Bacillus cereus strain RS87 was isolated from the rhizosphere of Green Kuang Futsoi (*Brassica chinensis* Jusl var *parachinensis* (Bailey) Tsen & Lee) at Tumbol Bung-Phra, Phitsanulok province, Thailand and was identified by fatty acid analysis (J.W. Kloepper personal communication, 2006). The bacterium was maintained in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD, USA) supplemented with 20% glycerol at -80°C for long-term storage.

For experimental use, the bacterial strain was transferred onto tryptic soy agar (TSA; Becton Dickinson) and incubated at 30°C for 24 hours. Then, the bacterial cells were transferred to 250-ml Erlenmeyer flask containing 100 ml of TSB and incubated at 30 °C for another 24 hours. TSB containing bacterial cells was centrifuged at 10,000*g* in a refrigerated tabletop centrifuge (SORVALL® Biofuge Stratos, Kendro Laboratoty Products, Germany) for 10 minutes at 4°C. The supernatant was discarded and bacterial cells were re-suspended in autoclaved double distilled water (ddH_2O). The bacterial concentration was then adjusted to 10^8 CFU/ml. Scanning electron microscopy was also performed to confirm the vegetative state of *Bacillus cereus* strain RS87 during 24 hours culture. Most cells were ranged from 2.5-2.6 μm in length by 1.2-1.4 μm in width. Additionally, several cells still undergo binary fission.

Bacterial spore medium, preparation, harvesting and handling

B. cereus strain RS87 was grown in a chemically defined medium modified from the work of de Vries et al. (2004), which contained the following components (final concentrations): D-glucose (10 mM), L-glutamic acid (20 mM), L-leucine (6 mM), L-valine (2.6 mM), L-threonine (1.4 mM), L-methionine (0.47 mM), L-histidine (0.32 mM), sodium-DL-lactate (5 mM), acetic acid (1 mM), $FeCl_3$ (50 μM), $CuCl_2$ (2.5 μM), $ZnCl_2$ (12.5 μM), $MnSO_4$ (66 μM), $MgCl_2$ (1 mM), $(NH_4)_2SO_4$ (5

mM), Na_2MoO_4 (2.5 μM), $CoCl_2$ (2.5 μM), and $Ca(NO_3)_2$ (1 mM). The medium was buffered at pH 7.2 with 100 mM dipotassium hydrogen phosphate. All chemical were obtained from commercial supplies.

Strain RS87 was grown for 24 hours on TSA and then transferred to 300 ml of the defined medium in a 500 ml Erlenmeyer flask. The medium was incubated at a constant temperature of 30°C in Environmental Shaker-Incubator ES-20 (BIOSAN, Riga, Latvia) with 200 rpm for four days. Spores were harvested by centrifugation at 10,000*g* in a refrigerated tabletop centrifuge for 10 minutes at 4°C. The supernatant was discarded and the solid pellet containing spores of *B. cereus* strain RS87 was re-suspended in sterile distilled water. Spores were washed at least twice with autoclaved distilled water. The spores were then suspended in 10 mM phosphate buffer (pH 7), adjusted to 10^9 spores/ml, and stored in the dark at 4°C until used in the experiment.

Preparation of seed coating solutions

Two percent low viscosity sodium alginate (ISP Alginates, San Diego, CA, USA) and 4% GENU pectin type LM104 AS-FS (CP Kelco, Lille Skenseved, Denmark) at the ratio of 2:1 were dispersed in deionized water and agitated for one hour. Subsequently, 2% propylene glycol was added to the solution under gentle agitation. The mixtures were left to stand until the air bubbles disappeared. Spore suspensions of *B. cereus* strain RS87 were added to the solution and gently mixed, giving a final spore concentration of 10^8 spores/ml.

Source of seeds, and seed treatment with bacterial vegetative cells and spores

Seeds of cucumber (*Cucumis sativus* L. cv. Thong) were obtained from CHIA TAI Co., Ltd., Thailand and long cayenne pepper seeds (*Capsicum annuum* L. var.

acuminatum Fingerh cv. 111 CHANYA) were obtained from Known-You Seed Company, Thailand. Prior to applying the seed treatment, the cucumber and pepper seeds were surface disinfested in a solution of 2.25% NaOCl for five minutes followed by repeated washings with sterile distilled water for another five minutes. Then the seeds were air-dried and stored at 4°C before use.

Cucumber and pepper seeds treated with bacterial vegetative cells, were soaked in bacterial cell suspensions (10^8 CFU/ml), maintained in 250-ml Erlenmeyer flasks containing 100 ml of TSB and were then incubated in the Environmental Shaker-Incubator ES-20 with 200 rpm at 30°C for 60 minutes. Seeds in the non-bacterized control treatment were soaked in autoclaved ddH_2O.

In preparation for the film coating of seeds, the seed coating solution containing 10^8 spores/ml of *B. cereus* strain RS87 was drenched onto the cucumber and pepper seeds in a sterile petri dish (1g seeds/0.5 ml coating solution). While seeds were mixed with the coating solution, a mixture of 0.5% $CaCl_2$ and 2% propylene glycol was sprayed onto the seeds to form water insoluble coating layer on the seed surface. Seeds were air-dried until the seed surface was completely dry before seeding. A control treatment with film coated seeds was mixed with the same solution mentioned above in the absence of bacterial spores.

To evaluate the spore size and spore distribution on the coated seed surface, both bacterial spores after being suspended in 10mM phosphate buffer and the seed coating film with spores of strain RS87 were investigated under scanning electron microscope LEO 1455 VP (LEO Electron Microscopy Ltd., Cambridge, England).

Greenhouse experiments

Previously, strain RS87 was successfully applied as seed treatment using its vegetative cells to enhance plant growth (Jetiyanon 2002). In this study, soaking seeds with vegetative cells of strain RS87 was included in the experiment as a positive control.

Three separate experiments were performed in the greenhouse including one each to assess seed emergence, root length, and plant height. Each experiment consisted of 5 treatments: a non-treated seed control (NC), a control with water-soaked seeds (WC), a control with film coating of seeds (FC), soaking seeds with vegetative cells of strain RS87 (VC-RS87), and film coating of seeds with strain RS87 spores (FC-RS87 spores). The temperature in the greenhouse was 33°C during the day and 27°C at night. The humidity was approximately RH 80-85%. Each experiment was conducted twice.

Seed emergence experiment

The seeds in each treatment were planted in a polyetyrene seedling tray (Thai Charoen Thong Karntor Co., Ltd., Thailand) containing a sterile soilless peat-based medium (Klasmann-Deilmann GmbH, Geeste-Groß Hesepe, Germany). There were 30 replications per treatment. Each hole of a polyetyrene seedling tray represented a replication. Three seeds were planted in each hole. The experiments were conducted twice. All of the cucumber and pepper seed emergences were observed and recorded at day four and day nine after seeding, respectively.

Root length experiment

Seeds in each treatment were planted in a polyetyrene seedling tray containing a sterile soilless peat-based medium. There were 50 holes per treatment with one seed/hole. Thirty cucumber and pepper seedling plants from each treatment were

sampled, observed and recorded at seven days and fourteen days after seeding, respectively, for root length measurement. Each seedling plant was gently pulled out from the tray and roots were gently rinsed with a tap water to discard the soilless peat. The root length was measured from the seed germination site to the end of the main root.

Plant height experiment

The experiment had a randomized complete block design consisting of 12 replications per treatment. Seeds in each treatment were planted in a polyetyrene seedling tray containing a sterile soilless peat-based medium. Cucumber and pepper seedlings were transplanted into 10-cm-diam plastic pots containing a soilless peat-based medium at ten days and twenty one days after seeding, respectively. Each pot contained one plant. The height of each cucumber and pepper plant was recorded seven days after transplanting.

Measurement of IAA production

Two known PGPR strains (*Bacillus amyloliquefaciens* strain IN937a and *Bacillus pumilus strains* IN937b) having the ability to promote growth in cucumber and pepper plants (Jetiyanon et al., 2003), were included in this study as reference strains. Both of them were obtained from the culture collection of the phytobacteriology laboratory of Auburn University (Auburn, AL, USA).

B. cereus strain RS87, *B. amyloliquefaciens* strain IN937a, and *B. pumilus* strains IN937b were grown in modified Nutrient Broth-M26 for 24 hours in the Environmental Shaker-Incubator ES-20 with 200 rpm at 30°C as seed culture. The medium contained 5 g NaCl, 10 g peptone, and 10 g beef extract in 1,000 ml distilled water. After overnight incubation, 200 µl of culture was inoculated to 20 ml minimal

salt (MS) medium amended with 5mM L-tryptophan modified from Frankenberger and Poth (1988) and grown again for 48 hours on the shaker. The MS medium contained 1.36 g KH_2PO_4, 2.13 g $NaHPO_4$, 0.2 g $MgSO_4.7H_2O$ in 1,000 ml distilled water. The pH of MS medium was adjusted to 7.0 before autoclaving. L-tryptophan solution was prepared as stock solution containing 10 g glucose, 1 g L-tryptophan, and 0.1 g yeast extract in 100 ml distilled water. The stock solution was filtered through a sterile 0.2µm membrane filter (Satorius Minisart®,Göttingen, Germany).

To measure the amount of IAA produced, 1.5 ml bacterial broth culture was centrifuged at 12,000 rpm for five minutes. One milliliter of the supernatant was added to 2 ml $FeCl_3$-$HClO_4$ reagent (Gordon and Weber, 1951). The $FeCl_3$-$HClO_4$ reagent was prepared by mixing 1 ml 0.5M $FeCl_3$ in 50 ml 35% $HClO_4$. After 25 minutes, the mixture was read in a UV-spectrophotometer at 530 nm absorbance. One milliliter of MS medium in 2 ml $FeCl_3$-$HClO_4$ reagent served as a blank. The amount of IAA produced per milliliter culture was estimated using the IAA standard curve. A standard absorption curve was obtained from authentic IAA (Sigma) dissolved in absolute ethanol at different concentrations. The test was replicated three times.

