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Evaluation of *Streptomyces*-biofungicide to control chili anthracnose in pot experiment

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The biofungicides from selected effective *Streptomyces* species were formulated to evaluate the efficacy to control chili anthracnose caused by *Colletotrichum gloeosporioides* in pot experiment. The biofungicides namely NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 were significantly reduced disease incidence on chili fruits. Disease incidences were significantly reduced after treated with biofungicides of NSP-1, NSP-3, NSP-5 and NSP-6 at concentration of 0.5-2.0 $g.L^{-1}$ and gave significantly lowest in the infected fruit per plant. Moreover, application of biofungicides exhibited the increased in plant growth parameters at harvesting by means of increased in plant height, plant stem fresh/dry weight, root fresh/dry weight, root length and fruit yield.

Key words: Biofungicide**,** *Streptomyces*, chili anthracnose

Introduction

Anthracnose is an economically important disease of chili caused by *Colletotrichum* spp. under favorable condition and the disease is mainly problem on mature fruits, caused marketable losses (Manandhar, *et al.*, 1995), the symptoms on chili plants include shoot dieback, damping-off, leaf spot, leaf spot and symptoms on immature fruits (Hong and Hwang, 1998; Shin *et al.*, 2000). The anthracnose disease managements are promising with the combination of cultural practices, resistant cultivar, chemical control and biological control (Than *et al*., 2008). The chemical control of anthracnose disease was recommended, the benzimidazole fungicide, has been used for

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control ripe rot of chili caused by *C. capsici* in Malaysia (Sariah, 1989). The pathogens were decreased sensitivity to benzimidazole fungicide which resulting in poor disease control and become resistant to the fungicide (Beresford, 1994; Ma and Michailides, 2005).

Although, chemical fungicide has high efficiency in disease control, environmental pollution and food safety are still concerned. The alternate approach in disease management has been interested in using biological control method to integrate in disease management to reduce chemical fungicides utilize.

The biofungicide might be used to instead of conventional fungicides (Karasuda *et al*., 2003). Seed-borne diseases of cereal caused by *Drechslera (Pyrenophora) graminea, D. teres, D. avenae, Ustilago avenae*, *U. hordei*, and *Tilletia caries* were suppressed after treated seeds with *Pseudomonas chlororaphis* strain MA 342 (Johnsson *et al*., 1998). The mixture of *Pseudomonas boreopolis* + *Brassica* seed pomace + glycerin + sodium alginate + *Streptomyces padanus* is an effective method to control damping-off of Chinese cabbage which *Rhizoctonia solani* infested soil (Chung *et al*., 2005), the combination of *Trichoderma harzianum* and *P. fluorescens* reported to against *R. solani, Sclerotium rolfsii* and *Macrophomina phaseolina*, root and stem rot disease of soybean (Mishra *et al.*, 2011). *Streptomyces*, the actinobacteria were known to produce a variety of antimicrobial compounds (Jiménez-Esquilín and Roane, 2005) and possessed antagonistic activity to various phytopathogenic fungi (Sabaratnam and Traquair, 2002), several biologically active compounds that have been developed for agricultural use which were majority produced from *Streptomyces* (Ilic *et al*., 2007). Moreover, the commercial products of antagonistic microorganisms were used in formulation of various biofungicides, the commercial fungicide formulated from actinomycetes was also available as Mycostop® (Kemira Agro Oy, Helsinki, Finland) that contains *Streptomyces griseoviridis* L. Anderson (K61) that was reported to suppress soilborne fungal pathogens, *Alternaria, Botrytis, Fusarium, Phomopsis* (Tahvonen and Avikainen, 1987), *Pythium, Phytophthora,* and *Rhizoctonia* in ornamental and vegetable crops (Tahvonen and Lahdenpera, 1988).

The objectives of this study were to screen the effective *Streptomyces* to control chilli anthracnose caused by *Colletotrichum gloeosporioides* and to formulate as biofungicide for evaluation of disease control in pot experiment.

Materials and methods

Preparation of pathogen-inocula

Inoculum of *Colletotrichum gloeosporioides* isolate TPCMCg60 was prepared by culturing on potato dextrose agar (PDA) plate and incubated at room temperature for 14 days. After 14 days, plates were flooded with sterile distillated water and conidia suspensions were harvested. Conidial suspension was determined by using haemacytometer and adjusted to $1x10^6$ conidial/ml with sterile distillated water for inoculation.

Preparation of antagonist

The six effective *Streptomyces* strains, NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 were cultured on ISP-2 medium which consisted of glucose 4 g, yeast extract 4 g, malt extract 10 g, $CaCO_3$ 2 g, agar 20 g/L (Suwan *et al*. 2012) and incubated for 21 days at room temperature spore mass were harvested by scraped from agar surface and determined by using dilution plates method.

