

CONFIDENTIAL

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**FIELD EFFICACY STUDY OF BACILLUS AMYLOLIQUEFACIENS IN HATAKE  
BIOFUNGICIDE AGAINST DURIAN CANKER**

Trial Done by:

**HATAKE GLOBAL SDN BHD**

Report Prepared by:

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## INTRODUCTION

Durian (*Durio zibethinus Murr.*) is a popular fruit tree grown extensively in Southeast Asia for its highly valued fruit. Durian canker (*Phytophthora palmivora*) is widespread in other countries in this region. The symptoms are reddish brown discoloration of the outer bark, areas of necrosis on the bark, and a reddish brown discoloration in the outer sapwood. While, symptoms can be observed by indicating tree canopy such as defoliation or dieback of twigs from the top of trees.

*Bacillus amyloliquefaciens* is a species of bacterium in the genus *Bacillus* and is used to fight some plant root pathogens in agriculture such as ralstonia, rhizoctonia, phythium, alternaria, fusarium and phytophthera. Therefore, this trial was done to determine the effective of *B. amyloliquefaciens* on control of *P. palmivora*.

## OBJECTIVE

1. To evaluate the efficacy of *Bacillus amyloliquefaciens* Hatake Biofungicide against durian canker (*Phytophthera palmivora*).

## MATERIALS AND METHODS

Table 1. Field trial details.

No.	Details
1	Location of trial Bentong
2	Design of trial RCBD
3	No. of treatment 5
4	No. of replication 3
5	No. of plot 15
6	Plot size 3 trees/plot
7	Treatment list Refer Table 2
8	Target crop Durian ( <i>Durio</i> sp)
9	Target pest Canker ( <i>Phytophthera palmivora</i> )
10	Type of sprayer -
11	Type of nozzle -
12	Spray volume/Water Volume -
13	Method of application Bark Painting

14	Spay interval/Application interval	2 weeks (3 times) and Monthly (2 times)
15	No. of application	5 application (7/7/20, 21/7/20, 4/8/20, 4/9/20, 4/10/20)
16	Weather	7/7/20 (Sunny at application, 0830; Sunny after application, 1100) 21/7/20 (Sunny at application, 0815; Cloudy after application, 1100) 4/8/20 (Sunny at application, 0800; Sunny after application, 1030) 4/9/20 (Cloudy at application, 0830; Sunny after application, 1100) 4/10/20, (Sunny at application 0900; Sunny after application, 1115)
17	Crop Stage	> 7 years
18	Trial Plot History	Durian
19	Percentage of Shade	Open area

Table 2. Treatment list

Treatments	Rate of Application (g or ml/L)	Replications		
Control	-	1	9	12
T1. Hatake Biofungicide	2.5 g	2	10	13
T2. Hatake Biofungicide	5.0 g	3	6	14
T3. Hatake Biofungicide	7.5 g	4	7	15
T4. Ridomil G 43.9 SL	2.4 ml	5	8	11

\* Hatake Biofungicide is a wettable powder (WP) bio-fungicide which contain *Bacillus amyloliquefaciens* ( $1.1 \times 10^9$  cfu/g). Ridomil G 43.9 SL is a chemical fungicide which contain 480 g/L metalaxyl-M.

## METHOD OF ASSESSMENT

- i. Pre-assessment were carried out at each applications.
- ii. Disease severity was observed by using the rate of canker severity (Anderson & Guest, 1990).

0 : no canker

1 : canker < 100 cm<sup>2</sup>

2 : canker > 100 cm<sup>2</sup> but < 70 % of girdling of the main trunk.

3 : canker > 70 % girdling of the main trunk/almost dead owing to canker.

iii. Assessment was done at 0DAT, 2WAT, 2WA2T, 2WA3T, 1MA3T, 1MA4T, 1MA5T.

## RESULTS

Table 3. Effect of different fungicide treatments on disease severity of canker attack.

Treatments	Rate (g or ml/L water)	Disease severity of Canker Attack, cm <sup>2</sup>						
		0WAT	2WAT	2WA2T	2WA3T	1MA3T	1MA4T	1MA5T
Control	-	166.3 a	182.3 a	287.3 a	369.0 a	-	-	-
T1	2.5 g	167.3 a	65.0 b	48.0 b	45.1 b	44.3 a	45.0 a	37.8 a
T2	5.0 g	256.0 a	101.9 b	50.7 b	13.6 b	13.2 b	12.8 b	13.2 b
T3	7.5 g	235.7 a	95.0 b	49.8 b	12.4 b	11.3 b	11.6 b	11.4 b
T4	2.4 ml	262.7 a	78.7 b	36.9 b	41.0 b	40.9 a	40.7 a	42.2 a

Notes: Mean followed by different letters are significantly different from one to another at the probability level of  $p=0.05$  by Least Significant Difference (LSD).

\* WAT = Week After Treatment; 2WA2T = 2 Week After Second Treatment; 1MA3T = 1 Month After 3 Treatment.

From the trial, Hatake Biofungicide gave significantly difference on canker disease severity. Before trial, disease severity was recorded. At 2WAT, durian canker can be controlled effectively by different fungicides. While, disease severity was increased for untreated durian trees and the observation was stopped at 3WA3T to avoid dying of durian trees.

Results showed that different fungicides had no significance at 2WA2T and 3WA3T. However, Hatake Biofungicide at the lowest rate (2.5 g/L water) and Ridomil (2.4 ml/L water) were less effective at 1MA3 as compared to Hatake Biofungicide at 5.0 g and 7.5 g respectively in 1 L of water. At the end of trial, T2 and T3 had better control on durian canker than T1 and T4.

