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Effect of Chitosan on Disease Control and Yield Parameters of Rambutan (*Nephelium Lappaceum* L.) Variety Malwana Special

E.R.S.P.Edirimanna[1], R.G.A.S.Rajapakse[2], M.P.T. Premarathna[2], K.J.P.K. Kahawaththa[2], Shyamalee Kohombange[2], A. K. Rathnayake[3], K.R.C.de Silva[3], D.C.K.K. Dissanayake[3]

[1]Fruit Research and Development Institute, Horana
[2]Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya
[3]Atomic Energy Board of Sri Lanka, Colombo
Corresponding Author: shyamaleekohombange@gmail.com

Abstract— *Rambutan (Nephelium lappaceum L.) is an extensively grown fruit crop in Sri Lanka. Powdery mildew incited by fungus Oidium nephelii attack young leaves, flowers and immature fruits caused heavy fruit losses in Rambutan. Chitosan is a natural biodegradable de-acetylated form of chitin has been proven to control numerous pre and post-harvest disease on various horticultural commodities. Therefore, the objective of the present study was to investigate the effect of chitosan compounds on control of powdery mildew disease and yield parameters of Rambutan (Nephelium lappaceum L.) variety Malwana Special. Different types of chitosan i.e. chitosan fungicide, chitosan oligomer, copper containing chitosan and wettable sulphur were used as treatments. Based on the green house test results, 40 ppm concentration of chitosan fungicide, chitosan oligomer and Copper chitosan was identified as economically effective concentration for field studies. Field experiment was conducted in WU_1 agro ecological zones at Gannoruwa in Central province of Sri Lanka. Experiment was arranged as Randomized Complete Block Design with 8 replicates. Treatments were sprayed by Knapsack sprayer. Disease severity Index of fruits, fruit weight and number of fruits per bunch were recorded at harvesting. Highest fruit weight was recorded in chitosan fungicide treated fruits. Higher number of fruits per bunch was recorded in all tested chitosan and wettable sulphur treated fruits compared to control. Chitosan fungicide (40ppm) and wettable sulphur 80 WP (4000ppm) has equally potential in controlling of powdery mildew pathogen of Rambutan variety Malwana Special.*

Keywords— Chitosan, Rambutan, Powdery mildew, Sulphur.

I. INTRODUCTION

Rambutan (*Nephelium lappaceum* L.) is an extensively grown fruit crop in Sri Lanka. Malwana Special is most widely grown high yielding, good quality variety of Rambutan recommended by the Department of Agriculture. This variety has high demand from consumers as well as growers especially due to their excellent quality as a fresh fruit.

Powdery mildew is a disease of Rambutan incited by fungus *Oidium nephelii* attack young leaves, flowers and immature fruits. When it attack to immature fruits these become discolored and dry off causing fruit quality deterioration and heavy fruit losses of all Rambutan varieties including variety Malwana Special. It has reported that this pathogen is obligatory parasite on Rambutan and unculturable on common artificial media [1]. Powdery mildew of Rambutan was first noticed in year 2000 in Sri Lanka and later it became a serious problem causing economic losses to growers [2], [3]. At present, this disease has reached epidemic level in Rambutan growing areas in the country. White colour powdery fungi appear first on the young leaves, and then these enlarge to cover the entire surface of the both side of the leaves and spread on to the fruits at any maturity stages. Infected splinters of the mature fruits become discolored and dry off causing fruit quality deterioration. Surface of the infected mature fruits become

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hard, turns black and may crack. All cultivars grown in Sri Lanka are susceptible to this disease with different severity levels [1]. It has reported that this disease is also prevalent in Thailand and Malaysia where the pathogen is identified as fungi *Oidium nephelii* [4], [5].

Powdery mildew disease of Rambutan trees can be effectively controlled with spraying of wettable sulphur [6] or any other fungicides such as Chlorothalonil, Thiophanate Methyl at 7 – 10 days intervals when disease is observed [7], [1]. As a habit, fruit peel of Rambutan removes with mouth and therefore, attention must be paid regarding the time of application and toxicity of the fungicides, which are used to control the powdery mildew disease. To date, research on powdery mildew disease in Sri Lanka was mainly directed towards the development of control methods using fungicides.