Preparation of biofungicide

Biofungicides were separately formulated as powder formulation by using six *Streptomyces* strains (NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6). The spore mass suspensions of each *Streptomyces* strain was separately mixed and homogenized. The homogenized powder were sieved through a nylon screen to obtain particles of a uniform size and sterilized at 121^o C, 15 lbs for 30 min and stored at room temperature before used.

Viability of Streptomyces- Biofungicides

Viability of biofungicides were periodically checked by using dilution plate method on ISP-2 medium at 1 and 16 weeks after formulation.

Evaluation of Streptomyces-biofungicide to control chili anthrcnose in pot experiment

The *Streptomyces*-biofungicide namely NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6, were evaluated to control chilli anthracnose caused by *Colletotrichum gloeosporioides*.

The thirty-day-old chili plants were grown in sterililized soil and inoculated the inoculum of *C. gloeosporioides* isolate TPCMCg60 at concentration of 10^6conidia/ml, the inocula were sprayed over the canopy (2 ml/plant).

Two factor factorial experiments in Randomized Completely Block Design (RCBD) were performed with four replications. Factor A was *Streptomyces*-biofungicide designed as A1=NSP-4, A2=NSP-1, A3=NSP-2, A4=NSP-6, A5=NSP-5 and A6=NSP-3. Factor B was application rates designed as B1 = 0, B2 = 0.5 $g{\cdot}L^{-1}$, B3 = 1.0 $g{\cdot}L^{-1}$ and B4 = 2.0 $g{\cdot}L^{-1}$ which sprayed at every 15 days until harvest. Data were gathered as plant height (cm), number of fruits per plant, fresh weight of fruit (g) per plant, plant fresh/dry weight (g) root fresh/dry weight (g) and root length (cm). The increased in plant growth parameters (IPG) percentages were calculated as follows:-

IPG = treated biofungicide – non-treated one / treated biofungicide X 100.

Number of infected fruits per plant was collected and expressed as per cent of infected fruit per plant. Disease incidence of infection on fruit per plant was evaluated on harvesting day based on the rating scale which modified from Gopinath *et al*. (2006) as follows: - level 1= non infected fruit per plant, level 2= 1-25% infected fruit per plant, level 3= 26-50% infected fruit per plant, level 4= 51-75% infected fruit per plant and level 5= 76-100% infected fruit per plant.

Data were subjected to analysis of variance and treatment mean were compared using Duncan's Multiple Range Test (DMRT) at p=0.05 and p=0.01.

Results

Six biofungicides from *Streptomyces* spp. were formulated as a wettable powder namely NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 and tested for their viability. Results revealed that at first week day of formulation the viable of living *Streptomyces* spp. in biofungicides NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 were 1.4 $x10^5$, 1.1 $x10^5$, 7.4 $x10^5$, 54.7 $x10^5$, 1.8 $x10^5$ and 2.8 $x10^5$ $cfu.g^{-1}$, respectively. After 16 weeks of storage viability of *Streptomyces* spp. in biofungicides NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 were 45.1 $x10^5$, 33.1 $x10^5$, 5.9 $x10^5$, 69.8 $x10^5$, 35.0 $x10^5$ and 79.2 $x10^5$ $cfu.g^{-1}$, respectively.

Evaulation of Streptomyces biofungicide to control chili anthrcnose in pot experiment

Six biofungicides from *Streptomyces* spp. namely NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 were separately treated on chili plants inoculated with spore suspensions of *C. gloeosporioides* in pot experiment.

Efficiency of biofungicides to control chili anthracnose were evaluated and the results revealed that all of biofungicides:- NSP-1, NSP-2, NSP-3, NSP-4, NSP-5 and NSP-6 had lower number of infected fruits per plant. The lowest infected fruits per plant were found in all concentration (0.5-2.0 $g.L^{-1}$) of NSP-1, NSP-3, NSP-5 and NSP-6 as zero percent and significantly lower than non-treated control.

Disease incidences were significantly reduced after treated with biofungicied and biofungicides NSP-1, NSP-3, NSP-5 and NSP-6 at concentration of 0.5-2.0 $g.L^{-1}$ and gave significantly lowest infected fruit per plant (Table 1, Figure 1).

