore, according to the results, the application of *L. camera* was identified to be the most effective treatment at scheduled time intervals of three occasions (flower bud initiation, fruit setting, and fruit maturation) to control papaya postharvest disease of anthracnose at the field level (Fig. 2). Rana et al. (2005) recorded that few or many of the compounds in *L. camera* leaf oil may be present in methanol-mediated leaf extracts with antifungal efficacy as well. They also pointed out that one or a few of those compounds acting as antifungal agents against *C. gloeosporioides* in papaya could be extracted and applied as a potential bio fungicide (Rana et al., 2005).

According to our research findings, it is clear that the application stage and frequency of application contribute very much to the successful control of post-harvest disease development in papaya fruits, particularly for the diseases like anthracnose. Maeda and Nelson, (2014) reported that anthracnose disease is invaded at the field level and it is also recorded as a foliar-level disease in cultivations. Optimum temperature (18-25 °C) and relative humidity facilitated at the field level can enhance fungal development rapidly. The fungal spores splashed and deposited in the immature green fruits and the pathogen is activated at the post-climacteric stage of the fruit which is called latent infection. These facts aligned with our results, so the application time may enhance the inhibition mechanism of the disease.

Table 5. DSI values for block-treatment interactions.

Block × Treatment	Number of replicates	DSI
Block A * T1	3	33.33 [abcd] ± 6.67
Block A * T2	3	53.33 [abc] ± 3.85
Block A * T3	3	15.56 [cd] ± 4.44
Block A * T4	3	31.11 [abcd] ± 12.37
Block A * T5	3	40.00 [abcd] ± 10.18
Block B * T1	3	68.89 [a] ± 2.22
Block B * T2	3	51.11 [abcd] ± 4.44
Block B * T3	3	6.67 [d] ± 3.85
Block B * T4	3	60.00 [abc] ± 6.67
Block B * T5	3	62.22 [ab] ± 5.88
Block C * T1	3	22.22 [bcd] ± 9.69
Block C * T2	3	46.67 [abcd] ± 13.88
Block C * T3	3	35.56 [abcd] ± 17.36
Block C * T4	3	60.00 [abc] ± 3.85
Block C * T5	3	44.44 [abcd] ± 5.88
Block D * T1	3	62.22 [ab] ± 9.69
Block D * T2	3	46.67 [abcd] ± 10.18
Block D * T3	3	48.89 [abcd] ± 2.22
Block D * T4	3	55.56 [abc] ± 9.69
Block D * T5	3	57.78 [abc] ± 9.69

T1 (Citronella oil treatment), T2 (Cinnamon oil treatment), T3 (*L. camara* leaf extract), T4 (*O. tenuiflorum* leaf extract) and T5 (control-distilled water). Block A (spray at four scheduled occasions within fruit development), block B (spray at three scheduled occasions within fruit development), block C (spray at two scheduled occasions within fruit development), block D (spray at one scheduled occasion within fruit development).
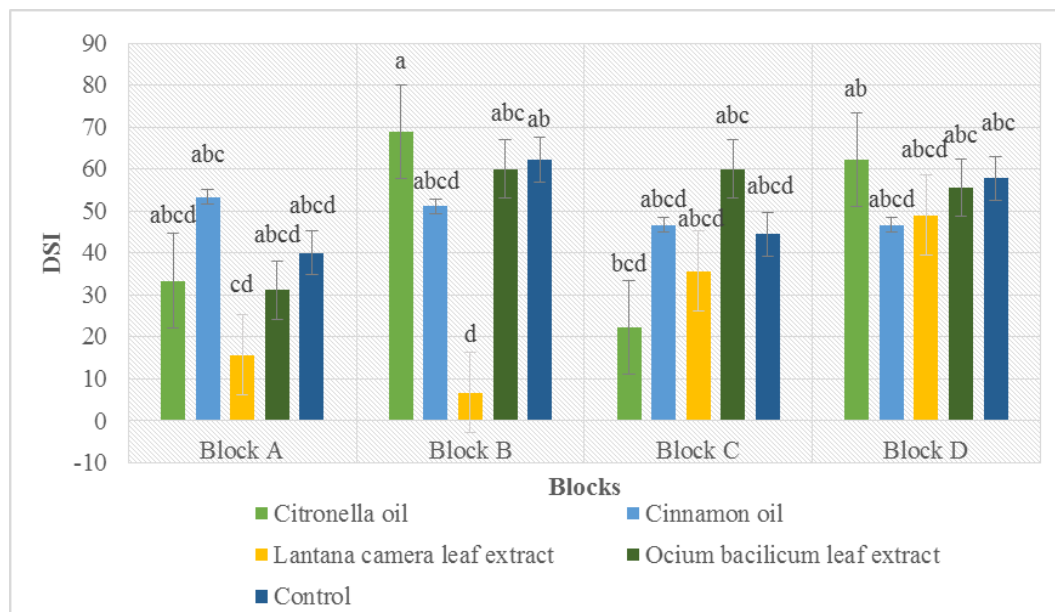
Fig. 2. Graphical representation of the DSI values against four treatments (T1 (Citronella oil), T2 (Cinnamon oil), T3 (*L. camera* leaf extract), T4 (*O. tenuiflorum* leaf extract), with control (T5) and the different time schedules of application during the fruit development stages (Blocks A-D).

According to the obtained results, though the tested essential oils; Citronella oil and Cinnamon oil and the leaf extract of *O. tenuiflorum* can control the anthracnose disease development compared to the control, they were not able to display significant antifungal effect against papaya postharvest disease of anthracnose caused by *C. gloeosporioides* in field level, compared to *L. camera* leaf extract.

