

CONFIDENTIAL

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**FIELD EFFICACY STUDY OF BACILLUS FIRMUS HG210 IN HATAKE
BIONEMATICIDE AGAINST NEMATODES ON ROSE**

Trial Done by:

HATAKE GLOBAL SDN BHD

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INTRODUCTION

Rose is a woody perennial flowering plant from Rosaceae family. Cultivated roses are often subject to severe damage from insect, arachnid and fungal pests and diseases. Rose cultivation at the same planting site lead to nematode damage. Nematodes cause galls on the roots and constrict the vascular system. Some symptoms such as stunt growth, yellowing, and reduced yield can be noticed, plant even die if infected heavily.

Bacillus firmus is a member of the genus *Bacillus*, a diverse group of gram-positive endospore-forming bacteria. It believes that the mode of action of *B. firmus* involves bacterial colonization of young roots that create a “living barrier” to promote healthy root growth and reduce nematode damage. Therefore, *B. firmus* can be used as biological control for nematode attack.

OBJECTIVE

1. To evaluate the efficacy of *Bacillus firmus* HG210 Hatake Bionematicide against nematode (*Meloidogyne incognita*) on rose.

MATERIALS AND METHODS

Table 1. Field trial details.

No.	Details
1	Location of trial Kampung Raja, Cameron Highland
2	Design of trial RCBD
3	No. of treatment 5
4	No. of replication 4
5	No. of plot 20
6	Plot size 1 m x 5 m
7	Treatment list Refer Table 2
8	Target crop Rose (<i>Rosa</i> sp)
9	Target pest Nematode (<i>Meloidogyne incognita</i>)
10	Type of sprayer -
11	Type of nozzle -
12	Spray volume/Water Volume 20 000 L/Ha
13	Method of application 2 liter per m ²

14	Spay interval/Application interval	Once after roses harvested
15	No. of application	1 application (2 December 2020)
16	Weather	2/12/20 (Sunny at drenching, 0830; Sunny after drenching, 1000)
17	Crop Stage	> 3 years
18	Trial Plot History	Rose
19	Percentage of Shade	< 90 % (under shelter)

Table 2. Treatment list

Treatments	Rate of Application (g or ml/m ²)	Rate kg or L/ha	Replications			
Control	-	-	1	9	12	18
T1. Hatake Bionematicide	0.05 g/m ²	0.5 kg	2	10	13	19
T2. Hatake Bionematicide	0.10 g/m ²	1.0 kg	3	6	14	20
T3. Hatake Bionematicide	0.15 g/m ²	1.5 kg	4	7	15	16
T4. Carbofuran 3 % (Carbotack)	2.0 g/m ²	20.0 kg	5	8	11	17

* Hatake Bionematicide is a wettable powder (WP) bio-nematicide which contain *Bacillus firmus HG210* (4.6×10^8 cfu/g).

METHOD OF ASSESSMENT

- i. Pre-assessment were carried out at each applications.
- ii. Percent of leaf yellowing (based on area of the each plot) was visually observed at 2 WAT (weeks after treatment), 4 WAT, 6 WAT and 8 WAT.

TRIAL DESIGN AND LAYOUT

16	17	18	19	20
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11	12	13	14	15
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6	7	8	9	10
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1	2	3	4	5
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RESULTS

Table 3. Effect of different nematicide treatments on rose's nematodes

Treatments	Rate (L/Ha or Kg/Ha)	Percent of Leave Yellowing				
		0WAT	2WAT	4WAT	6WAT	8WAT
Control	-	20.0 a	21.25 a	23.75 a	28.75 a	31.25 a
T1	0.5 kg	23.75 a	23.75 a	23.75 a	26.25 a	25.0 b
T2	1.0 kg	20.0 a	17.5 a	15.0 b	12.5 b	11.25 c
T3	1.5 kg	23.75 a	21.25 a	13.75 b	12.5 b	11.25 c
T4	20.0 kg	22.5 a	18.75 a	15.0 b	12.5 b	11.25 c

Notes: Mean followed by different subscripted letters are significantly different from one to another at the probability level of $p=0.05$ by Duncan's Multiple Range Test (DMRT).

* WAT = Week After Treatment

Before trial, phytotoxicity test was carried out by comparing the standard rate (x) and double rate (2x) which were 0.1 g/m^2 and 0.15 g/m^2 of Hatake Bionematicide in 2 liter water respectively. After 7 days of soil drenching, there was no phytotoxicity observed in x and 2x plots. Therefore, trial was carried out to determine its efficacy on control of *Meloidogyne javanica* by visual observation on percent of leave yellowing.

Pre-assessment before first application showed that the percent of leave yellowing were not significantly difference from all the plots. At 2 week after treatment (2 WAT), no difference was observed between the treatments too.

However, rose plants were healthier at 4 WAT for T2, T3 (1.0 kg/Ha and 1.5 kg/Ha Hatake Bionematicide) and T4 (20.0 kg/Ha carbofuran 3.0%). At the lowest rate of Hatake Bionematicide, 0.5 kg/Ha, yellowing on leave still remained the same when comparing to control (untreated plot). Similar observations were observed at 6 WAT.

At the end of the trial (8 WAT), treatments were significantly affected the rose plants. Percent of leave yellowing was slightly lower for T1 when comparing to Control. But in term of efficiency, T2 was the optimal rate which had similar efficacy to T4.

CONCLUSION

The result showed Hatake Bionematicide at 1.0 kg/Ha gave a good performance and the recommended rate in controlling the nematode (*Meloidogyne javanica*) at tea.

APPENDIX

Anova Table

Nematode (0 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	97.5	13.9285714	0.4	0.8873
Error	12	422.5	35.2083333		
Corrected Total	19	520			

R-Square	Coeff Var	Root MSE	pre Mean
0.1875	26.97119	5.933661	22

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	4	57.5	14.375	0.41	0.7993
rep	3	40	13.33333333	0.38	0.7701

Nematode (2 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	150	21.4285714	1.05	0.4483
Error	12	245	20.4166667		
Corrected Total	19	395			

R-Square	Coeff Var	Root MSE	1DAT Mean
0.379747	22.04137	4.518481	20.5

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	4	95	23.75	1.16	0.3747
rep	3	55	18.33333333	0.9	0.4705

Nematode (4 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
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Model	7	411.25	58.75	3.48	0.0282
Error	12	202.5	16.875		
Corrected Total	19	613.75			

R-Square	Coeff Var	Root MSE	3DAT Mean
0.670061	22.50915	4.107919	18.25

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	4	407.5	101.875	6.04	0.0067
rep	3	3.75	1.25	0.07	0.9728

Nematode (6 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1117.5	159.642857	13.93	<.0001
Error	12	137.5	11.458333		
Corrected Total	19	1255			

R-Square	Coeff Var	Root MSE	5DAT Mean
0.890438	18.29738	3.385016	18.5

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	4	1092.5	273.125	23.84	<.0001
rep	3	25	8.333333	0.73	0.5551

Nematode (8 WAT)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1455	207.857143	15.12	<.0001
Error	12	165	13.75		

Corrected Total 19 1620

R-Square	Coeff Var	Root MSE	7DAT Mean
0.898148	20.60055	3.708099	18

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	4	1445	361.25	26.27	<.0001
rep	3	10	3.333333	0.24	0.8651
