
Application of mycofungicide to control late blight of potato

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Late blight of potato in northern Thailand is caused by *Phytophthora infestans* and leads to economic damage in large areas of potato planted. The pathogen infects all stages of plant growth which show symptoms of late blight, stem rot and tuber rot and can result in 100% yield loss even with the use of chemical fungicides. Laboratory based bi-culture antagonistic tests showed that Chaetomium-mycofungicide inhibited pathogen growth by more than 53% over 10 days. In field trials, we used a Chaetomium-mycofungicide for disease control in combination with biological fertilizers comprising 12 effective strains of cellulose degrading fungi and specific fungi for plant growth stimulants. The mixture significantly reduced disease indices in infected fields when compared with the non-treated control and the difference between Chaetomium-mycofungicide and chemical pesticide treated fields was not significant. Three Chaetomium-mycofungicide/biological fertilizer treatments were tested and compared with no treatment and a chemical pesticide treatment. Bio-technique 1 reduced late blight incidence by 34% and pathogen colonization by 56.5% leading to an increase in yield of 49%. Bio-technique 2 reduced late blight incidence by 15.5% and reduction colonization by 29% leading to an increased yield of 49%. Bio-technique 3 reduced late blight incidence by 19% and pathogen colonization by 29% leading to an increased yield of 45.5%. Chemical pesticide treatment reduced late blight incidence by 34.5% and pathogen colonization of 51.5% leading to an increased yield of 52.5%. Non-treatment gave the lowest yield due to high late blight incidence. It is recommended that the use of Bio-IPM techniques could solve the problem of disease epidemics in the infested areas planted to potato in the cool tropics.

Key words: biological fertilizers, *Chaetomium*, late blight of potato, mycofungicide

Introduction

The main problem of growing potato worldwide are economic losses due to late blight, which is caused by *Phytophthora infestans* which can destroy all parts of potato plants (*Solanum tuberosum* L.) within two weeks in wet conditions (Hooker, 1981; Fry *et al.*, 1993; van der Zaag, 1996). *Phytophthora infestans* can survive under adverse conditions and over winter in the form of

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oospores. The pathogen however, invades and infects potato plants in the field via zoosporangia which disperse via soil water, rain splash and wind (van der Zaag, 1996). The zoosporangia may directly germinate on potato organs or produce zoospores in sporangium, which are motile and disperse, following encystment, germination and host penetration within 2-3 hours under favorable conditions of high relative humidity, rain or sprinkler irrigation. Infection occurs when leaves are moist for at least 5 hours at 15-20°C. Spore germination results in colonization and infection causing symptoms on leaves, stem or tubers and production of new spores within 4-5 days (Rich, 1983). Potato plants infected with *Phytophthora infestans* may also show wilt symptoms which start in younger leaves leading to stunted plants and leaf chlorosis. If the tuber seed potatoes are infected, the emerging seedlings wilt after emergence, becoming infected through the vascular tissue, and finally gummosis occurs from the tuber buds after harvest (van Derzaag, 1996).

Late blight disease has been controlled using chemical fungicides at seed dressing and from interval spraying until harvest e.g. Metalaxyl (systemic fungicide) (Milgroom and Fry, 1988), Fostyl A-1, Mancozeb, Fentin-acetate phosphate, Chlorotalonyl and Captafol (Samoucha and Cohen, 1986). The use of chemical fungicides has resulted in an increased degree of pathogen resistance (Levy *et al.*, 1983). Control of the pathogen population below economic damage levels is then still not possible and has led to low yields or even no yield (Rotem and Bashi, 1983). There has been research to control the disease problem using microbial antagonists e.g. *Trichoderma harzianum* and *T. viride* to control *P. infestans* late blight of potato (Singh, 1986; Mukerji and Garg, 1988). Some reports have stated that *Aspergillus terreus* and *Penicillium oxalicum* could inhibit the growth of *P. infestans* in potato (Roy *et al.*, 1991). Other promising microbial antagonists are *Chaetomium* spp. As they can degrade cellulolytic plant debris in high organic soils and specific isolates can inhibit several plant pathogens (Soytong and Quimio, 1989). For example, *C. globosum* and *C. cochlioides* can inhibit the growth of *Fusarium* sp. and *Helminthosporium* sp. (Tveit and Moore, 1954). Seed dressing with *C. globosum* can prevent seedling blight of corn caused by *Fusarium roseum* f. sp. *cerealis* 'graminearum' (Chang and Kommedahl, 1968). It was also stated that spraying the ascospore suspension of *C. globosum* to apple trees can reduce apple scab caused by *Venturia inaequalis* (Heye and Andrews, 1983; Cullen and Andrews, 1984; Boudreau and Andrews, 1987). It has also been reported that *C. globosum* produces metabolites that inhibit the growth of *Pythium ultimum* which causes damping-off of sugar beet (Di-Pietro *et al.*, 1991), *Rhizoctonia solani* (Walter and Gindrat, 1988), leaf blight of brassicas caused by *Alternaria brassicicola* (Vannacci and Harman, 1987) and can reduce the pathogenic