Statistical analysis

All data was analyzed by analysis of variance (ANOVA) and the treatment means were separated by using Fisher's protected least significant difference (LSD) test $P \leq 0.05$ using SAS software (SAS Institute, Gary, NC, USA).

Results

Scanning electron microscopy

Spores of *Bacillus cereus* strain RS87 ranged from 1.6-1.9 μm in length by 760-980 nm in width as shown in Fig 1. The surface of the coated cucumber seeds (Fig 2a) and coated pepper seeds (Fig 2b) was covered with a smooth-thin film containing spores of strain RS87. Spores were attached and uniformly distributed over the coated seeds surface (Fig 2c and 2d).

Percentage of seed emergence

In general, most seeds in the bacterized treatments either soaked with the vegetative cells or coated with spores of strain RS87 germinated faster than seeds in control treatments. Cucumber and pepper seeds treated with *B. cereus* strain RS87 had significantly greater emergence ($P \leq 0.05$) than all of the control treatments. There were no differences in the seed emergence between the VC-RS87 and FC-RS87spores treatments. Additionally, the percentage of seed emergence was similar among control treatments (Table 1).

Root length

The main root length of the cucumber and pepper plants in PGPR treatments were significantly ($P \leq 0.05$) longer; approximately 25% longer than the plants in the control treatments. Moreover, better root proliferation was also observed in the PGPR treatments when compared with the control treatments. Vegetative cells of strain RS87 promoted the longest main roots of the cucumber and pepper plants. However, there was no significant difference of main root length between seeds treated with vegetative cells and spores. Main root lengths among the control treatments were the same (Table 2).

Plant Height

Cucumber and pepper plants pretreated with either vegetative cells or spores of strain RS87 showed better plant growth at 10 and 21 days after seeding, respectively, compared to control treatments. After transplanting, the cucumber and pepper plants treated with strain RS87, both the vegetative cells and the spores generally developed faster than the plants in the control treatments. Both vegetative cells and spores of strain RS87 had a significant affect on plant height ($P \leq 0.05$). It was about 50% greater than plants in the control treatments (Table 3). Additionally, an increase in leaf size and numbers of fully developed leaves were also observed in PGPR treatments comparing with that of the control treatments (Fig 3). There was no difference in plant height among the control treatments.

IAA production

The appearance of a pink color in the solutions after reaction time represents the existence of IAA. The more intense the pink color in the solution is the higher the IAA amount will be. The results showed that *B. cereus* strain RS87 produced a significant amount of IAA; approximately 31% or 1.5 fold greater than the *Bacillus amyloliquefaciens* strain IN937a and *Bacillus pumilus strains* IN937b (Table 4).

Discussion

It was shown that the film coating of seeds alone did not promote plant growth compared to the non-treated seed control treatments, suggesting that the coating solutions may function as only a microbial carrier. This study revealed that the film coating of seeds with spores of *Bacillus cereus* strain RS87or with vegetative cells showed similar significant increases in seed emergence, root development, and plant height when compared to the control treatments. This may be due to the ability of strain RS87 spores becoming vegetative cells within a short period of time after receiving the optimal conditions as described by Hashimoto et al. (1969).

The method to deliver PGPR as a plant growth promoting agent should be compatible with the grower's use. The advantage of this film coating of seed process is helping spores of strain RS87 to bind onto the seeds' surface until use. This method is a mimic of chemical seed treatment. Therefore, film coating of seeds with a resistant life stage of strain RS87 would be a new practical means to deliver microorganisms functioning as biofertilizing agent. This may result in a reduction rate of using chemical fertilizer during early plant establishment.

For early plant growth enhancement, root proliferation has been related to IAA-producing PGPR (Barazani and Friedman 1999). In this study, *B. cereus* strain

RS87 is able to produce IAA in an amount which is possibly involved in root development. This increased rooting in the PGPR treatment may enhance the plant mineral uptake resulting in better plant growth compared to the control treatment. Fallik et al. (1994) also reported that the dose-response curve of roots to cultures with increasing concentrations of *Azospirillum* fit the dose-response curve of roots to increasing concentrations of IAA. Nevertheless, the strain RS87 produced higher IAA amounts than the concentration of authentic IAA (0.1μM) needed to promote root growth, in agreement with Barazani and Friedman (1999). Environmental factors dilution, leaching, and oxidation, may reduce inhibitory levels of IAA concentrations that are effective in stimulating growth. As reported by Ryu et al. 2003, *Bacillus amyloliquefaciens* strain IN937a, one of the two tested reference strains in this study, released a blend of volatile components that promoted growth of *Arabidopsis thaliana*. Acceleration of seed emergence and the enhancement of plant height observed in seeds treated with *B. cereus* strain RS87 indicate that other bacterial metabolites released from strain RS87 may be involved with plant growth enhancement. It is postulated that IAA produced by *B. cereus* strain RS87 would be one of the modes of actions for plant growth promotion. Other bacterial metabolites produced by *B. cereus* strain RS87 will be further investigated to explore the multi-mechanisms of this organism functioning as biofertilizing-PGPR.

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Table 1 Efficacy of film coating of seeds with *Bacillus cereus* strain RS87 spores for promoting cucumber and pepper seed emergence

Treatment[x]	Means percentage of cucumber emergence 4 days after seeding*	Means percentage of pepper emergence 9 days after seeding
NC	87.77 b[y]	87.22 b
WC	89.44 b	89.44 b
FC	88.89 b	87.77 b
VC-RS87	96.11 a	93.89 a
FC-RS87 spores	96.66 a	94.44 a
$LSD_{0.05}$	5.40	5.32

*Means percentage of seed emergence are from two separated experiments

[x]NC=non-treated seed control, WC=control with water-soaked seeds, FC=control with film coating of seeds, VC-RS87=soaking seeds with vegetative cells of strain RS87, FC-RS87 spores=film coating of seeds with strain RS87 spores.

[y]Numbers with different letter show significant differences at $P \leq 0.05$ according to least significant difference (LSD) test.

Table 2 Efficacy of film coating of seeds with *Bacillus cereus* strain RS87 spores for promoting cucumber and pepper root elongation

Treatment[x]	Means main root length of cucumber 7 days after seeding* (cm)	Means main root length of pepper 14 days after seeding (cm)
NC	8. 61 b[y]	6.83 b
WC	8.66 b	6.85 b
FC	8.90 b	7.00 b
VC-RS87	11.86 a	9.60 a
FC-RS87 spores	11.72 a	9.36 a
$LSD_{0.05}$	0.38	0.32

*Means main root length are from two separated experiments

[x]NC=non-treated seed control, WC=control with water-soaked seeds, FC=control with film coating of seeds, VC-RS87=soaking seeds with vegetative cells of strain RS87, FC-RS87 spores=film coating of seeds with strain RS87 spores.

[y]Numbers with different letter show significant differences at $P \leq 0.05$ according to least significant difference (LSD) test.

Table 3 Efficacy of film coating of seeds with *Bacillus cereus* stain RS87 spores for promoting cucumber and pepper plant height*

Treatment[x]	Means plant height of cucumber 17 days after seeding * (cm)	Means plant height of pepper 28 days after seeding (cm)
NC	11.45 b[y]	8.41 b
WC	11.16 b	8.75 b
FC	11.20 b	8.50 b
VC-RS87	22.62 a	17.25 a
FC-RS87spore	22.54 a	17.08 a
$LSD_{0.05}$	0.85	0.68

*Means plant height are from two separated experiments.

[x]NC=non-treated seed control, WC= control with water-soaked seeds, FC=control with film coating of seeds, VC-RS87=soaking seeds with vegetative cells of strain RS87, FC-RS87 spores=film coating of seeds with strain RS87 spores.

[y]Numbers with different letter show significant differences at $P \leq 0.05$ according to least significant difference (LSD) test.

Table 4 Indoleacetic acid (IAA) production by *Bacillus cereus* strain RS87, *Bacillus amyloliquefaciens* strain IN937a and *Bacillus pumilus strains* IN937b

Bacterial isolates	**IAA production* (μmol ml^{-1})**
Bacillus cereus strain RS87	30.85 a**
Bacillus amyloliquefaciens strain IN937a	20.76 b
Bacillus pumilus strains IN937b	21.14 b
$LSD_{0.05}$	2.83

*IAA production from each bacterial isolate was performed at least three times and the numbers shown in the table are means of IAA production in each isolates.

**Numbers with different letter show significant differences at $P \leq 0.05$ according to least significant difference (LSD) test.

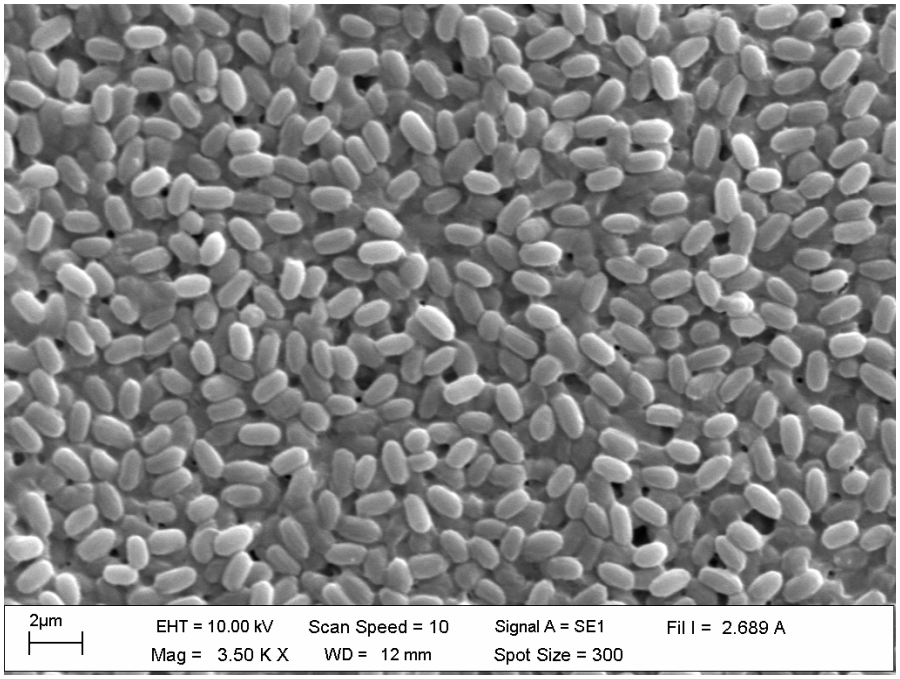

Fig. 1 Scanning electron micrograph of *B. cereus* strain RS87 spores after being suspended in 10 mM phosphate buffer at pH 7