Table 1. Efficiency of biofungicides to control chili anthracnose

Treatment		Infected fruit (fruit/plant)		Infected fruit per plant (%)		Disease incidence[1/]	
NSP-1	0 g/L	1.00[2/]	c	100.00	a	5.00	a
	0.5 g/L	0.00	d	0.00	c	1.00	d
	1.0 g/L	0.00	d	0.00	c	1.00	d
	2.0 g/L	0.00	d	0.00	c	1.00	d
NSP-2	0 g/L	1.50	b	83.33	ab	4.50	ab
	0.5 g/L	0.25	d	5.00	c	1.25	d
	1.0 g/L	0.00	d	0.00	c	1.00	d
	2.0 g/L	0.00	d	0.00	c	1.00	d
NSP-3	0 g/L	1.75	ab	87.50	a	4.50	ab
	0.5 g/L	0.00	d	0.00	c	1.00	d
	1.0 g/L	0.00	d	0.00	c	1.00	d
	2.0 g/L	0.00	d	0.00	c	1.00	d
NSP-4	0 g/L	1.00	c[3/]	62.50	b	3.50	c
	0.5 g/L	0.25	d	12.50	c	1.50	d
	1.0 g/L	0.25	d	8.33	c	1.50	d
	2.0 g/L	0.00	d	0.00	c	1.00	d
NSP-5	0 g/L	1.75	ab	87.50	a	4.25	b
	0.5 g/L	0.00	d	0.00	c	1.00	d
	1.0 g/L	0.00	d	0.00	c	1.00	d
	2.0 g/L	0.00	d	0.00	c	1.00	d
NSP-6	0 g/L	2.00	a	93.75	a	4.75	ab
	0.5 g/L	0.00	d	0.00	c	1.00	d
	1.0 g/L	0.00	d	0.00	c	1.00	d
	2.0 g/L	0.00	d	0.00	c	1.00	d
CV (%)		85.81		70.20		27.12	

[1/] Disease incidence based on a disease rating scale: 1= non infected fruit per plant, 2= 1-25% infected fruit per plant, 3= 26-50% infected fruit per plant, 4= 51-75% infected fruit per plant and 5= 76-100% infected fruit per plant

[2/] Average of four replications. Means with the same common letter in each column are not significantly different according to Duncan's multiple range test at p = 0.05.

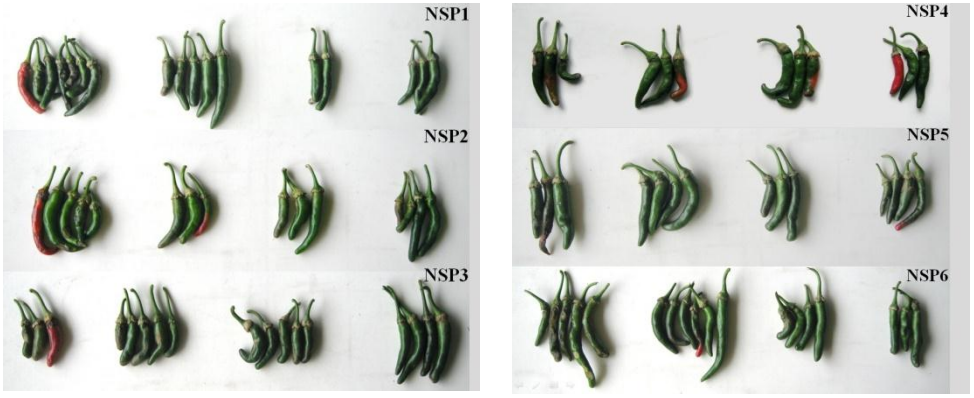

Fig. 1 Fruits of long cayenne chili after treated with biofungicide. T1=non bio-fungicide treated, T2= bio-fungicide treated at the rate of 0.5 g·L^{-1}, T3= bio-fungicide treated at the rate of 1.0 g·L^{-1}and T4= bio-fungicide treated at the rate of 2.0 g·L^{-1}.

Plant growth parameters at harvested were collected and the results revealed that after treated chili plants with NSP-2 at 2.0 g.L^{-1} significantly gave highest stem fresh weight as 89.25 g and biofungicide NSP-3 concentration at 0.5 g.L^{-1}gave 50.54% increasing when compared with non-treated control. The highest stem dry weight was found after treated with 0.5 g.L^{-1} of NSP-6 as 26.0 g and treated with 0.5 g.L^{-1} of NSP-3 gave highest stem dry weight increasing as 52.25% (Table 2). After treated chili plants with NSP-2 at 2.0 g.L^{-1} significantly gave highest root fresh weight as 21.25 g and treated with 0.5 g.L^{-1}of NSP-2 also gave 63.87% increasing when compared with non-treated control. The highest root dry weight was found after treated with 0.5 g.L^{-1} of NSP-2 as 5.5 g and treated with 0.5 g.L^{-1} of NSP-6 gave highest stem dry weight increasing as 79.17% (Table 3). Moreover, the longest root length were found after treated with NSP-3 at 2.0 g.L^{-1} as 34.50 cm and treated with 2.0 g.L^{-1} of NSP-6 gave 46.40% increasing when compared with control (Table 2).