inoculum of *Botrytis cinerea* on deadly lily leaves in the field (Kohl *et al.*, 1995). *Chaetomium cupreum* is also an interesting species as it has been reported to control soybean plant pathogens e.g. *Phomopsis* and *Colletotrichum* spp. (Manandhar *et al.*, 1986).

The International Foundation for Science in Sweden has supported research on biological control of plant diseases in Thailand using *Chaetomium* spp. as microbial antagonists since 1991. Strains of *C. cupreum* and *C. globosum* have been reported to reduce leaf spot disease of corn caused by *Curvularia lunata*, rice blast caused by *Pyricularia oryzae* and sheath blight of rice caused by *Rhizoctonia oryzae* (Soytong, 1989, 1992a). These strains have also been shown to reduce tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* in the greenhouse and field (Soytong, 1990, 1992b) and reduce basal rot of corn caused by *Sclerotium rolfsi* (Soytong, 1991). Twenty-two strains of *C. cupreum* and *C. globosum* were formulated in the form of biopellets and biopowder as a new broad spectrum mycofungicide for plant disease control (Soytong and Soytong, 1997). This can be used instead of the chemical fungicides to control tomato wilt and basal rot of corn (Soytong, 1991). *Chaetomium*-mycofungicide completely prevented root rot caused by *Phytophthora* spp., e.g. root rot of durian (Pechprome and Soytong, 1997), black pepper (Sodsaard and Soytong, 1999) and tangerine (Soytong *et al.*, 1999). The objectives of this study were to identify the main diseases of potato crops in Thailand agriculture and to develop biological integrated pest management (BioIPM) techniques to control potato diseases without the use of chemical pesticides.

Materials and methods

Isolation, identification and pathogenicity testing

Soil samples were collected at random in the field trial covering an area of 3.2 hectares in Chiang Mai Province. Potato blight caused by *Phytophthora infestans* had seriously destroyed potato harvests leading to 100% loss over the previous few years. Thirty soil samples (200 g) at the soil depth of 10-20 cm were collected every 30 days until harvest and brought to laboratory at King Mongkut's Institute of Technology Ladkrabang, Bangkok. Soil samples were dried and ground to fine particles; 10 g of each sample was then placed to sterilized Petri dishes; 20 ml of sterilized distilled water and leaf disks of potato cv. Atlanta (1 × 1 cm) were added; and incubated at 27°C for 3-5 days. Leaf pathogens were observed and isolated into pure culture and identified where possible. Isolation of pathogens from disease plant parts was also carried

out using a soil plate assay for fungal isolation and cross streak technique for bacteria. Isolates were confirmed to be pathogenic using Koch's Postulate.

Testing of Chaetomium mycofungicide to control the disease in laboratory

Chaetomium-mycofungicide was tested for its ability to inhibit mycelium growth and spore production of *P. infestans* causing potato late blight. Tests were done *in vitro* using a bi-culture antagonistic test as described by Soyong, (1989). The percent inhibition (PI) of mycelium growth and spore production was calculated after incubation of the plates at 27°C for 10 days. There were five replications with controls consisting of either Chaetomium mycofungicide or the pathogen alone on PDA. The experiments were arranged in a completely randomized design (CRD) and the analysis of variance of colony diameter and spore production was computed, then treatment means were compared using the LSD test at P = 0.05 and P = 0.01.