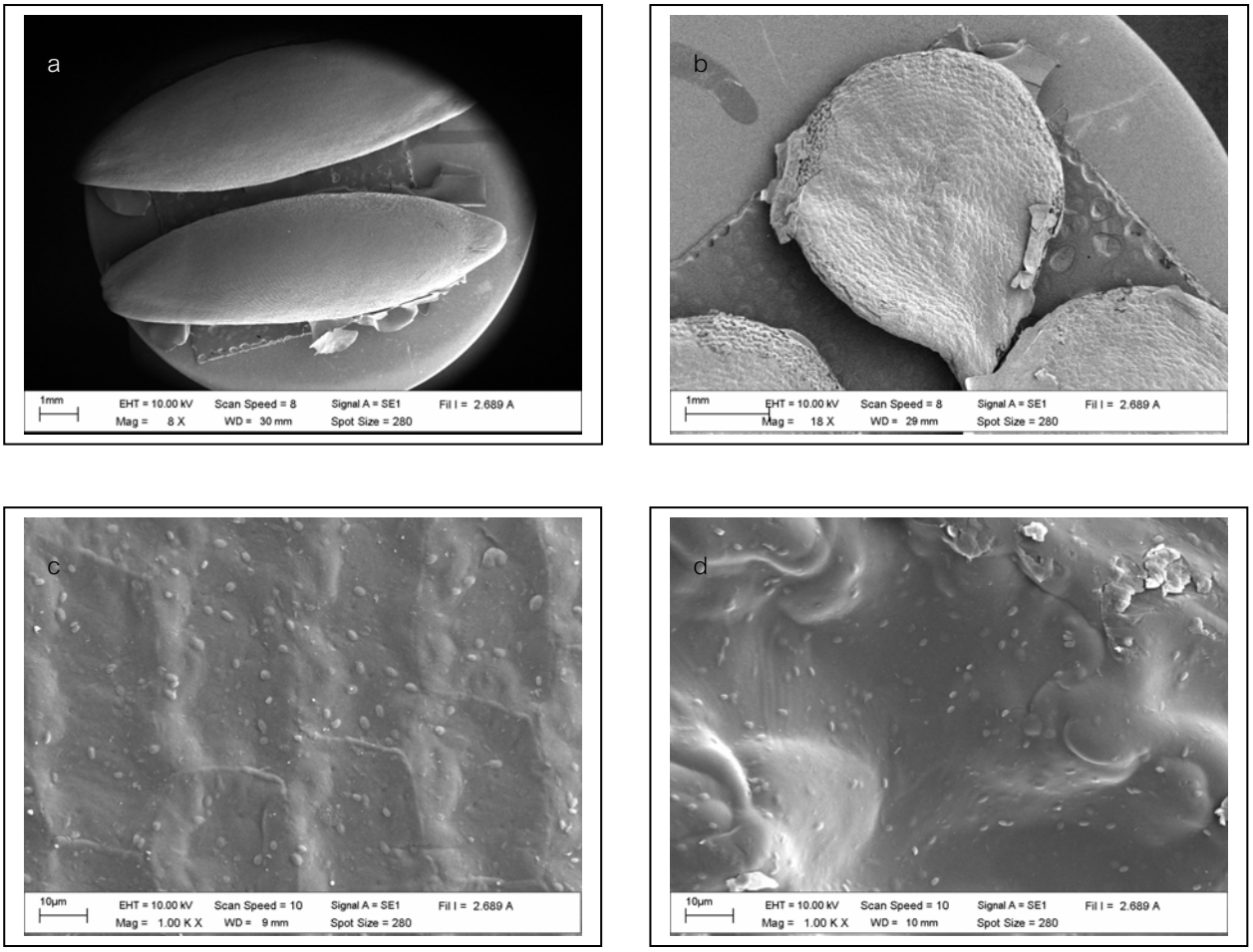

Fig. 2 Scanning electron micrograph of film coating of cucumber and pepper seeds with *B*. *cereus* strain RS87 spores. [an overview of coated cucumber seed (a); an overview of coated pepper seed (b); uniform distribution of strain RS87 spores over cucumber seed surface (c) and pepper seed surface (d)].

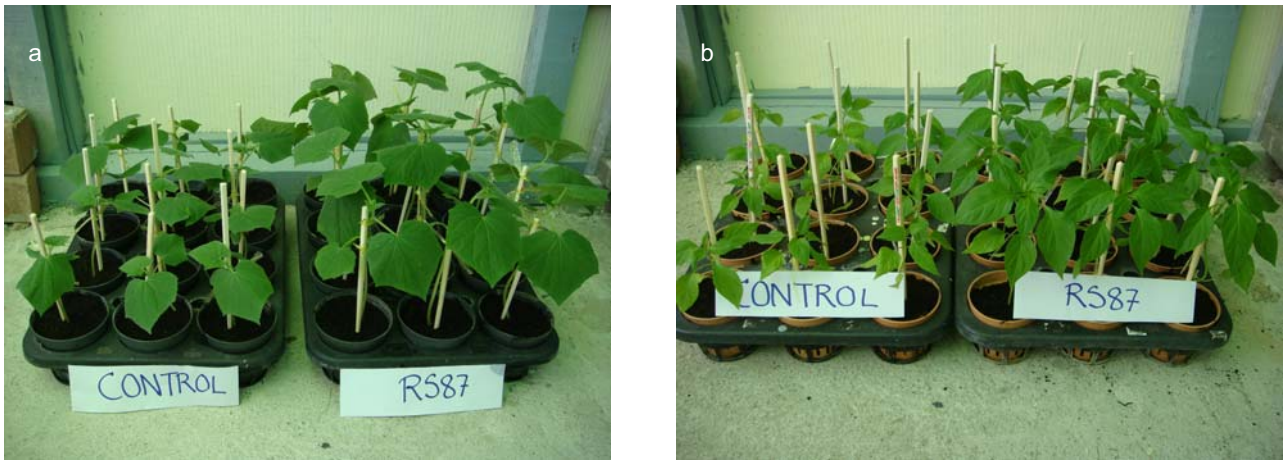

Fig 3 Growth enhancements on cucumber (a) and pepper (b) pretreated with film coating of seed with *B*. *cereus* strain RS87 spores comparing with non-treated seed control 7 days after transplanting.

ภาคผนวกที่ 4

Title

Pesticide use patterns among small-scale farmers in Phitsanulok, Thailand

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Abstract

The inappropriate use of pesticides is extremely harmful to farmers. In addition, such practice is potentially detrimental to the environment and the health of consumers. In this study, 130 small-scale farmers of pesticide use patterns were surveyed in the rural area of Phitsanulok in northern Thailand using a structured questionnaire administered via personal interviews. The results indicated that pesticides were readily available and heavily used in crop production, including

endosulphan which has been banned by the government since 2004. Overall, pesticide use was inappropriate. Farmers did not wear suitable personal protection, applied pesticides irrationally in preventive fashion, and discarded the wastes carelessly. Farmers relied on commercial information for the best use of the pesticides. Recommendations from governmental personnel were rarely mentioned as a resource. In conclusion, pesticide use patterns among small-scale farmers still need much improvement. Educational interventions are essential for promoting safety during all phases of pesticide handling. Public policies should also be developed to encourage farmers to change their pest management methods from chemical-based to methods that are healthier and more environmental-friendly.

Keywords

Pesticides, Small-scale Farmers, Agro-chemicals, Thailand

Introduction

Thailand covers about 513,000 square kilometers with a population of some 63 million citizens. Of those 63 million people, 64.1% live in the rural areas. Over the years, the Thai economic structure has slowly shifted from an agricultural sector to an industrial sector. With an exception of Bangkok's highly concentrated industrial sector, Thailand's major source of income and occupation is agriculture. Agricultural contribution to the total GDP was approximately 10.5% in 2000.

In recent years, concern has been growing all over the world that improper agro-chemical use can create hazards for humans and the environment. Thailand is no exception. Coupled with the green revolution policy of the Thai government, the use of pesticides has skyrocketed over the past 40 years (Health Systems Research

Institute, 2005, pp.3-4). In 2002, the amount of pesticides consumed in the country was 39,904 metric tons (World Health Organization, 2006, p.12). The heavy use of pesticides resulted in various negative health, environmental and economic consequences. In 2005, 2.12:100,000 Thai citizens were occupationally poisoned by pesticides (Department of Disease Control, 2006, pp.444-445).

Past environmental studies unanimously found pesticides contamination in the soil and water throughout the country. This contamination resulted in the reduction of natural insect habitats, earthworms, micro-organisms and cover crops. Pesticide residue in agricultural product not only affect the health of consumers, but also cause rejection of export goods, which can lead to economically damage for the country (Health Systems Research Institute, 2005, pp.22-26).

In order to intervene and promote safe and appropriate use patterns of pesticides, it is critical to understand the real situation among small-scale farmers who are the majority of Thai agricultural labor force. Until now, there has been no published report on the actual behavior of small-scale farmers regarding their pesticide use patterns. For this reason, this study was conducted to explore pesticide use patterns among small-scale farmers in Phitsanulok, Thailand.

Materials and Methods

The study was carried out in the area of Phitsanulok, which is located in the northern part of Thailand and covers a total area of 10,815.854 square kilometers. This is mainly a rural province where the population heavily depends on rice, horticultural (fruits and vegetables), and field (corn and soy) crop production. The survey sites were selected based on the proportion of full-time small-scale farm populations, cooperation from local leaders, and the willingness of the farmers to

participate. The study protocol was approved by Naresuan University's Ethics Committee.

The data was collected by means of a structured questionnaire administered via personal interviews. The data collected included farmers' demographic information, farm system and practices, and pesticide use practices. The instrument was pre-tested with farmers in the nearby area who did not participate in the final survey.