Application of agro-chemicals are the major plant protection method over decades even though they are associated with many disadvantages including their expensive applications, environmental pollution and human health hazards due to excessive usage. This has emerged a worldwide huge trend to explore other environmental friendly alternative methods for plant protection. Chitosan a natural biodegradable de-acetylated form of chitin has been proven to control numerous pre and post-harvest disease on various horticultural commodities [8], [9], [10]. It has reported that different chitosan molecules such as Oligochitosan induces a series of defense reactions in plants correlated with enzymatic activities and chitosan fungicide compounds has a direct effect on microbes by fungistatic or fungicidal potential [8], [11], [12], [13]. Coating fruits with chitosan could be an effective treatment to control weight loss and increase postharvest life [14], [15]. Therefore, the objective of the present study was to investigate the effect of chitosan compounds on control of powdery mildew disease and yield parameters of Rambutan (*Nephelium lappaceum* L.) variety Malwana Special.

II. MATERIAL AND METHODS

2.1 Isolation and identification of pathogen:

Mildew affected leaves were collected from Rambutan plants from different locations in the major Rambutan growing areas. Powdery mildew disease affected leaf samples were microscopically observed for detection of morphological characters of mycelia and conidia of pathogen isolates and then these were compared with published data of *Oidium nephelii*. Conidia of pathogen were collected from powdery mildew affected leaves of Rambutan plants and conidia suspensions of pathogen isolates ($2.4x10^4$conidia per ml of water) were prepared in distilled water. Then potted Rambutan seedlings were artificially inoculated with these conidia suspensions by spraying conidia suspensions to study the development of symptoms on leaves. Conidia suspension of pathogen was cultured on potato dextrose agar and potato dextrose agar + 1% Rambutan leaf extract media to detect the growing ability of pathogen on artificial media.

2.2 Green house Experiment:

Different concentrations of chitosan fungicides, chitosan oligomer and Copper chitosan (20, 40, 60, 80 ppm) was sprayed on to the disease affected Rambutan seedlings by hand sprayer and continued 4 times with 7 days interval to identify the effective concentration of each chitosan molecules in controlling powdery mildew disease. Disease suppression ability of each concentration of chitosan compounds was visually inspected by comparison with control treatment.

2.3 Field experiment:

Experiments were conducted in WU_1 agro ecological zones at Gannoruwa in Central province of Sri Lanka. Two types of chitosan, one compound is oligo-chitosan with selected molecular mass (Viscosity Average Molecular Mass, M_V = 3000 – 10000 Da), possess plant growth promoter/elicitor properties, while other one is low molecular weight Chitosan in M_V range 10000 – 100000 Da possess significant fungicidal activity for many common diseases in plant pathology were obtained by Atomic Energy Board, Colombo, Sri Lanka and used for this study program. Effect of chitosan molecules on powdery mildew disease were compared with recommended practice i.e. spraying of wettable sulphur (4000ppm) and untreated i.e. control. Commercial grown variety Malwana Special was used for this study. Experiment was arranged as Randomized Complete Block Design with 8 replicates. Different types of chitosan i.e. chitosan fungicides, chitosan oligomer, copper containing chitosan and wettable sulphur were sprayed as in table 1 by Knapsack sprayer. Disease severity Index (DSI) of fruits was recorded at leaf flush stage, four times during fruiting stage and at harvest using rating scale given below. Chitosan oligamer (40 ppm) was sprayed on fruit surface one day before harvesting of respective treatments to detect the effect of chitosan compounds on post-harvest fruit quality of Rambutan. Mean weight per fruit and mean fruit number per bunch of different treatments was also recorded.