Evaluation of Chaetomium mycofungicide to control late blight of potato in the infested field soils

Potato seeds cv. Atlanta were used in this study. The experimental plots covered 3,200 m² of infested field-soil in Chiang Mai Province. The experiments were carried out using a randomized complete block design (RCBD) with four replications, and five treatments as follows:

T1 = control (without chemical pesticides, only apply chemical fertilizers 19-19-19, 15-15-28)

T2 = chemical pesticides with chemical fertilizers 19-19-19, 15-15-28)

T3 = bio-technique 1: mycofungicide (Chaetomium) and biofertilizer combination with chemical fertilizer either 19-19-19 or 15-15-28 at the ratio of 2:1

T4 = bio-technique 2: mycofungicide (Chaetomium) and biofertilizer combination with chemical fertilizer either 19-19-19 or 15-15-28 at the ratio of 1:1 and

T5 = bio-technique 3: mycofungicide (Chaetomium) and biofertilizer without chemical fertilizer.

Application of biofertilizer and chemical fertilizer was separately carried out in each treatment. The biofertilizer was applied during soil preparation before planting the tuber seeds at the rate of 600 kg/hectares. Chaetomium

mycofungicide in powder form was applied at the rate of 10 g/20 L of water. Weeds were removed by hand. The harvesting time was 90 days after planting.

Data collections were maintained to evaluate the disease index (DI) of late blight infection using a modification of the method of Sanyong (1992) as follows: level 1 = green and healthy leaves, level 2 = blight symptom on leaves 1-25%, level 3 = blight symptom on leaves 26-50%, level 4 = blight symptom on leaves 51-75%, and 5 = blight symptom on leaves 76-100% or dead plants and also plant height (cm) and yield (kg) was recorded. The diseased plant parts from leaves, stem and tubers were collected to diagnose and isolate the pathogen. Soil samples were collected every 30 days after planting to evaluate pathogen colonization of the soils. The experiment was repeated two times.

Results

Isolation, Identification and Pathogenicity tests

Late blight and dry rot of potato caused by *P. infestans* rapidly infected plants, spreading to all parts of potato plants and completely destroying the whole plants within 2 weeks. The pathogen infected above and belowground parts of the plant, with infection usually starting on the leaves, stems, and then tubers leading to reduction in tuber yield. The symptoms on the leaves started as small spots, which become water-soaked and then the lesions expanded to 1-2 cm diameter in favorable environments, especially with early morning dew. Sporangiohores and sporangia were produced from the lower surface of the leaves. Lesions expanded into irregular water-soaked circular areas, eventually expanding to become irregular lesions with leaf chlorosis and eventually blighted. When the weather was less favorable for disease development (e.g. temperatures over 25°C during the dry season, the pathogen first infected leaf tissue, with leaves turning brown at the beginning of plant growth. The fungal mycelia produced less spores, and lesions showing chlorosis and late blight