The raw data was coded and entered into SPSS. Relative frequencies were calculated for each question.

Results

Participants

One hundred and thirty small-scale farmers voluntarily participated in this study. The majority were females (61.2%). Respondents were between 20-80 years of age with an average age of 52 and a standard deviation of 13.3 years. A considerable number either finished primary school or received no formal education. All farmers reported growing more than one kind of crop on their lands. Rice was found to be the major produce, followed by mango and vegetables and corn, respectively (Table 1).

[Insert Table 1 about here]

Pesticides utilization

The vast majority of respondents reported using pesticides in crop production (123, 94.6%), with 66 (50.8%) used chemicals only and 57 (43.8%) combined pesticides with biological/organic pest control methods.

The use of pesticides in the area was heavy. Various formulations were reported. All were stated by their trade names without any awareness of the common names. Among them, the most frequently mentioned were insecticides, followed by herbicides and fungicides (Table 2). Some of the pesticides were extremely hazardous or highly hazardous (World Health Organization, 2005, pp.16-20). Chlorpyriphos, a pesticide in the organophosphates family was the most frequently used by farmers, followed by cypermethrin, which is in the family of pyrethroids. Glyphosate was the most popular herbicides, whereas the combination of difenoconazole and propiconazole was frequently mentioned fungicidal agent. Alarmingly, endosulphan which was officially banned in October 2004 due to its extreme danger was found to be used. Additionally, a number of reported pesticides were obtained in small re-packaged containers without appropriate labeling. Farmers reported consulting pesticide vendors about their pest problem, and receiving the agents without any other accompanying information except how to administer it. From our investigation, no farmer had specific storage for their pesticides. The agents were stored casually with fertilizers and farm equipment.

[Insert Table 2 about here.]

Availability of pesticides

Pesticides were readily available for purchase by the farmers. All participants reported obtaining pesticides from more than one place. The primary source of pesticides in the area was the agro-chemical shops in the community that were located within one or two kilometers of their home (60%). Co-operative shops in the

community and agro-chemical shops in the municipal markets were also frequently mentioned.

[Insert Table 3 about here.]

Frequency of pesticide application

All farmers used knapsack sprayers for pesticide application. The majority of them reported routine application of pesticides to prevent an incoming pest invasion. Some farmers even sprayed more frequently than once a week on a routine basis. Only a small number of farmers would observe the manifestation prior to their pesticide use (Table 4).

[Insert Table 4 about here.]

Pesticide practices

The majority of farmers (74.1%) based their decisions about pesticide use on multiple external sources. The most frequently mentioned source of information was from commercial media/public broadcasts such as television, newspaper and community broadcasting. The second most frequently mentioned source was from governmental agricultural extension officers, followed by village leaders and finally the opinions of other community leaders (Table 5).

[Insert Table 5 about here.]

Amongst 123 farmers who reported using pesticides, about 80% said that they read the labels on pesticide containers before use. However, not everyone paid attention on every aspects of the content, with the majority focusing only on directions (Table 6). Moreover, all participants did not feel that it is necessary to strictly follow the direction. As a result, it was found that the actual practice varied greatly, from using much less than the recommended dose to save costs, to using a 100% more than the recommended dose to accelerate the results.

More than half of the farmers used at least one kind of personal protection when handling pesticides. The most frequently mentioned protection included face masks, followed by gloves. The use of boots and long-sleeved shirts were much less stated. Approximately 30% of the respondents took wind condition into account while spraying the pesticides. Only 9% reported cleaning up after handling the pesticides (Table 6). Interestingly, none of the farmers completely protected themselves. The reasons given for poor protection were the lack of awareness of pesticide hazards (52.4%), the high price of the equipment (25%), and the discomfort due to the hot and humid climate (22.6%).

A great proportion of the farmers (75.6%) reported selling the empty pesticide containers. Some farmers kept them for various uses and still others buried or burnt them (Table 6). No mention was made of rinsing or cleaning empty containers prior to disposal.

[Insert Table 6 about here.]

Discussion and Conclusion

The results of this survey indicated that pesticide use patterns among small-scale farmers in Phitsanulok posed an alarming concern. The manner in which pesticides are used in Phitsnaulok is most likely representative of other agricultural areas in Thailand and thus likely these patterns reflect a true estimate of a national problem.

The use of extremely and highly hazardous insecticides including an agent which was officially banned since 2004 for its extreme hazard was observed in this study. Other less hazardous agents created health risks to the farmers as well. Paraquat, one of the frequently mentioned herbicides, for example, has a lethal dose of only one teaspoonful if ingested. Yet, the agent has been very popular as a herbicide throughout Thailand (Health Systems Research Institute, 2005, p.7).

In general, the frequencies of pesticide applications were unreasonably high. This was a result of calendar spraying as a preventive measure as opposed to a curative application approach without much health and environmental consideration. Such practices are very common among Thai farmers (Tienmar, 2004, p.15), and have even been found in developed countries (Epstein & Bassein, 2003). Frequent applications might be due to a lack of knowledge about the proper pesticide application schedule. Together with the fact that farmers relied mainly on commercial sources for information about the pesticides, along with the influence of suppliers whose goal was to maximize their sale volumes, the negative impacts of the pesticides might be downplayed.

Personal protective equipment as well as personal hygiene was inadequate. The main concern was to cover their mouth and nose, and this was found to be practiced by just more than half of the farmers, indicating poor knowledge of pesticide routes of absorption. This finding is consistent with may other studies that

found very little concern regarding precautions taken while handling pesticides (Berg, 2001; Burleigh et al., 1998; Isin & Yildirim, 2007; Matthews et al., 2003). In less developed countries, adequate protective clothing was oftentimes neglected for the reasons of discomfort and high costs. In addition, there are no national regulations that require farmers working with pesticides to observe specific precautions (Wilson & Tisdell, 2001).

Proper pesticide waste disposal is also an important part of responsible pesticide use. Accidental release or uncontrolled discharge of pesticide waste into the environment can harm people and contaminate the environment (Damalas et al., 2008). In this study, the disposal of pesticide containers was found to be careless. Empty pesticide containers may often retain unacceptable quantities of pesticide residue if not rinsed properly (Miles et al., 1983). As in many other developing countries where empty pesticide containers are highly valued and sold or exchanged as storage containers for other materials, the majority of farmers in this survey sold empty containers to the buyers who picked up the waste from the community. Unfortunately, it was unclear what the buyers would do with such containers. Dalamas and associates (2008) were strongly against such practice, and recommended puncturing any empty containers to prevent their re-use.

The national policy is a very critical issue that determines the trend of pesticide use among farmers. Unfortunately, mixed messages have constantly been conveyed from the Thai government. While the government seems to be trying to encourage organic agriculture, it imposes zero taxation on imported agrochemicals, and grants “lifetime” license to hazardous materials without a proper reviewing system. Moreover, organic farming was introduced only as an alternative to the conventional chemical-based practice, not as a serious substitution.

Federal agricultural extensionists also have not played a significant role in persuading farmers to reduce pesticide use. Even though there have been many projects to introduce alternative farming techniques, most of the programs are organized at the training centers. Only a few interested farmers who can afford to travel long distance attend such training sessions. The majority of small-scale farmers would like to attend but do not have the time and resources to do so. In addition, the main focus of such training sessions usually is on introducing new advanced methods of farming. However, basic knowledge on appropriate use, and the dangers of agro-chemicals are rarely emphasized. For this reason, farmers still hold to lay beliefs about agro-chemicals and resort mainly to the commercial sources for chemical information.

Thai farmers often have no other option other than consulting with the agro-chemical vendors who are readily available in the community, or seeking information from commercial mass media. Misleading promotional strategies have been reported to be associated with commercial mass media promotion (Health Systems Research Institute, 2005, pp.13-15). For example, hidden promotional messages heavily promoted on radio broadcast programs, mainly conveyed by a DJ who has absolutely no formal training in agriculture, and aimed only at changing farmers' beliefs and attitudes toward pesticides. Promotion of pesticides by brand names instead of common names are also widely practiced, especially in the form of sponsors of entertainment or charity events, causing redundant application of the same pesticides in the same field.

In conclusion, the study indicated inappropriate pesticide use patterns among Thai farmers. Findings of this study clearly suggest that it is necessary to reduce possible health and environmental risks associated with pesticide use by documenting

risk perceptions and developing ways to address them. However, health and environmental factors cannot be isolated from economic concerns. Since the majority of farmers in Thailand are low-income, the initial cost of switching from pesticides to more environmentally friendly and healthy methods should be seriously investigated. A recent study found cost to be an important predictor for small-scale farmers to switch to a biological fertilizer (Jetiyanon et al., 2007). Further studies are highly warranted to generate appropriate date on which to base policy.