Table 2 Growth parameters after treated of *Streptomyces* biofungicides formulation

Treatments		Stem				Root				Root length	
		Fresh weight		Dry weight		Fresh weight		Dry weight			
		(g)	(%)[1/]	(g)	(%)	(g)	(%)	(g)	(%)	(cm)	(%)
NSP-1	0 g/L	48.50ef[2/]	-	13.00jk	-	10.00fg	-	2.00g-j	-	20.00ij	-
	0.5 g/L	43.00fg	16.19cd	10.75kl	10.54d	10.00fg	10.75de	1.38ij	25.63cd	21.00h-j	8.69e
	1.0 g/L	70.25a-d	40.96a-c	24.50ab	19.64b-d	15.50b-e	34.92c	3.00c-h	42.40bc	24.75f-i	8.87e
	2.0 g/L	54.00c-f	31.61a-c	18.50e-h	24.66b-d	15.25b-e	28.71c	2.38e-j	41.67bc	25.25e-i	24.07 b-d
NSP-2	0 g/L	52.50de-	-	19.50c-g	-	14.50b-f	-	3.50c-f	-	24.38f-i	-
	0.5 g/L	71.50a-d	42.05ab	21.25b-f	35.29a-d	18.25a-c	63.87a	5.00ab	34.38bc	26.00d-h	14.23 de
	1.0 g/L	67.00b-e	44.94ab	17.25f-j	23.44b-d	17.50a-d	47.97b	3.75b-e	29.69bc	31.25a-d	23.51 b-d
	2.0 g/L	89.25a	46.53ab	23.25a-d	28.30a-d	21.25a	50.58b	5.50a	29.69bc	29.00a-f	22.10 c-e
NSP-3	0 g/L	51.75d-f	-	13.75i-k	-	12.75d-g	-	3.25c-g	-	27.25b-g	-
	0.5 g/L	73.25a-c	50.54a	24.50ab	52.25a	15.50b-e	52.97ab	3.50c-f	74.46a	32.50ab	25.85 b-d
	1.0 g/L	58.00b-f	44.27ab	19.00d-g	29.38a-d	16.25a-e	53.61ab	3.50c-f	45.57bc	26.75c-g	14.81 de
	2.0 g/L	68.00b-e	35.68a-c	22.00a-e	42.80ab	18.50ab	50.73b	3.25c-g	48.44b	34.50a	37.18 ab
NSP-4	0 g/L	48.50ef	-	16.25g-j	-	10fg	-	2.625d-i	-	22.75g-i	-
	0.5 g/L	40.75fg	27.99bc	16.50g-j	27.50a-d	13.50b-g	33.36c	1.75h-ij	7.03de	22.88g-i	7.76e
	1.0 g/L	27.50g	27.46bc	6.75l	38.54a-c	8.75g	24.23c	2.25f-j	28.91b-d	20.88h-j	26.98 b-d
	2.0 g/L	48.25ef	2.03d	10.75kl	30.21a-d	10.00fg	9.72e	1.00j	1.56e	17.00j	15.92 de
NSP-5	0 g/L	57.50b-f	-	17.00f-j	-	14.25b-f	-	3.50c-f	-	27.25b-g	-
	0.5 g/L	77.25ab	25.34bc	23.75abc	40.28abc	15.25b-e	23.14cd	2.75d-i	45.83bc	25.00e-i	35.92 a-c
	1.0 g/L	48.25ef	14.21cd	18.00e-i	26.59b-d	14.50b-f	22.36c-e	3.00c-h	40.00bc	27.50b-g	25.47 b-d
	2.0 g/L	60.00b-f	14.68cd	23.50a-c	35.65a-c	18.25a-c	30.94c	3.00c-h	41.67bc	30.38a-e	24.87 b-d
NSP-6	0 g/L	68.00b-e	-	22.00a-e	-	16.50a-e	-	4.00b-d	-	31.75a-c	-
	0.5 g/L	75.50ab	32.02a-c	26.00a	38.61a-c	17.25a-d	48.53b	3.13c-h	79.17a	25.25e-i	33.53 a-c
	1.0 g/L	52.25d-f	14.41cd	14.50h-k	17.77cd	11.50efg	29.88c	3.25c-g	74.17a	27.88b-g	35.89 a-c
	2.0 g/L	57.50b-f	29.05a-c	15.50g-j	33.91a-d	13.25c-g	55.46ab	4.25a-c	70.83a	27.50b-g	46.40 a
CV.(%)		24.05	52.75	17.44	56.77	24.26	24.31	32.60	36.86	14.93	42.47

[1/] Increased in growth parameter (IPG) percentages (IPG = treated biofungicide – non-treated one / treated biofungicide X 100)

[2/] Average of four replications. Means with the same common letter in each column are not significantly different according to Duncan's multiple range test at p = 0.05

The yield of chili fruits were collected and the results demonstrated that the highest fruit number were found after treated with 0.5 $g.L^{-1}$ of NSP-3 as 8 fruits/plant and treated with 2.0 $g.L^{-1}$ of NSP-2 gave 79.17% increased in yield when compared with non-treated control, significantly (Table 3).

Fruit weight were significantly increased after treated with NSP-3 at 0.5 $g.L^{-1}$ and NSP-6 at 2.0 $g.L^{-1}$ gave highest percent increased as 21 g/plant and 71.37%, respectively when compared with control. Besides, the longest fruit length were significantly increased after treated with NSP-2 at 0.5 $g.L^{-1}$ and as 7.45 cm and NSP-6 at 0.5 $g.L^{-1}$ gave highest percent increased as 50.81%, respectively when compared with control (Table 3).