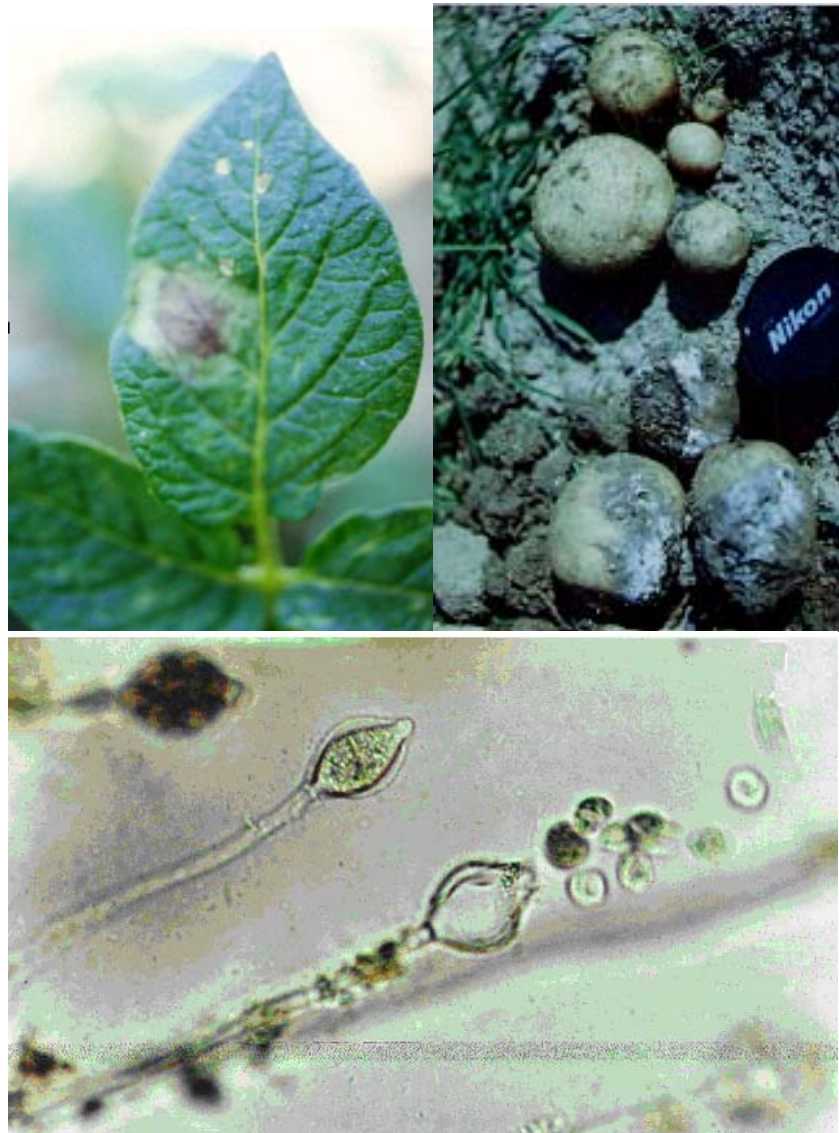


Fig. 1. Symptoms of late blight caused by *Phytophthora infestans* on leaves (upper left) and dry rot on potato tuber (upper right). Sporangiphore, sporangium of *Phytophthora infestans* (lower part).

were not clearly seen. When these diseased plant parts were, however, collected and placed in a moist chamber, the leaf infected with *P. infestans* produced white mycelia within 24 hours. The symptoms on stems were grayish brown to blackish-brown elongate lesions. In moist weather, the fungal mycelia grew well on the infected leaves and produced a lot of spores, and spread to other plant parts such as tubers.

Potato tubers were infected by the pathogen through lenticels, eyes and cracks on the tuber surface. The lesion first appeared on the tuber and caused cell death, penetrating from the surface into the tubers (Fig. 1). Late blight of potato tubers occurred in the field and after harvest; lesions appeared during storage and invaded healthy potato tubers leading to dry rot. Secondary pathogens, such as bacterial soft rot, usually infected the lesions of blighted tubers. The infected potato tuber may appear to be caused by nematode tuber rot or pink eye including herbicide injury. *Phytophthora infestans* can survive under unfavorable conditions, such as cold or hot weather; the resistant oospores over season in soil.

However, the pathogen can spread out and directly infect to the potato plants in the field via zoosporangia which disperse by wind or raindrops or by zoospores that are released from sporangium (Fig. 1). Infection occurs under moist conditions, heavy dew, rain or sprinkler irrigation, the cysts of zoospores germinating within 3 hours and colonizing plant tissues. When potato leaves remain moist for 4-5 hours at 15-20°C, they easily become infected by the pathogen. The life cycle starts from spore germination until symptoms appear and produce a new spore generation within 4-5 days.

Phytophthora infestans has been found in the soil in high populations. The colony on PDA is flattened, forming a grayish-white colony within 3 days. The hyaline hyphae are non-septate; sporangia release zoospores after being transferred on agar plugs into sterilized distilled water and incubated at 10-12°C. The resistant structures are oospores. All isolates have been shown to be virulence to potato cv. Atlantic which shows blight symptoms and dry rot on the potato tubers. Other pathogens have been found to be secondary invaders e.g. *Fusarium* sp., *Erwinia* sp. and *Pseudomonas solanacearum*.

Testing of Chaetomium mycofungicide to control the disease in laboratory

Results show that *Chaetomium* mycofungicide inhibits mycelium growth of *P. infestans* by 42.91% and inhibits inoculum production by 53.3% percent within 10 days. Mycelium growth and inoculum production were completely inhibited within 30 days (Table 1).



Fig. 2. Experimental plot: with application of chemical fungicide (left) and bio-technique plot (right).



Fig.3. Potato seeds are healthy in bio-technique plot after 90 days harvesting.

Table 1. Application of *Chaetomium* mycofungicide for inhibition of *Phytophthora infestans* causing late blight of potato in bi-culture test for 5 days.