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Table 1 General information about the participants

Variable	Number (%)
Level of education	
No education	19 (14.6)
Primary school	97 (74.6)
Secondary school	14 (10.8)
Crops (multiple answers possible)	
Rice	120 (92.3)
Horticultural (mango and vegetables)	73 (56.1)
Corn	19 (14.6)

Table 2 Types of pesticides used

Group of pesticides/Common name	Chemical family	Toxicity class*	Status	Number of farmers mentioned using
Insecticides:				
Parathion-methyl	Organophosphates	Ia	Registered (on watched list)	1
Methomyl	Carbamates	Ib	Registered (on watched list)	6
Chlorpyriphos	Organophosphates	II	Registered	40
Cypermethrin	Pyrethroids	II	Registered	19
Endosulphan	Organochlorines	II	Banned (Oct 2004)	5
Fenobucarb	Carbamates	II	Registered	3
Abamectin	-	U	Registered	5
Captan	-	U	Registered	1
Unidentifiable insecticides in re-packaged containers	unk	unk	Unk	29
Herbicides:				
Butachlor+Propanyl	-	II (Propanyl	Registered	6

70		70) and U (Bulachlor)		
Paraquat dichloride	Paraquats	II	Registered	6
2,4-D, isobutyl ester	-	U	Registered	8
Atrazine	Triazine derivatives	U	Registered	2
Butachlor	-	U	Registered	1
Glyphosate	-	U	Registered	17
Oxadiazon	-	U	Registered	1
Unidentifiable herbicides in re-packaged containers	unk	unk	unk	2
Fungicides:				
15% w/v Difenoconazole + 15% w/v Propiconazole	Azole derivatives	U	Registered	11
Cabendazim (benzimidazole)	-	U	Registered	3
Mancozeb	Carbamates	U	Registered	2
Unidentifiable fungicides in re-packaged	unk	unk	unk	4

containers

Note:

* Toxicity class as classified by World Health Organization (2004) where Ia = extremely hazardous, Ib = Highly hazardous, II = moderately hazardous, III = slightly hazardous, U = unlikely to present acute hazard in normal use

unk = unknown

Table 3 Sources of pesticides

Sources (multiple answers possible)	Number (%)
Agro-chemical shops in the community	210 (60)
Co-operative shops in the community	65 (18.6)
Agro-chemical shops in the municipal markets	54 (15.4)
Convenience stores in the community	15 (4.3)
Direct sale of the agro-chemical companies	4 (1.1)
Village leaders	2 (0.6)

Table 4 Frequency of pesticide application

Frequency of pesticide application (multiple answers possible, depending on the types of pesticides)	Number (%)
Less than once a month (approximately 1-2 times/season)	59 (40.7)
Once or twice a month	24 (16.5)
Three to four times a month	32 (22)
More frequently than once a week	6 (4.1)
Depends on the pest manifestation	24 (16.6)

Table 5 Sources of information about pesticide use

Sources of information (multiple answers possible)	Number (%)
Commercial media/public broadcast (including television, community broadcasting, radio, newspaper, leaflets & pamphlets, billboards)	201 (37.6)
Governmental agricultural personnel (including agricultural extension officers, and local administrative officers)	141 (26.4)
Village leaders, opinion leaders, and community healthcare volunteers	134 (25)
Neighbors	30 (5.6)
Sales persons from agro-chemical companies	29 (5.4)

Table 6 Pesticide practices

Variables	Number (%)
Label read	
Yes	99 (80.5)
Every topic on the label	32 (32.3)
Direction only	40 (40.4)
Indication only	3 (23.2)
Caution only	23 (23.2)
No	24 (19.5)
Personal Protection (multiple answers possible)	
Mouth and nose cover	79 (64.2)
Gloves	51 (41.5)
Taking wind condition into account while spraying	38 (30.9)
Boots	26 (21.1)
Long-sleeves shirts	26 (21.1)
Taking a shower after handling	11 (8.9)
Pesticide empty containers disposal	
Selling them	93 (75.6)
Keeping them home for other uses	20 (16.3)
Burying them	5 (4.1)
Burning them	4 (3.2)
Leaving them randomly by the field	1 (0.8)

ภาคผนวกที่ 5

Seed Coating for Vegetative Cells of Plant Growth-Promoting Rhizobacteria using Alginate/Pectin Wet Film Formulations

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Abstract

Plant growth-promoting rhizobacteria (PGPR) have been traditionally introduced in liquid bacterial suspensions or in the form of dry seed coatings. However, dry seed coatings still have limitation in where they are not readily applicable for vegetative cells of PGPR and generally require specific instruments for coating. The objective of this study was to develop the practical formulation for application of PGPR vegetative cells. Alginate and pectin were used as film formers and propylene glycol was used as a plasticizer. The formulation was developed with various concentrations of the film components and evaluated for some physical film properties. The films were further cross-linked using calcium chloride solution to improve the film strength. The optimum film formulation was selected for evaluation of appropriate coating method and PGPR survival in the film components using cucumber seeds as a model for seed coating. Results showed that PGPR can grow in all of the film formulations. The optimum film formulation contained 2% w/v alginate and 4% w/v pectin (ratio2:1), and 2% w/v propylene glycol using 0.1% w/v calcium chloride and 2% w/v propylene glycol as a cross-linking solution. This film formulation showed even film formation with excellent water adsorption and water vapor penetration. The coating method conducted by pouring 5 mL of polymer mixtures over 10 grams of cucumber seeds and then spraying with 0.5 mL of cross-linking solution resulted in good film coating feature with minimal coating duration. It was suggested that this seed coating formulation could be benefit for vegetative cells of PGPR and would be a practical preparation for growers.

1. Introduction

Rhizobacteria are root-colonizing bacteria that form a symbiotic relationship with many plants. The term “rhizobacteria” usually refers to bacteria that form a relationship beneficial for plants. These bacteria are often referred to as plant growth-promoting rhizobacteria or PGPR [Bushan, 1998; Benizri et al., 2001; Jetiyanon and Kloepper, 2002; Kloepper et al., 2004]. Although rhizobacteria inoculants are indisputably beneficial for various plants, they are not widely used in industrial agriculture, especially for biofertilizer, since large-scale application techniques have not been economically available [Ciccillo et al., 2003]. Several requirements have to be fulfilled in order to induce a beneficial effect on plants such as the bacterial deposit around seeds should be homogenously distributed around the seed at an optimal concentration [Amiet-Charpertier et al., 1998; Ryu et al., 2006], ranging between the biosaturation and the biostimulation threshold [Hartley et al., 2004]. This deposit will be effective, if it takes place in the direct environment of the seeds. These remarks bring out the need for direct bacterization of seeds, so as to reduce the distance between bacteria and roots.

Currently, rhizobacteria has been traditionally introduced in liquid bacterial suspensions or in the form of dry seed coatings [Amiet-Charpertier et al., 1998; Hartley et al., 2004; Ryu et al., 2006]. However, dry seed coatings still have limitation where they are not readily applicable for vegetative cells of free living rhizobacteria and generally require specific instruments for coating. Wet film coating for application of PGPR vegetative cells using spontaneous cross-linking reaction between alginate or pectin and calcium chloride can resolve a crucial step toward preserving the vegetative cells viability to produce homogenously distributed bacterial cells over the seed surface.

Alginate is a salt of alginic acid, a polymer of β-D-mannuronic acid and α-L-guluronic acid, which is isolated from brown seaweeds [Sime, 1990]. Pectin is commonly derived from fruit waste mainly apple and citrus peel. Alginate films can be prepared by cross-linking with $CaCl_2$ to generate a film with barrier properties [Olivas et al., 2008]. The main pectin component is a linear chain of α-(1-4)-linked D-galacturonic acid that forms the pectin backbone, a homogalacturonan. In low-ester pectins, ionic bridges are formed between calcium and carboxylic acid of the galacturonic acid. Low ester pectins need calcium to form a gel, but can do so at lower soluble solids and higher pH-values than high-ester pectins [Sriamornsak et al., 2006]. Both alginate and pectin are idealistic for preparing films and easily forming insoluble gel with calcium ions. The objective of this study was to develop the practical formulation and method of wet film coating for vegetative cells of free living PGPR. The basic film properties required for good barrier such as water vapor penetration, water adsorption, coating method, and survival of PGPR in wet film and on coated seeds will be evaluated to obtain good film properties and easy to use for growers.

2. Material and methods

2.1 Materials

Low viscosity sodium alginate (viscosity 250 cps, 2% w/v) containing mannuronate (M) and guluronate (G) in a M/G ratio of 0.45 (Manugel® DMB) was purchased from ISP Alginates, San Diego, CA, USA. GENU pectin type LM104 AS-FS was obtained from CP Kelco (Lille Skenseved, Denmark). All other chemicals were obtained from commercial suppliers and were used as received.

2.2 Preparation of the free films

The free films were prepared by casting and solvent evaporation technique using sodium alginate, pectin, and mixture of sodium alginate and pectin solutions as film formers and propylene glycol (PG) as a plasticizer. For sodium alginate solution, a specified amount of sodium alginate was dispersed in deionized water and agitated for 1 hr. PG was then added to the sodium alginate solution under gentle agitation. The mixed solution was left to stand until air bubbles have disappeared and the solution was then poured on a dry glass petri dish in a dust-free environment and allowed to air dry at 40°C for 24 hr. Then the obtained films were tested for their physical properties.

The pectin solution was prepared in the same manner as preparing sodium alginate solution. The mixed solutions at various ratios of sodium alginate and pectin solution were prepared by mixing various volumes of sodium alginate and pectin solution, followed by gentle agitation.

To improve film integrity by cross-linking with $CaCl_2$, the dry glass petri dish containing sodium alginate, pectin, or mixture solutions were immersed in $CaCl_2$ solution for cross-linking reaction at 1, 5, and 10 minutes (Figure 1). Then, the cross-linked films were drained and allowed to air dry at 40°C for 24 hr. The obtained films were tested for their physical properties.

2.3 Characterization of the free films

2.3.1 Water vapor penetration

To measure the water vapor penetration, the films were cut and placed on top of open 2.5 cm bottles containing 5 g of silica gel and held in place with a screw lid (test area: 4.9 cm^2). The bottles were conditioned in a desiccator containing silica gel for 12 hours (0% RH) to ensure complete dryness of film and silica gel before conducting an experiment. The bottles were then placed in a desiccator containing a saturated solution of NaCl solution at 30°C (75% RH) [Nyqvist, 1983]. The equilibrium moisture penetration was determined by weighing the bottles at 0, 12, 24, and 48 hrs, respectively.

2.3.2 Water uptake

The water uptake was determined gravimetrically. The weight of a strip of completely dried film (2.5 x 2.5 cm^2) was determined directly with an analytical balance. Then the strip of film was immersed into deionized water at room temperature (25°C) for 2 hr. The resultant swollen film was gently blotted with filter paper to remove excess surface water and weighed again. The water uptake of the film is expressed as the percentage of weight increased.

2.4 Wet film coating on cucumber seeds

Spontaneous wet film coating was performed using cucumber seeds as a model for seed coating. Various amount of coating solution containing vegetative cells of *Bacillus cereus* strain RS87 (10^6 cfu/mL) were first dispersed on cucumber seeds, then mixture solution of $CaCl_2$ and PG was sprayed on the coated seeds to form water insoluble coating layer. The determination of coated cucumber seeds was examined by visual inspection of coating material around the seed surface.