Table 3 Efficiency of biofungicides on yield of chilli at harvesting day of 120 days

Treatment		Fruit number (/plant)		Fruit weight(/plant)		Fruit length	
		(fruit)	(%)[1/]	(g)	(%)	(cm)	(%)
NSP -1	0 g/L	2.25f-i[2/]	-	4.75j	-	3.45j	-
	0.5 g/L	2.00g-i	52.50a-c	7.50e-j	14.88i	3.13j	32.06b-g
	1.0 g/L	3.25d-h	20.00c-e	9.50d-i	23.07g-i	5.83b-e	29.34c-g
	2.0 g/L	4.50b-e	61.91ab	6.50g-j	58.10a-c	6.00a-d	44.75a-d
NSP -2	0 g/L	2.75e-i		11.00def		5.50c-g	-
	0.5 g/L	4.25b-f	62.50ab	15.25bc	40.66d-f	7.45a	17.77g
	1.0 g/L	5.00b-d	63.75ab	10.50d-g	18.48hi	6.90a-c	22.79e-g
	2.0 g/L	3.25d-h	79.17a	12.00cd	60.94ab	4.18f-j	36.99a-f
NSP -3	0 g/L	5.25b-d	-	16.75b	-	7.10ab	-
	0.5 g/L	8.00a	27.50b-e	21.00a	36.61e-g	5.18d-h	23.19e-g
	1.0 g/L	5.50bc	56.19a-c	13.25b-d	33.65f-h	5.08d-i	21.73fg
	2.0 g/L	4.00b-fg	48.33a-d	17.00ab	53.08b-d	6.13a-d	47.78a-c
NSP -4	0 g/L	1.75hi	-	7fghij	-	4.225fghij	-
	0.5 g/L	2.00g-i	36.56b-d	5.50ij	24.17ghi	3.90hij	25.62efg
	1.0 g/L	1.00i	42.71a-d	6.25hij	36.46efg	4.40efghij	29.30cdefg
	2.0 g/L	2.25f-i	56.98a-c	5.50ij	40.83def	3.60ij	31.34bcdefg
NSP -5	0 g/L	3.25d-h	-	9.50d-i		6.75a-c	
	0.5 g/L	5.00b-d	51.25abcd	11.50c-e	54.48bcd	4.03g-j	47.94a-c
	1.0 g/L	4.25b-f	3.13e	12.00cd	49.53bcde	6.20a-d	48.15ab
	2.0 g/L	6.00ab	58.75ab	13.50b-d	61.74ab	7.38a	28.06d-g
NSP -6	0 g/L	3.75c-h	-	11.50c-e	-	6.25a-d	-
	0.5 g/L	2.50e-i	28.96b-e	7.25f-j	61.16ab	5.63b-f	50.81a
	1.0 g/L	3.25d-h	14.06de	7.88e-j	44.74c-f	6.03a-d	40.56a-e
	2.0 g/L	3.25d-h	55.12a-c	10.25d-h	71.37a	6.15a-d	28.57d-g
CV (%)		39.48	59.25	27.43	25.18	19.37	39.06

[1/] Increased in yield (IPG) percentages (IPG = treated biofungicide – non-treated one / treated biofungicide X 100)
[2/] Average of four replications. Means with the same common letter in each column are not significantly different according to Duncan's multiple range test at p = 0.05

The plant height rate of chili at harvested were increased when treated with the six biofungicides (Table 4). The highest plant height rate was demonstrated when treated with 2.0 $g.L^{-1}$ of NSP-1 biofungicide as 89.73% and followed by treated with 1.0 $g{\cdot}L^{-1}$ of NSP-4, 2.0 $g{\cdot}L^{-1}$ of NSP-3, 0.5 $g{\cdot}L^{-1}$of

NSP-6, 1.0 g·L^{-1} of NSP-2 and 1.0 g·L^{-1} of NSP-5 as 88.76%, 88.02%, 87.38%, 86.48% and 85.89%, respectively (Table 5).

Discussion

The viability of *Streptomyces* in six biofungicide after 16 weeks were markedly reduced after survived up at 4 weeks for biofungicide from *Streptomyces* strain NSP-3, NSP-4, NSP-5 and NSP-6 and biofungicide from *Streptomyces* strain NSP-1, NSP-2 were survived up at 2 weeks after formulation. This result was in agreement with Vidhyasekaran and Muthamilan (1995) which reported that *Pseudomonase fluorescens* in different carriers were survived up to 20 days without any dramatic declined from initial population although declined after 120 days storage. Similarly to report of Sriram *et al*., (2011) imply that the addition of glycerol at 3 and 6% resulted in significantly higher viability of *T. harzianum* with an average of 8.13 and 8.06 Log CFUs g^{-1} of formulation during the shelf-life for up to 8 months, respectively. According to declined of *Streptomyces* in six biofungicide after storage were depended on low stability of propagules in kaolin base-carrier as the report of Sabaratnam and Traquair (2002) found that populations of *Streptomyces* sp. were stable in talcum powder and starch granules over the 10–14 weeks test period and that they were more stable at 4^{o}C than at 24^{o}C.