Table 4. Effect of different fungicides on percent reduce of durian canker.

Treatments	Rate (g or ml/L water)	Percent Reduce of Durian Canker, %					
		2WAT	2WA2T	2WA3T	1MA3T	1MA4T	1MA5T
Control	-	-9.4	-75.9	-127.6	-	-	-
T1	2.5 g	60.9	70.9	72.8	73.3	72.4	75.9
T2	5.0 g	60.2	80.3	94.7	94.8	95.0	94.8

T3	7.5 g	59.6	78.1	94.5	94.7	94.5	94.6
T4	2.4 ml	70.0	85.9	83.7	83.7	83.8	82.7

The disease severity was converted to percent reduce of durian canker. Negative value in the Control row showed the increasing of area of canker.

From Table 4, fungicide treatments gave the optimal control after third treatment (3WA3T) which had 72.8%, 94.7%, 94.5% and 83.7% respectively for T1 to T4. And the percent reduce of durian canker was not significant at 1MA3T until the end of trial.

## CONCLUSION

The result showed Hatake Biofungicide at 5 g/L water gave a good performance and the recommended rate in controlling the durian canker (*Phytophthora palmivora*).

## APPENDIX

### Anova Table

Durian Canker (0 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
<b>Model</b>	6	37775.3333	6295.8889	0.8	0.5937
<b>Error</b>	8	62656.2667	7832.0333		
<b>Corrected Total</b>	14	100431.6			

R-Square	Coeff Var	Root MSE	0WAT Mean
0.37613	40.67039	88.49878	217.6

Source	DF	Type I SS	Mean Square	F Value	Pr > F
<b>trt</b>	4	26960.93333	6740.23333	0.86	0.5265
<b>rep</b>	2	10814.4	5407.2	0.69	0.5289

Durian Canker (2 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
<b>Model</b>	6	25927.428	4321.238	2.55	0.1102
<b>Error</b>	8	13552.16133	1694.02017		
<b>Corrected Total</b>	14	39479.58933			

R-Square	Coeff Var	Root MSE	2WAT Mean
0.65673	39.35096	41.15848	104.5933

Source	DF	Type I SS	Mean Square	F Value	Pr > F
<b>trt</b>	4	25138.88267	6284.72067	3.71	0.0542
<b>rep</b>	2	788.54533	394.27267	0.23	0.7975

Durian Canker (2 WA2T)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
<b>Model</b>	6	140325.7972	23387.6329	27.13	<.0001
<b>Error</b>	8	6896.6902	862.0863		
<b>Corrected Total</b>	14	147222.4874			

<b>R-Square</b>	<b>Coeff Var</b>	<b>Root MSE</b>	<b>2WA2T Mean</b>
0.953155	31.06335	29.36131	94.52073

<b>Source</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>trt</b>	4	139782.2324	34945.5581	40.54	<.0001
<b>rep</b>	2	543.5649	271.7824	0.32	0.7383

Durian Canker (2 WA3T)

<b>Source</b>	<b>DF</b>	<b>Sum of Squares</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>Model</b>	6	282798.932	47133.1553	86.05	<.0001
<b>Error</b>	8	4382.024	547.753		
<b>Corrected Total</b>	14	287180.956			

<b>R-Square</b>	<b>Coeff Var</b>	<b>Root MSE</b>	<b>2WA3T Mean</b>
0.984741	24.3185	23.40412	96.24

<b>Source</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>trt</b>	4	281726.356	70431.589	128.58	<.0001
<b>rep</b>	2	1072.576	536.288	0.98	0.4165

Durian Canker (1 MA3T)

<b>Source</b>	<b>DF</b>	<b>Sum of Squares</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>Model</b>	5	3084.124167	616.824833	3.83	0.0664
<b>Error</b>	6	966.585	161.0975		
<b>Corrected Total</b>	11	4050.709167			

<b>R-Square</b>	<b>Coeff Var</b>	<b>Root MSE</b>	<b>1MA3T Mean</b>
0.761379	46.25236	12.69242	27.44167

<b>Source</b>	<b>DF</b>	<b>Type I SS</b>	<b>Mean Square</b>	<b>F Value</b>	<b>Pr &gt; F</b>
<b>trt</b>	3	2791.8225	930.6075	5.78	0.0334
<b>rep</b>	2	292.301667	146.150833	0.91	0.4526

Durian Canker (1 MA4T)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
<b>Model</b>	5	3138.868333	627.773667	4.51	0.0472
<b>Error</b>	6	835.528333	139.254722		
<b>Corrected Total</b>	11	3974.396667			

R-Square	Coeff Var	Root MSE	1MA4T Mean
0.789772	42.88537	11.80062	27.51667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
<b>trt</b>	3	2856.936667	952.312222	6.84	0.0231
<b>rep</b>	2	281.931667	140.965833	1.01	0.418

Durian Canker (1 MA5T)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
<b>Model</b>	5	2537.045	507.409	9.69	0.0077
<b>Error</b>	6	314.341667	52.390278		
<b>Corrected Total</b>	11	2851.386667			

R-Square	Coeff Var	Root MSE	1MA5T Mean
0.889758	27.66158	7.238113	26.16667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
<b>trt</b>	3	2341.313333	780.437778	14.9	0.0035
<b>rep</b>	2	195.731667	97.865833	1.87	0.234