This is however contradictory to previous studies which reported their higher efficacy on the fungal pathogen *C. gloeosporioides* (Maqbool et al., 2011). This may be because essential oils are highly volatile compounds and at the time of preparation and at the time of spraying, those volatile compounds responsible for antifungal efficacy could be degraded even by adding any surfactant (tween 80) to the solution causing the compounds to highly release from the substrate as well. During this experiment, the treatments were applied in the morning and at noon when intensive sunlight was present, it is expected that more or less active agents can be released causing the effect to be vastly reduced.

During this research, we experienced that 10 g of leaf powder dissolved in 100 ml of methanol was fair enough to completely spray for 60 experimental units (375 m^2) and only 107 g is required for application at four times per acre. Hence the application of *L. camera* leaf extract contributes to reducing the cost of the product when it is used as a fungicide. Nevertheless, *L. camera* is identified as one of the most destructive and invasive plants in Sri Lanka (Fernando et al., 2016). Therefore, it is very effective to use this extract as an alternative antifungal agent for *C. gloeosporioides*. Although here we used methanol as the solvent to extract *L. camera*, other suitable solvents can be used. Mdee et al. (2009) suggested another alternative method to extract leaf saps under the field condition at a significant level of efficacy is by preparing them with hot water instead of methanol. So, such approaches can be easily recommended and adopted for rural areas in the country with limited resources instead of organic solvents.

Development of disease severity index to monitor anthracnose in the papaya
Disease severity indices are very important to determine and assess the damage of any fresh product. The disease severity score was arranged into a visual key to determine the disease severity index of the papaya red lady variety. The visual key may be useful to assess disease severity scores, and pathogenicity assessments regarding the papaya variety Red Lady.

The visual assessment depended on a few parameters of the fruit peel such as the size of the fruit, lesion type (stage of initiation, radian of the lesion, severity of decay), and spreading area of the lesion. According to that estimate, the percentage values of anthracnose disease spread in a fruit treated by both selected essential oils and leaf extracts were decided and indicated in the developed disease severity index shown below in Figure 3. The prepared disease severity index consists of the sequential development of the anthracnose disease in papaya Red Lady variety fruits. Anthracnose development in selected papaya fruits can be divided into 5 severities scores and three selected fruits are shown under each severity score. Considering one particular stage/severity score, the calculated percentage development of anthracnose disease symptoms as a range is shown below the respective photograph (Fig. 3). For example; at severity score 1, the percentage development of anthracnose disease symptoms covering the fruit surface is 6-10%.
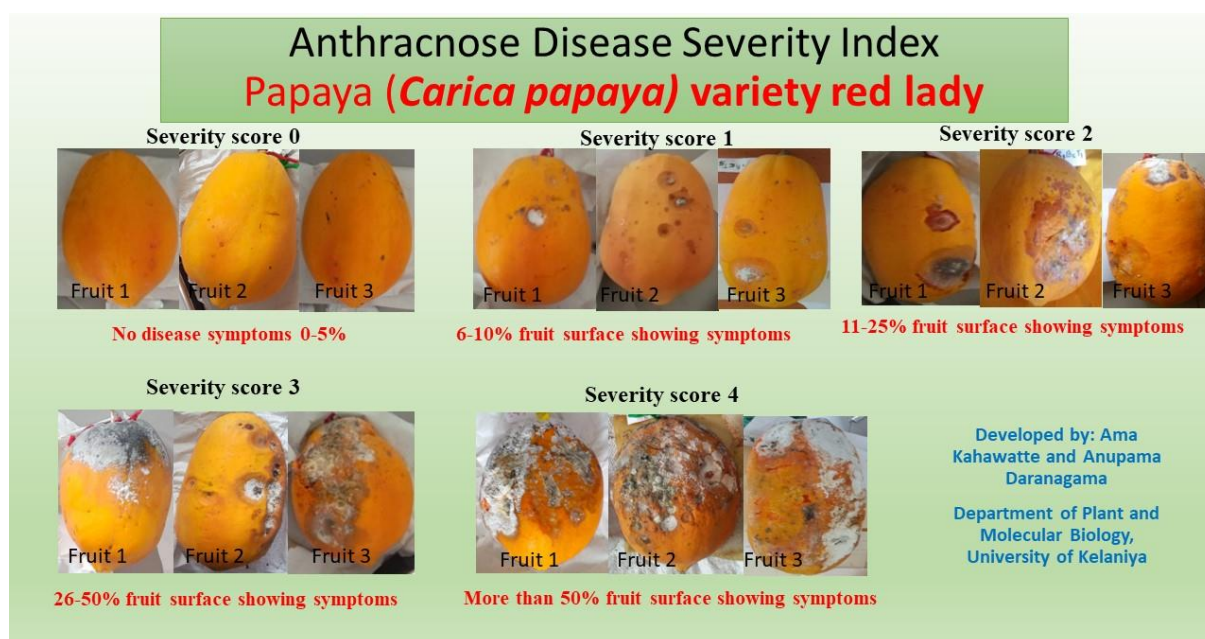

Fig. 3. The disease severity index prepared to monitor anthracnose development on the papaya Red Lady variety.

CONCLUSION

L. camera leaf extract was identified as the best treatment to control papaya (*C. papaya*) anthracnose disease caused by the fungal pathogen *C. gloeosporioides* with the least disease severity index (DSI) value. The most appropriate time schedule and frequency for the application of the selected treatment at the field level were identified to be the application on three scheduled occasions; flower bud initiation, fruit setting, and fruit maturation of papaya. This is the first report on identifying *L. camera* leaf extract used as an anti-fungal natural compound and the appropriate time schedule and frequency for the pre-treatments at the field

level in Sri Lanka for the papaya red lady variety. Compounds as the pre-treatments which experimented at the field level in Sri Lanka for the papaya red lady variety.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest to report.

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