The six formulations of biofungicide from *Streptomyces* spp. were tested for induced immunity in chili plants which challenged with *C. gloeosporioides* isolate TPCMCg60. The chili plants were treated with the biofungicides exhibited fascinating results in disease control due to reduction of disease incidence and enhance percent of disease reduction. The biofungicide from *Streptomyces* strain NSP-1 at concentrations 0.5 to 2.0 g.L^{-1} gave terrific results for plant immunity induction before pathogen invasion. One possibly mode of action was production of hydrolytic enzyme from antagonistic bacteria. As described previously (Suwan *et al*., 2012) the six *Streptomyces* isolates were provided antifungal activity through affecting colony growth and conidia production of *C. gloeosporioides in vitro*. The *Streptomyces* were exhibited production of hydrolytic enzyme activity that provided probably acting in synergism in the lysis of the fungal cell-wall, chitinase (Yano *et al*., 2008), β-glucanase and cellulase, lead to inhibition of pathogen mycelia growth and disease suppressed (Gomes *et al*., 2000). Chitinase provides antifungal activity and are related to the systemic acquired resistance (SAR) pathway and also bring about liberation of molecules that trigger the first steps of resistance induction, phytoalexins and phenolic compounds (Silva *et al*., 2004). In this study anthracnose symptom and disease severity on chili plants were reduced after treated with the biofungicides from the *Streptomyces* and it was previously

reported that treated of *S. melanosporofaciens* EF-76 on potatoes tuber be able to reduce disease incidence of common scab in the greenhouse and field assay (Beauśejour *et al*., 2003) though, *Streptomyces* were effectively possessed control plant pathogens in laboratory or controlled-environment however in green house or field scale showed less successful to antagonize the pathogens (Doumbou *et al*., 2001). In spite of high capacity to suppress disease in laboratory scale but in large scale the conditions were variable and the biofungicides agents required good preservation method and food base in extended shelf-life (Kolombet *et al*., 2008). Based on viability and shelf-life, the biofungicide *Streptomyces* were tested for viability every 30 days. The result revealed that 30 days after formulations the highest amount were determined and after 60 days were declined gradually in number. At 120 days after formulation, the propagules still remained as same amount of formulation preparation simultaneously.

The six formulation of biofungicide from six *Streptomyces* were clearly demonstrated that chili growth promoting potential and perhaps are referred to as plant growth–promoting rhizobacteria (PGPR) (Van Loon *et al*., 1998). Besides, the stimulating of other growth parameters and yields of chili were excessively observed after treated with the biofungicide formulations. Similarly to report of Ratanacherdchai (2010) imply that biological fungicides such as Bio-CG, Bio-CLT and Bio-T from *Chetomium globosum* N0802, *Ch. Lucknowense* CLT and *Trichoderma harzianum* PC01, respectively were increased in yields of chili in organic crop production and reduced disease incidences. In agreement with the biofungicide from *Coniothyrum minitans* was able to suppressed growth of sclerotia forming fungi i.e. *Sclerotinia sclerotiorum, Sclerotium rolfsii* and *S. cepivorum* in soil infested and caused great increase of root and plant length and fresh weight of survival plants initiating flowering and reproduced pods of bean plants as well as the weight of bean pods (Embaby, 2006). Dependence on pathogen suppressed by antagonistic microbes as biological agents the plant growth was improved (Weller, 1988). Perhaps from this study the biofungicides performed antifungal activity in controlling disease, *Streptomyces* NSP-1 is most likely referred to as plant growth–promoting rhizobacteria (PGPR) (Van Loon *et al*., 1998). The PGPR was able to induce of systemic resistance against plant pathogens in bean, carnation and cucumber (Viswanathan and Samiyappan, 1999). Induced systemic resistance (ISR) was elicited by PGPR induced systemic resistance (ISR) is similar to pathogen-induced systemic acquired resistance (SAR) (Sticher *et al*., 1997; Van Loon *et al*., 1998) in non-infected parts and lead to induced plants more resistant to pathogens infection and are effective against a broad spectrum of root and foliar pathogens (Zhang *et al*., 2002). This study

revealed the successfully efficient of biofungicides from *Streptomyces* spp. in controlling of chili anthracnose in pot experiment. Among the formulation, NSP1 provided significantly good efficiency included increase percent of disease reducing, stimulate plant growth and high fruit quality. Hence, using of NSP1 formulation as biofungicide for control of chili anthracnose in field may be feasible and practical.