2.5 Survival of *Bacillus cereus* strain RS87 in the wet film and on the coated seeds

Coated cucumber seeds from wet film coating experiment were randomly sampling. Four coated seeds were placed onto a petri dish containing tryptic soy agar and incubated at room temperature for 24 hrs. The experiment was repeated three times. The determination of bacterial growth on seeds was done with visual inspection of colony forming on and around the base of the seeds

2.6 Scanning Electron Microscopy (SEM)

The coated seed samples were mounted directly onto the SEM sample holder using double-sided sticking tape and were gold spray-coated. Then, the samples were examined using scanning electron microscopy (LEO 1455VP, Cambridge, UK). to investigate the distribution of vegetative cells of *B. cereus* strain RS87 over the seed surface.

2.7 Statistical analysis

All experiments for the characterization of the composite film were done in triplicate. One-way analysis of variance (ANOVA) was performed to determine the significant difference in each property among the formulated films. The differences were considered to be significant at a level of $P < 0.05$.

3. Results and discussion

3.1 The free film basic properties

The compositions of free film preparing from various concentration of sodium alginate (1-3% w/v) and pectin (3-5% w/v) was shown in Table 1. The free films prepared from sodium alginate were translucent, smooth and water-soluble. The free films prepared from pectin were slightly opaque, yellowish, smooth and water-soluble. However, both alginate and pectin wet films were simply washed away with gentle rinse, while the dried films were easily broken apart and washed away shortly after rehydration. During the film casting step, the polymer solution (alginate/pectin solution) was immersed in 0.5-2% w/v calcium chloride solution to form weakly cross-linked films. Calcium ions (Ca^{2+}) diffused into the polymeric solution during cross-linking reaction forming either intermolecular or intramolecular linkages [Sriamornsak et al., 2006]. The cross-linked films were not soluble in water and showed substantially strong membrane properties both before and after the rehydration process. Higher calcium chloride concentrations at 2% w/v, while keeping the sodium alginate concentration constant at 2% w/v resulted in highly contraction of the films to form thick lens instead of insoluble thin films. High total solid contents of the films (3% w/v sodium alginate or 5% w/v pectin) resulted in uneven surface, whereas lower concentrations (1.5% w/v sodium alginate or 2% w/v pectin) resulted in poor structural integrity and hard to remove from petri dish.

The formulations of 2% w/v sodium alginate or 4% w/v pectin with 2% w/v propylene glycol (PG) found to be the optimal concentrations with good overall basic properties of the films. The free films preparing from sodium alginate produced translucent films but severely contracted during the cross-linking reaction, while the free films prepared from pectin produced yellowish, slightly opaque films but only slightly contracted during the cross-linking reaction. Therefore, various ratios (v:v) of

2% w/v sodium alginate and 4% w/v pectin were examined for the following experiments to obtain films with good overall basic properties both before and after the cross-linking reaction. The composition and physical properties of the films are summarized in Table 2.

The films prepared from 2% w/v sodium alginate and 4% w/v pectin at a ratio of 2:1 (v:v) with 2% w/v PG using 0.5, 1, and 2% w/v $CaCl_2$ provided translucent films with relatively uniform thickness. Increasing $CaCl_2$ concentrations (2% w/v) in the cross-linking solution resulted in excessive contraction of the wet film. The applied small molecular weight plasticizers (propylene glycol and glycerol) caused not only increase the resistance to the mechanical effect, but also decrease in the surface free energy of the films [Bajdik et al., 2007]. Addition of 2% w/v PG in $CaCl_2$ as a cross-linking solution produced slow gel formation and resulted in substantially decreasing film contraction after cross-linking. The films formulation containing 2% w/v sodium alginate and 4% w/v pectin at a ratio of 2:1 with 2% w/v PG using 0.5, 1, and 2% w/v $CaCl_2$ and 2% w/v PG as a cross-linking solution was further prepared and characterized for their specific properties.

3.2 Characterization of the free films

The evaluation of the free films has been established as an effective step in the development of film coating systems, since it can be readily used to characterize and evaluate fundamental properties of the coating. The results of the free films properties in various conditions will then be applied in order to get the practical coating formulations and conditions.

3.2.1 Water vapor penetration

The water vapor penetration across the films at 6, 12, 24, and 48 hours were measured and expressed as % weight increased of dried silica gel. The free films showed a zero order water penetration profile as a function of time. The vapor transmission was measured under steady state conditions. Therefore, the contribution of the moisture absorbed by the film can be considered negligible. Figure 2 shows water vapor penetration at 48 hours of the film containing 2% w/v sodium alginate and 4% w/v pectin at ratio of 2:1 with 2% w/v PG using 0.5, 2% w/v $CaCl_2$ and 2% w/v PG as a cross-linking solution, cross-linking at 1, 5, and 10 minutes, respectively. Using 0.5% w/v $CaCl_2$ resulted in higher vapor penetration than 2% w/v $CaCl_2$ due to less cross-linking in the polymer chains. No statistically difference vapor penetration after additional cross-linking time at 5 and 10 minutes. The loosely cross-linking of the gel structure at low $CaCl_2$ concentration resulted in porous film after drying and the vapor penetration was independent of time. Because of these advantages of the film, water vapor from planted seeds surroundings can penetrate through the film and help the distributed rhizobacteria and cucumber seed grow.

Several studies reported that anionic polysaccharides (e.g. sodium alginate and low methoxy potassium pectin) can react with calcium ions to form insoluble gels in the hydrated states. Therefore, the dried films can be applied as film coating for sustained release [Sriamornsak, 1997]. The cross-linking reaction was originally described by Grant et al. [1973] in terms of an egg-box model for the mechanism of binding involving two or more units. In case of alginate and pectin, the gel formation resulted from a specific interaction between calcium ions and blocks of galacturonate and

guluronate units, respectively. However, recent studies suggested that mannuronate-guluronate blocks also involved in gelation of alginate [Braccini and Perez, 2001; Donati et al., 2005]. This ability to form an insoluble network could explain how its concentration affects the water adsorption, making films with higher concentration acted as higher barriers to water vapor. Sriamornsak and Kennedy [2006] showed that the effect of hardening time on the puncture strength and elongation of calcium pectinate (GENU pectin type LM-104 AS FS) films required at least 30 min to reach the maximum mechanical strength, while a much shorter time was required for calcium alginate films. The results were consistent with our experiment where the combination of alginate and pectin solutions produced the films with only slightly contraction, consequently, better film features than the films produced from only alginate solution. In this experiment, a much shorter reaction time was employed so that we obtained the insoluble films within a practical time frame for further application regardless of the maximum gel strength.

3.2.2 Water adsorption

Figure 3 shows the equilibrium water uptake of the films at 0.5 and 2% w/v of $CaCl_2$ in cross-linking solution. At 0.5% w/v $CaCl_2$, the films showed no significant different in equilibrium water uptake with increasing cross-linking time. Increasing concentration of $CaCl_2$ resulted in higher water adsorption at 1 minute cross-linking time, while decreasing significantly at 5 and 10 minutes cross-linking time. Pavlath et al. [1999] assumed that there are two reactions occurring when immersing an alginate film in a calcium solution. One is the precipitation of the film by linkage of calcium with carboxyl groups on the film surface, and the other is dissolution of alginate by the solution. This could explain the results at 1 minute cross-linking time where at

low calcium ion concentration (0.5% w/v) where the dissolution of polymers was faster than the precipitation of polymers. Figure 4 shows that higher concentration of calcium chloride in cross-linking solutions at 1% w/v and 2% w/v produced no significantly different film thickness compared with the film without cross-linking reaction. The film thickness was significantly lower than other groups at 0.5% w/v calcium chloride probably due to dissolution of polymers higher than precipitation or gel structural change. Figure 4 shows that higher concentration of calcium chloride in cross-linking solutions at 2% w/v produced no significantly different film thickness compared with 0.5% w/v calcium chloride at 1, 5, and 10 minutes cross-linking time. The film thickness of 0.5 and 2% w/v calcium chloride at 1 and 5 minutes cross-linking time was significantly lower than the film without cross-linking reaction due to dissolution of polymers higher than precipitation or gel structural change. Rhim [2004] also found that thickness of alginate films decreases when immersed in calcium solutions and attributed that to polymer dissolution during soaking. However, Sriamornsak and Kennedy [2006] suggested that the changes in calcium content did not substantially influence the microscopic structure of the hydrated films in scanning electron micrographs. Our results showed that although the calcium content and crosslinking time did not significantly influence the microscopic structure, they substantially influence the water adsorption properties of the films.

3.3 Wet film coating on cucumber seeds

The seed coating procedure was first, application of 5 mL coating solution containing 2% w/v alginate and 4% w/v pectin (ratio 2:1) with 2% w/v PG on 10 grams (50 seeds) of cucumber seeds (approximately 10^5 cfu/seed), then 0.5 mL of cross-linking solution was sprayed on the coated seeds. The results were consistent with the free

film properties where cross-linking solution with more than 2% w/v $CaCl_2$ or without PG resulted in high contraction and separation of wet film from the seeds. Spraying the cross-linking solution provided good distribution over the coated seeds and easy to perform. This method of coating and spraying was modified to be easily prepared and practical on the field using plastic tub. Figure 5a shows the morphology of vegetative cells of strain RS87 after separation of cells from the medium. Figure 5b shows the vegetative cells of strain RS87 were evenly distributed over the coated cucumber seed. This result implied that the vegetative cells of strain RS87 were homogenously distributed around the seeds.

3.4 Survival of *B. cereus* strain RS87 in the wet film and on the coated seeds

Figure 6a shows that strain RS87 can grow well in the composition of the films. Figure 6b shows that the film coating on the seeds appeared uniform and colony of strain RS87 appeared around the base of each cucumber seed. It was found that strain RS87 can survive on the coated seeds over … days at room temperature and over … days at 40°C. Amiet-Charpertier et al. [1998] also suggested that M3.1 rhizobacterium strain survival was found to be rather high over 2 days, since the bacterial concentration in the dried polymer microparticles remained at a value of ~6×10^3 cfu/g, but all the encapsulated bacteria were dead after 6 days. Further work in greenhouse experiments will be performed in order to justify efficacy of using wet film coating comparing with bacterial suspension application.