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2.4 Treatments:

Treatments	Spraying Schedule		
	Leaf flush stage	Fruiting stage	Ripen fruits
T1	Spraying of chitosan fungicide (40 ppm) in two weeks intervals and spraying was continued two times	Four spraying of chitosan fungicide (40ppm) at two weeks intervals	One spraying with chitosan oligomer (40ppm) (one day before harvest)
T2	Alternative spraying of chitosan oligomer (40ppm) and chitosan fungicide (40ppm) in two weeks intervals and spraying was continued two times	Four spraying of chitosan oligomer(40ppm) at two weeks intervals	One spraying with chitosan oligomer (40ppm) (one day before harvest)
T3	Alternative spraying of chitosan fungicide (40ppm) and Cu – Chitosan (40ppm) in two weeks intervals and spraying was continued two times	Four alternative spraying of chitosan fungicide (40ppm) and Cu –chitosan (40ppm) at two weeks intervals	One spraying with chitosan oligomer (40ppm) (one day before harvest)
T4	Spraying with wettable Sulphur (4000ppm) in two weeks intervals and spraying was done at one time	Two spraying of wettable Sulphur (4000ppm) at two weeks intervals	One spraying of wettable Sulphur (4000ppm) (14days before harvest)
T5	Control	Control	Control

2.5 Rating scale for Disease Severity Index of powdery mildew on fruits:

0 - No disease symptoms on fruits

1- Thin white colour mycelia were observed on less than 50% fruits and fruits did not become shriveled or blackened (Mild disease severity)

3 - Thin white colour mycelia were observed on fruit surfaces but fruits did not become shriveled and blackened (Moderately high disease severity)

5 - White colour mycelia were observed on fruit surfaces and fruits become shriveled and blackened (High disease severity)

III. RESULTS AND DISCUSSION

Symptoms developed on leaves which are artificially inoculated with *Oidium* isolates were similar to typical powdery mildew symptoms. Microscopic observations indicated that pathogen produced conidia openly at the end of hyphae and exposed to air. Conidia were unicellular, oval shape, 20-30µ diameters in size, arranged as long chains. Mycelia were septate and grown only on the surface of plants. These are the characteristics features of *Oidium* species [16]. Pathogen did not culture on artificial media and disease symptoms developed only on leaves of inoculated Rambutan plants. All isolates tested possessed common features which described as typical for *Oidium nephelii* a causal agent of powdery mildew disease of Rambutan in Phillippines [4].

Green house experiment indicated that 40 ppm and higher concentration of chitosan fungicides, chitosan oligomer and Copper chitosan were suppressed development of powdery mildew disease compared to 20 ppm concentration. Based on the green house test results, 40 ppm concentration of chitosan fungicides, chitosan oligomer and Copper chitosan was identified as economically effective concentration for field studies.

Field test results indicated that all fruits were shriveled and blackened in control treatments. T2 & T3 treatments showed moderately high disease severity on fruits (Figure 1). It was observed white colour mycelia on fruit surface of T2 and T3 treatments. Finally splinters were became brownish color resulting no marketable values for T2 &T3 treated fruits. Application of chitosan fungicide (T1) showed mild disease severity on mature fruits. Thin white colour mycelia were observed on few fruits and fruits or splinters did not show any colour abnormality after ripening. Spraying of wettable sulphur (T4) did not show powdery mildew symptom on mature fruits or ripen fruits of Rambutan.