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Table 4 Plant height of long cayenne chili after treated with biofungicides

Treatment		Plant height (cm)															
		0 day		15 days		30 days		45 days		60 days		75 days		90 days		105 days		120 days	
NSP-1	0 g/L	7.63	fghijk[1/]	16.38	cdef	25.25	cde	33.25		38.25	abc	54.00	abc	65.75	a	71.00	a	74.50	ab
	0.5 g/L	6.50	k	11.38	i	21.50	defgh	21.25	f	26.50	fg	40.00	ef	47.38	ef	50.75	efg	53.50	ef
	1.0 g/L	8.13	cdefghi	16.25	cdef	27.50	abc	28.00	cdef	30.00	cdefg	38.50	ef	49.88	cdef	49.25	fg	53.25	ef
	2.0 g/L	6.63	jk	14.38	efgh	24.50	cdefg	31.00	abcd	37.50	abcd	52.25	abcd	58.00	abcde	61.50	abcde	65.00	bcde
NSP-2	0 g/L	7.63	fghijk	16.25	cdef	24.75	cdef	28.50	cde	33.25	bcdefg	48.00	abcde	51.00	cde	59.25	abcdef	62.50	bcdef
	0.5 g/L	7.75	efghijk	15.88	cdefg	19.50	h	28.75	cde	34.00	bcdef	44.25	bcdef	55.00	abcde	60.00	abcdef	56.75	def
	1.0 g/L	7.88	defghij	18.13	bc	22.25	defgh	30.25	abcd	37.25	abcd	47.25	bcde	53.88	bcde	59.25	abcdef	63.00	bcdef
	2.0 g/L	8.25	bcdefgh	16.63	cde	24.75	cdef	31.00	abcd	35.75	abcde	46.75	bcde	55.50	abcde	54.00	bcdefg	58.50	cdef
NSP-3	0 g/L	8.88	abcdef	15.00	defgh	22.00	defgh	27.00	cdef	29.75	defg	40.50	ef	49.63	cdef	53.00	cdefg	57.75	cdef
	0.5 g/L	9.00	abcde	17.50	c	25.75	bcde	33.00	abc	38.00	abcd	49.75	abcde	55.25	abcde	62.75	abcd	67.00	bcd
	1.0 g/L	9.13	abcd	16.38	cdef	23.50	cdefgh	29.25	bcd	33.50	bcdefg	45.25	bcde	53.00	cde	58.50	bcdef	66.00	bcd
	2.0 g/L	7.88	defghij	15.75	cdefg	23.25	cdefgh	31.25	abcd	35.75	abcde	49.50	abcde	60.13	abc	58.25	bcdef	64.75	bcde
NSP-4	0 g/L	7.33	hijk	14.50	efgh	21.75	defgh	26.25	cdef	30.75	cdefg	46.00	bcde	52.38	cde	62.75	abcd	66.25	bcd
	0.5 g/L	6.88	ijk	13.75	ghi	20.00	fgh	25.00	def	30.25	cdefg	41.50	def	48.50	def	56.00	bcdefg	59.75	cdef
	1.0 g/L	6.75	jk	13.13	hi	21.75	defgh	27.00	cdef	28.75	efg	40.75	def	48.00	def	54.25	bcdefg	60.00	cdef
	2.0 g/L	6.63	jk	12.88	hi	19.75	gh	22.00	ef	25.25	g	32.75	f	39.75	f	45.50	g	52.25	f
NSP-5	0 g/L	9.50	ab	20.13	ab	31.00	a	36.50	a	38.25	abc	49.50	abcde	58.13	abcde	63.75	abc	73.00	ab
	0.5 g/L	8.50	abcdefgh	17.13	cd	25.00	cde	32.00	abcd	38.00	abcd	49.00	abcde	53.75	bcde	58.00	bcdef	64.25	bcdef
	1.0 g/L	9.13	abcd	18.00	bc	26.25	abcd	32.00	abcd	36.25	abcde	44.50	bcde	56.25	abcde	57.00	bcdefg	60.00	cdef
	2.0 g/L	9.50	ab	17.88	bc	25.25	cde	31.50	abcd	34.00	bcdef	42.75	cdef	48.88	def	52.25	cdefg	55.00	def
NSP-6	0 g/L	9.75	a	21.13	a	30.50	ab	36.00	ab	41.50	ab	59.50	a	64.25	ab	70.75	a	80.25	a
	0.5 g/L	8.75	abcdefg	17.38	cd	27.50	abc	36.00	ab	42.75	a	55.25	ab	58.88	abcd	65.00	ab	69.50	abc
	1.0 g/L	9.38	abc	15.00	defgh	22.75	cdefgh	30.50	abcd	32.75	cdefg	44.25	bcdef	50.38	cdef	53.00	cdefg	58.75	cdef
	2.0 g/L	7.50	ghijk	14.00	fgh	21.25	efgh	28.50	cde	32.25	cdefg	44.75	bcde	48.75	def	51.75	defg	57.00	def
CV (%)		11.28		11.05		14.02		16.73		17.31		18.05		14.87		14.47		13.88	