4. Conclusions

Alginate/pectin films immersed in $CaCl_2$ appear to generate films with good water vapor penetration and water adsorption. Spreading the film coating solution

containing 2% w/v sodium alginate and 4% w/v pectin (ratio 2:1) followed by spraying the cross-linking solution of 0.5% w/v $CaCl_2$ and 2% w/v PG on the coated seeds was the quick and practical method for application in the field using farm utensils. Coated cucumber seed containing vegetative cells of strain RS87 showed good distribution of bacteria around every seeds.

5. Acknowledgements

This work was supported by a grant from the Thailand Research Fund (grant no. DBG4980001) and PERCH-CIC. A gratitude to Prof. Hans E. Junginger, Naresuan University, for his valuable advice in preparing this manuscript.

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List of Tables

List of Figures

No.	Sod.Alginate (% w/v)	Pectin (% w/v)	PG (% w/v)	Film characteristics
1	1.0	-	-	Translucent, smooth, brittle
2	1.5	-	-	Translucent, smooth, brittle
3	2.0	-	-	Translucent, smooth, brittle
4	3.0	-	-	Translucent, uneven, brittle
5	2.0	-	1.0	Translucent, smooth, flexible
6	2.0	-	2.0	Translucent, smooth, flexible
7	2.0	-	3.0	Translucent, uneven surface, flexible, sticky
8	-	3.0	-	Yellowish, translucent, smooth, brittle
9	-	4.0	-	Yellowish, translucent, smooth, brittle
10	-	5.0	-	Yellowish, translucent, uneven, brittle
11	-	4.0	1.0	Yellowish, translucent, smooth, flexible
12	-	4.0	2.0	Yellowish, translucent, smooth, flexible
13	-	4.0	3.0	Yellowish, translucent, uneven, flexible, sticky

Table 1 - Wittaya-areekul et al.

No.	Sod. Alginate : pectin (v:v)	PG (% w/v)	$CaCl_2$ (% w/v)	Film characteristics
1	2:1	2	-	Translucent, smooth, soluble
2	1:1	2	-	Translucent, smooth, soluble
3	1:2	2	-	Translucent, smooth, soluble
4	2:1	2	0.5	Translucent, smooth, shrink, insoluble
5	1:1	2	0.5	Translucent, smooth, shrink, insoluble
6	1:2	2	0.5	Translucent, smooth, slightly shrink, insoluble
7	2:1	2	1.0	Translucent, smooth, shrink, insoluble
8	1:1	2	1.0	Translucent, smooth, shrink, insoluble
9	1:2	2	1.0	Translucent, smooth, slightly shrink, insoluble
10	2:1	2	2.0	Translucent, smooth, shrink, insoluble
11	1:1	2	2.0	Translucent, smooth, shrink, insoluble
12	1:2	2	2.0	Translucent, smooth, shrink, insoluble

Table 2 - Wittaya-areekul et al.

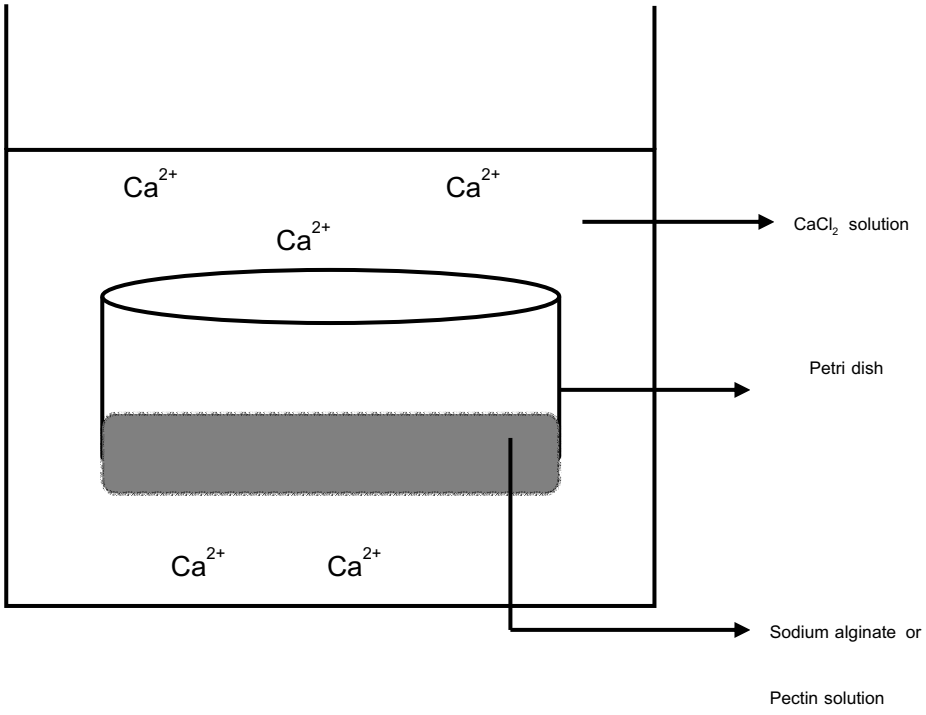


Figure 1 - Wittaya-areekul et al.

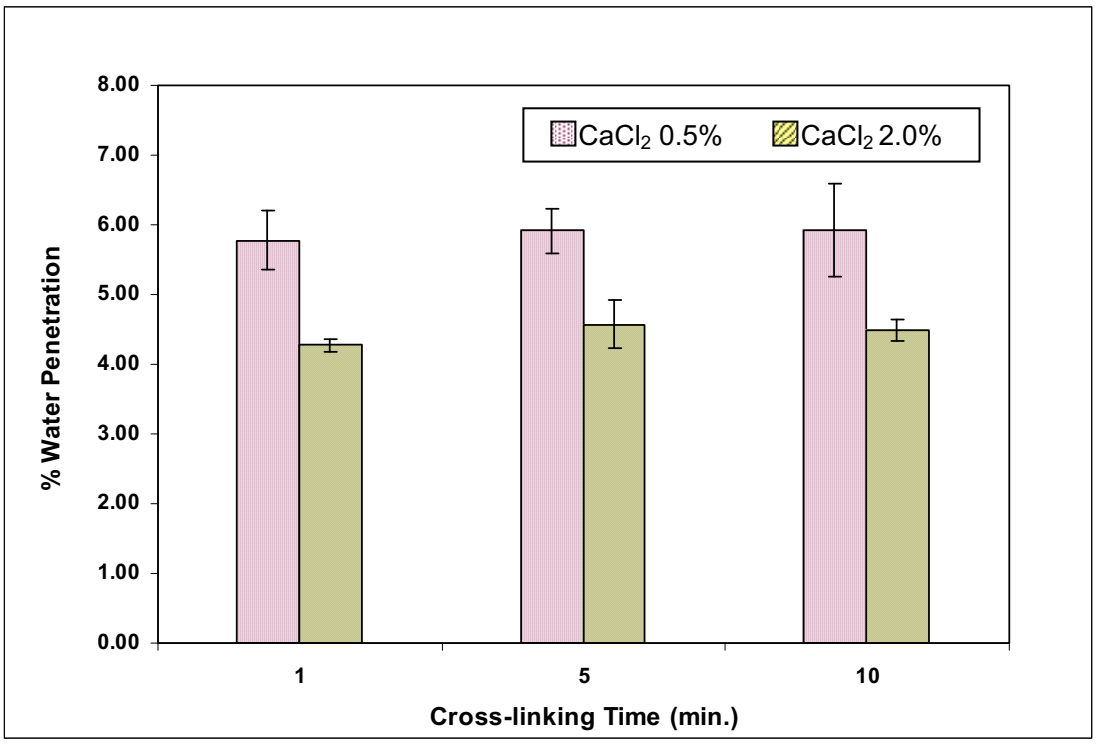


Figure 2 - Wittaya-areekul et al.

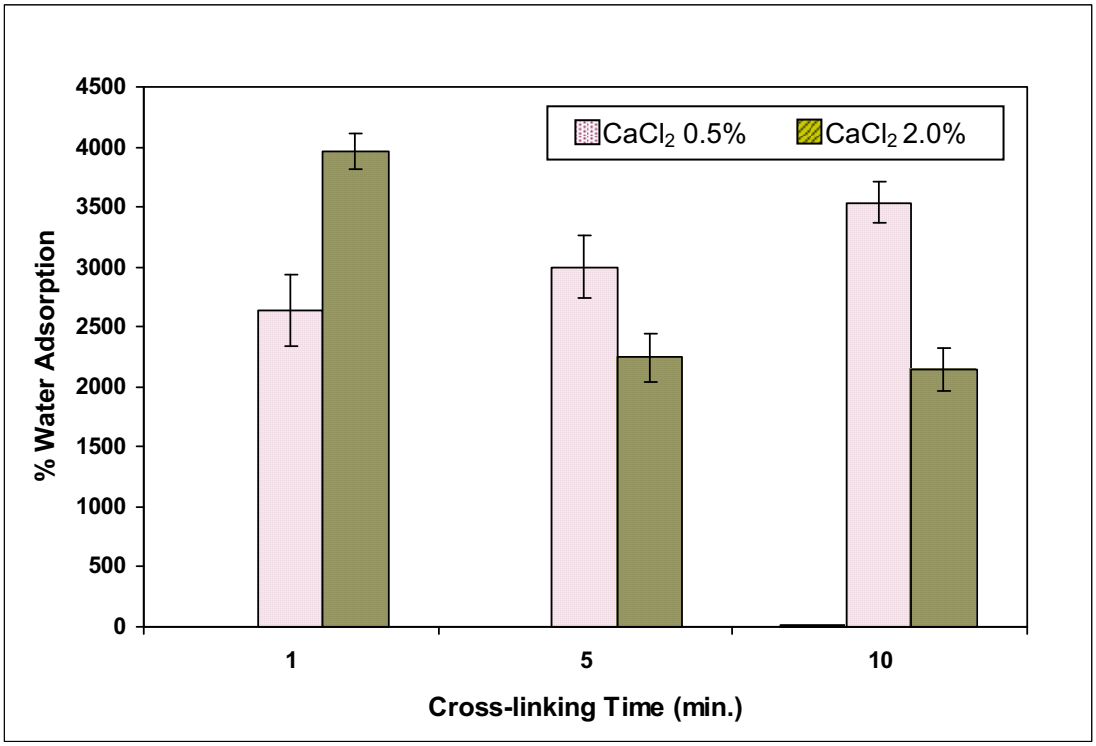


Figure 3 - Wittaya-areekul et al.