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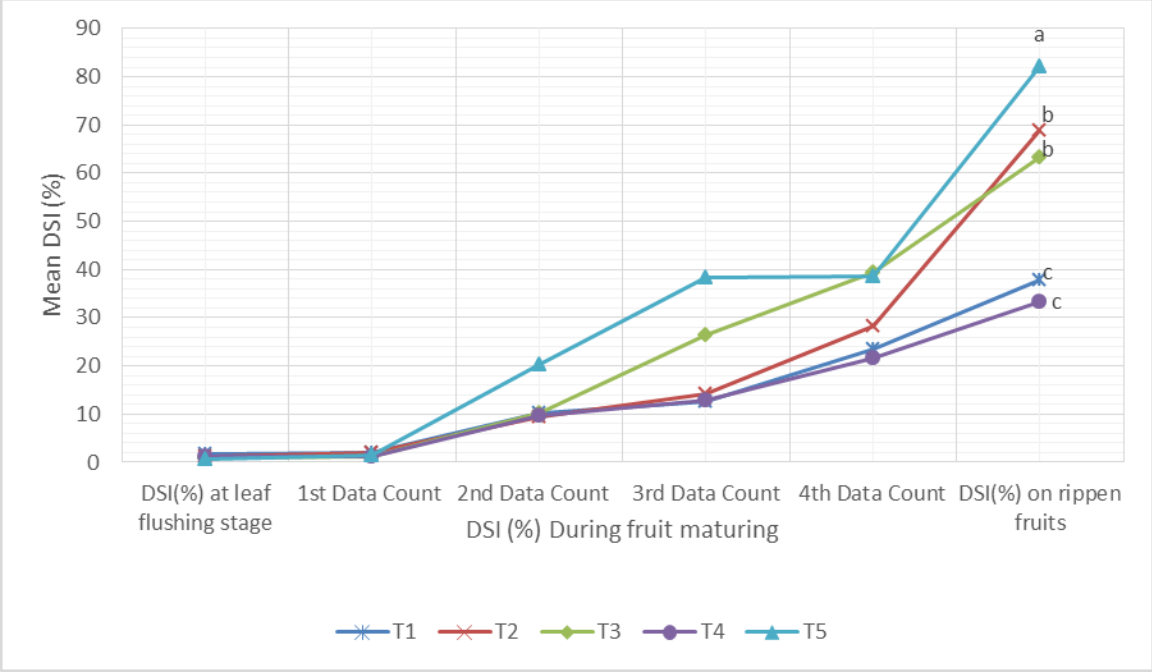

Fig.1: Disease severity index (%) of powdery mildew disease of young leaves and Rambutan fruits during the period of chitosan and wettable sulphur spraying. (Note: Means with the same letter(s) on the ripen fruit stage are not significantly different at P= 0.05)

Table 1. Effect of spraying of different chitosan treatments and wettable sulphur on mean fruit weight and mean number of fruits per bunch of Rambutan.

Treatments	Mean fruit weight (g)	Mean no. of fruit per bunch
1	39.4[a]	6.7 [a]
2	37.9 [b]	6.6 [a]
3	34.6 [d]	6.8 [a]
4	37.4[bc]	6.9 [a]
5	36.5 [c]	6.0 [b]

Note: Means with the same letter(s) on the column are not significantly different at P= 0.05

Results numerated in figure 1 and table 1 indicated that, treatment T1 show the higher disease control ability as well as highest mean weight per fruit compared to other treatment tested. It has reported that Chitosan has excellent film-forming property, making it easy to form a semipermeable film on the surface which may maintain the higher fruit weight [14], [15], [17], [18]. However, all tested chitosan compounds as well as wettable sulphur treatments significantly increased mean number of fruit per bunch compared to control (Table 1). It was also observed that chitosan as well as wettable sulphur treatments significantly decreased severity of powdery mildew compared to control treatment (Figure 1) which can reduce the immature fruit drop due to powdery mildew and resulting higher number of fruits per bunch. It has known that chitosan has nontoxic, biodegradable, biocompatible properties and more environmental friendly compared to the sulphur [11]. There were some reports showed that chitosan oligomers have ability in increasing post-harvest period and decreased post-harvest losses due to its fungicidal ability and minimize water losses due its semipermeable film forming ability on fruit surface [10]. All the fruits of treatment T2, T3 and T5 were fully infected with powdery mildew pathogen and completely rotten within one day after harvesting. However, tested concentration of chitosan compounds and wettable sulphur treated fruits did not show any difference in post-harvest period (Data not presented).

IV. CONCLUSION

Pathogen of powdery mildew of Rambutan was identified as *Oidium nephelii*. Highest fruit weight recorded in chitosan fungicide treated fruits. Chitosan fungicide (40ppm) and wettable sulphur 80 WP (4000ppm) has

equally potential in controlling of powdery mildew as well as increase number of fruits per bunch of Rambutan of var. Malwana Special.

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