Treatments	Mycelium growth	Inhibition of mycelium growth (%)	Spore production	Inhibition as spore production(%)
Control	9 ¹	-----	1.05×10^6	-----
<i>P. infestans</i>	5.13	42.91	0.49×10^6	53.36
C.V. (%)	2.66	-----	3.99	-----
L.S.D. 0.05	0.42	-----	6.92	-----
L.S.D. 0.01	0.77	-----	0.12	-----

¹average of four replications.

Evaluation of Chaetomium mycofungicide to control late blight of potato in the infested field-soil

The field experiment was carried out in a 0.32-hectare field in Chiang Mai province where the soil was infested with *P. infestans* and where the disease had previously completely destroyed the potato plants and resulted in total loss of tuber yield. In this study disease ratings from bio-technique 1, bio-technique 2 and bio-technique 3 were 2.10, 2.77 and 1.99 respectively, and a reduction of late blight incidence by 34.37, 15.62 and 37.81%, respectively. The yields using the bio-techniques were significantly different ($P = 0.01$), as compared to the non-treated ones where the disease rating was 3.20 and late blight incidence was much higher. Chemical fungicide treatment produced a disease rating of 2.10 and late blight incidence was reduced by 34.37% which was not significantly different when compared to the bio-techniques (Fig. 2).

In bio-technique 1, 2 and 3, the pathogen colonized baits placed in the soils with a disease index of 2.12, 3.43 and 1.62 respectively and could reduce the pathogen presence in the soils by 56.46, 29.95 and 66.73%, respectively. This difference was significant ($P = 0.01$) when compared to the non-treatment where the pathogen colonized the soil with a disease rating of 4.87, revealing increased populations of the pathogen in the soil. The soil with chemical fungicide treatment was colonized by the pathogen with a disease rating of 2.37 and reduced late blight incidence by 51.33% which was not significantly different as compared to all bio-techniques (Table 2). It was interesting that bio-technique 1, 2 and 3 treatments gave tuber potato yield of 12, 12.5 and 16.75 tubers, respectively which weighed 1,718.75, 1,103.75 and 1,450 g, respectively. This was significantly different ($P = 0.01$) as

Table 2. Disease index and colonization of *Phytophthora infestans* after using bio- techniques.

Treatments	Disease index ¹	Inoculum	Disease reduction (%)	Inoculum reduction (%)	Increasing yield (%)
Control	3.2	4.87	-----	-----	-----
Fungicide	2.1	2.37	34.37	51.33	52.3
Bio-technique1	2.1	2.12	34.37	56.46	49.09
Bio-technique2	2.7	3.43	15.62	29.95	28.94
Bio-technique3	2.6	1.62	18.75	66.73	45.39
C.V. (%)			-----	-----	-----

¹Disease Index of potato late blight, 5 levels: 1= healthy leaves, 2 = leaf blight 1-25%, 3 = leaf blight 26-50%, 4 = leaf blight 51-75%, and 5 = leaf blight 76-100% (modified from Sanyong, 1992).

compared to non-treatment which produced 9.63 tubers at 732.50 g. The chemical fungicide treatment produced 19.63 tubers (1,726.25 g); this is not significantly different as compared to all bio-techniques (Table 3).

Table 3. Potato yield when collected randomly from 3 × 0.6 meter plots (18 m²) following various biotreatments.

Treatments	Plant number	Tuber number	Weight of big size tuber (g)	Small size tuber	Weight of small size tuber (g)
Control	8.25 ab ¹	9.63 b	732.5 b	8.5 ab	221.25 a
Bio-technique1	8 ab	12 ab	1718.75 a	6.25 b	155 b
Bio-technique 2	7.63 b	12.50 ab	1103.75 ab	8.63 ab	238 ab
Bio-technique 3	9.88 a	16.75 ab	1450 a	10.13 a	295 a
C.V.(%)	9.83	33.92	28.30	24.92	30.13
Prob.	P=0.01	P=0.05	P=0.05	P=0.05	P=0.05

¹Average of four replications. Means followed by a common letter were not significantly different by DMRT.

Random collection of potato tubers in 34 square meters revealed that bio-technique 1, 2 and 3 produced larger tubers of 8.1, 7.4 and 7.5 kg, respectively which is significantly higher than the 5.9 kg of non-treated ones (P = 0.05). The chemical fungicide treatment gave a yield of 13.9 kg which is not significantly different as compared to all bio-technique treatments (Table 4). The bio-techniques can control late blight epidemics in the planted area and gave a high yield quality averaging 18.7% starch (Fig. 3).