[1/]Average of four replications. Means with the same common letter in each column are not significantly different according to Duncan's multiple range test at p = 0.05

Table 5 Plant height rate of long cayenne chili after treated with biofungicides

Treatments		Plant height rate (%)[1/]													
		15 day		30 days		45 days		60 days		75 days		90 days		105 days		120 days	
NSP-1	0 g/L	46.06	defgh	57.29	efg	63.87	ef	64.97	fgh	77.11	efg	80.92	def	82.524	fg	83.351	def
	0.5 g/L	50.97	abcdef	67.72	abc	69.66	bcde	74.93	abcde	83.72	abcd	85.94	abc	86.968	abcde	87.533	abcd
	1.0 g/L	51.09	abcdef	70.09	ab	70.31	bcde	72.00	bcdefg	78.67	cdef	83.43	bcde	83.605	defg	84.722	bcdef
	2.0 g/L	54.19	abc	72.76	a	78.50	a	82.13	a	87.19	a	88.50	a	89.124	ab	89.732	a
NSP-2	0 g/L	49.52	abcdefg	60.99	cdefg	64.95	def	64.41	gh	76.07	fg	78.37	f	81.498	g	83.411	def
	0.5 g/L	55.52	abc	63.00	bcdef	72.63	abc	76.74	abcd	83.12	abcde	84.59	abcd	87.028	abcde	84.139	cdef
	1.0 g/L	56.88	a	65.69	abcd	73.97	ab	78.40	ab	82.92	abcde	83.55	bcde	90.238	a	86.479	abcdef
	2.0 g/L	56.03	ab	68.89	abc	74.72	ab	78.11	ab	83.91	abc	85.00	abcd	86.412	abcdef	85.955	abcdef
NSP-3	0 g/L	50.78	abcdef	65.15	abcde	69.45	bcde	69.45	cdefgh	78.69	cdef	81.55	cdef	83.399	defg	84.571	bcdef
	0.5 g/L	51.38	abcdef	66.14	abcd	72.82	abc	77.06	abc	82.71	abcde	82.64	cdef	85.005	bcdefg	85.445	bcdef
	1.0 g/L	53.44	abcd	66.26	abcd	72.85	abc	76.13	abcd	81.90	abcdef	84.25	abcde	85.468	bcdefg	85.888	abcdef
	2.0 g/L	52.57	abcde	66.04	abcd	71.76	abcd	74.90	abcde	80.71	bcdef	82.36	cdef	83.829	defg	84.093	cdef
NSP-4	0 g/L	42.59	gh[2/]	54.39	g	59.77	f	63.75	h	72.25	g	78.34	f	81.189	g	83.555	def
	0.5 g/L	55.70	abc	66.69	abcd	73.62	abc	76.87	abc	83.74	abc	85.81	abc	87.692	abcd	88.487	ab
	1.0 g/L	51.50	abcdef	68.41	abc	74.64	ab	76.15	abcd	83.14	abcde	85.94	abc	87.557	abcd	88.755	ab
	2.0 g/L	45.85	efgh	66.12	abcd	69.07	bcde	73.03	bcdef	79.39	cdef	83.07	cdef	85.286	bcdefg	87.248	abcde
NSP-5	0 g/L	40.65	h	55.43	fg	60.98	f	66.90	efgh	77.11	efg	81.79	cdef	82.992	efg	83.552	def
	0.5 g/L	52.64	abcde	66.30	abcd	72.83	abc	76.64	abcd	82.37	abcde	84.53	abcde	86.333	abcdef	86.142	abcdef
	1.0 g/L	48.26	cdefg	64.11	bcde	69.34	bcde	73.57	bcde	79.13	cdef	82.35	cdef	84.278	cdefg	84.598	bcdef
	2.0 g/L	54.73	abc	67.02	abc	74.96	ab	78.51	ab	85.76	ab	87.91	ab	88.569	abc	88.018	abc
NSP-6	0 g/L	44.79	fgh[1/]	58.59	defg	66.13	cdef	68.64	defgh	77.64	defg	79.79	ef	81.486	g	83.134	ef
	0.5 g/L	51.49	abcdef	68.17	abc	75.68	ab	79.49	ab	84.17	abc	85.20	abcd	86.524	abcdef	87.382	abcde
	1.0 g/L	50.50	abcdef	63.23	bcdef	71.47	abcde	74.88	abcde	82.52	abcde	85.26	abcd	85.919	abcdef	82.995	f
	2.0 g/L	48.90	bcdefg	64.27	bcde	73.25	abc	76.18	abcd	83.05	abcde	85.11	abcd	86.105	abcdef	86.752	abcdef
CV.(%)		10.49		8.92		7.70		7.89		5.32		4.07		3.66		3.52	

[1/] Plant height rate (%)=(plant height at recored day- plant height at initial day)/ plant height at record day x 100

[2/]Average of four replications. Means with the same common letter in each column are not significantly different according to Duncan's multiple range test at p = 0.05