Table 4. Potato yield (random collection) over 34 m² following Bio-IPM applications.

Treatments	Big size (kg)	Small size (kg)	Total yield (kg)
Control	5.9 b ¹	2.5 a	8.4
Pesticides	13.9 a	2.8 a	16.7
Bio-IPM 1	8.1 ab	2.1 a	10.2
Bio-IPM 2	7.4 ab	2.4 a	9.8
Bio-IPM 3	7.5 ab	2.6 a	10.1
C.V.(%)	53.65 (P=0.05)	38.29 (NS)	-----

¹Average of four replications. Means followed by a common letters were significantly different by DMRT.

Discussion

Late blight caused by *Phytophthora infestans* is the major disease problem of potato causing large economic losses. The disease usually infects leaves, stems and tubers and is responsible for infection by secondary invaders such as *Erwinia carotovora*, *Pseudomonas solanacearum* and *Fusarium* spp. (van der Zaag, 1996). *Phytophthora infestans* was isolated and proved to be pathogenic to potato plants using Koch's postulate. It was found that the pathogen occurred in the soil and is a soil borne pathogen. Late blight of potato has seriously infected potato worldwide and rapidly destroys potato leaves and tubers in the field and after harvest. The disease caused serious problems to potatoes planted in Ireland leading to food shortages and famine in 1845-1850 (Talburton and Smith, 1975). Potato late blight has also been reported from northern Thailand by Sanyong (1992) where *P. infestans* race 1 and 4, mating type A1 was recorded. The pathogen can be transmitted through potato seeds as a seed borne fungus and the pathogen can survive in the soil. Disease control has utilized several chemical fungicides for years and the pathogen has become more resistant to these fungicides (Samoucha and Cohen, 1986).

Our experiment revealed that the bio-techniques when applied to the potato plots for pesticide-free production has been successfully used to control the disease. The applications of bio-technique 1, 2 and 3 could reduce potato late blight of 34.37, 15.62 and 37.81%, respectively which is not significantly different when compared to the chemical fungicide treatment that reduced potato late blight by 34.37%. However, these bio-techniques must be integrated with other disease control methods such as adjusting soil pH by applying bio-fertilizer (pH 8-9) that consists of high organic matter, humus, and other elements required for plant growth (Soytong, 2004).

Cellulose degrading fungi (a mixture of 10 strains:- *AO*, *As0.*, *Ast*, *CL*, *EN*, *ER*, *EC*, *EH*, *MC*, *PV*, and 2 strains of antagonistic fungi:- *BIO-1* and *BIO-2*) were added in biofertilizer for our experiments (Soytong, 2004). These saprobic fungi help to degrade cellulose materials from plant debris leading to better soil aeration and water drainage, high soil fertility and absorption of the remaining chemical fertilizers, all useful for plant absorption. Our results show that the potato late blight can be controlled using *Chaetomium* mycofungicide. The *Chaetomium* mycofungicide is a biological fungicide formulated from *C. cupreum* strains CC01-CC10 and *C. globosum* strains Cg1-Cg12 which consist of 1,500,000 CFU/g, the application rate is 10 g/ 20 litres of water spraying to the soil to control the pathogen, especially *P. infestans* after adjustment of the soil with bio-fertilizer. This experiment revealed that *Chaetomium* mycofungicide can control potato dry rot and reduce late blight symptoms on the leaves and stems after spraying regularly on the plants. Other recommended biological products are the crude extract of *Chaetomium* sp. and *Trichoderma* sp., antagonistic fungi which can induce plant immunity from disease. Trichotoxin A50 is a brown liquid formulation of natural substances that can induce immunity of plants such as tomato, cucumber and potato to disease (Suwan *et al.*, 2000) by interval spraying at 7-10 days to the plant at the rate of 50 cc/20 liters of water. Formulated *Chaetomium* sp. is a biological fungicide that has been successfully used to control root rot of durian and black pepper caused by *Phytophthora palmivora* (Pechprome and Soyong, 1997; Sodsard and Soyong, 1999), and root rot of citrus caused by *P. parasitica* (Soyong *et al.*, 1999).

Our results indicate that *Chaetomium* mycofungicide could reduce incidence of late blight of potato caused by *P. infestans* and reduce the population in the soil with significant reduction the potato late blight when compared to non-treatment. Biological products used in plant disease control must be integrated with other control measures for pest management.

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