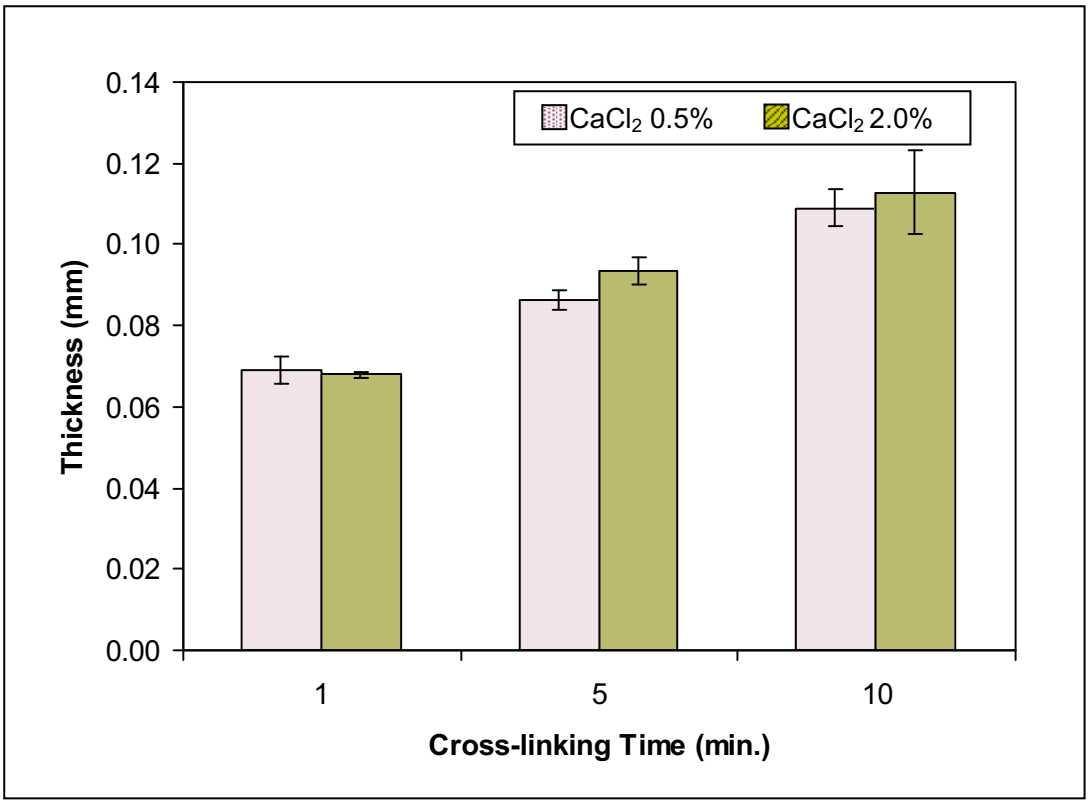
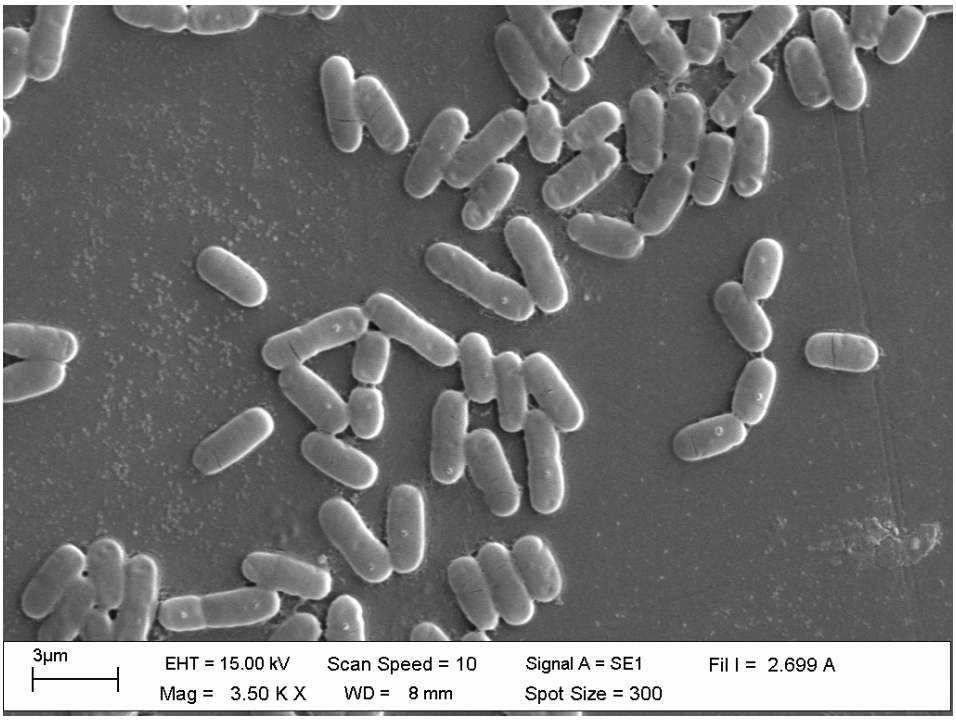

0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Thickness (mm)
CaCl2 0.5%
CaCl2 2.0%
1
5
10
Cross-linking Time (min.)

Figure 4 - Wittaya-areekul et al



(a)

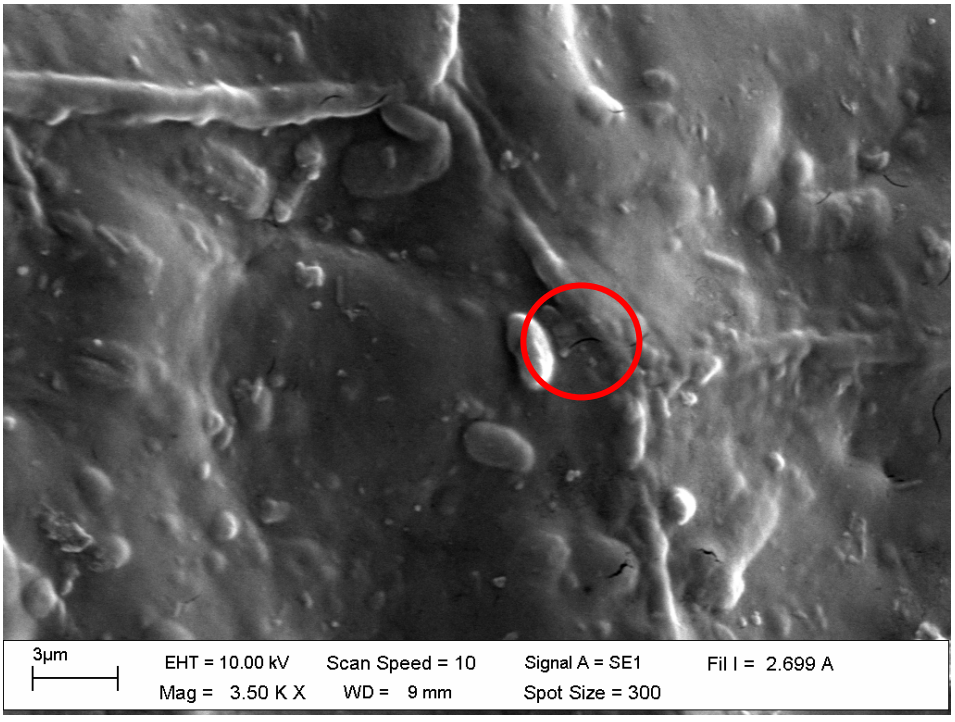


Figure 6 - Wittaya-areekul et al.

ภาคผนวกที่ 6

Development of Seed Coating Formulation for Live Cells of Free-living Plant Growth-Promoting Rhizobacteria

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Abstract

Currently, formulation containing live cells of free-living plant growth-promoting rhizobacteria (PGPR) is in a liquid form. Most products require intensive apparatus and certain conditions for preparation. The objective of this study was to develop the practical formulation for live cells of free-living PGPR. Alginate and pectin was used as film formers and propylene glycol was used as a plasticizer. The formulation was developed with various concentrations of the film components and evaluated for basic film properties, i.e., flexibility, water adsorption and vapor penetration. The films were further cross-linked using calcium chloride solution to improve the film strength. The optimum film formulation was selected for evaluation of appropriate coating method and rhizobacterial survival in the film components using soybean seeds as a model for seed coating. The result showed that rhizobacteria can grow in all of the film formulations. The optimum film formulation contained 2% w/v alginate 4% w/v pectin, and 2% w/v propylene glycol using 0.1 %w/v calcium chloride and 2% w/v propylene glycol as a cross linking solution. This film formulation showed even film formation with excellent water adsorption and vapor penetration. The coating method consisted of spreading 4 mL of polymer mixtures on 10 grams of soybean seeds and then spraying with 0.6 mL of cross linking solution resulted in good film coating feature with minimal coating duration. It was suggested that this seed coating formulation could be benefit for using live cells of PGPR and would be a practical preparation for growers.

Keywords: Rhizobacteria, Growth Promotion, Seed Coating, Wet Film

Outputs:

1. Submitted petty patent to Thailand Intellectual Property Department.

2.Wittaya-Areekul, S., Jetiyanon, K., Plianbangchang, P. Development of Seed Coating Formulation for Live Cells of Free-living Plant Growth-Promoting Rhizobacteria**.** Carbohydrate Polymers (manuscript in preparation).

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ภาคผนวกที่ 7

Seed Coating Film of *Bacillus cereus* strain RS87 for Plant Growth Promotion

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Abstract

Plant growth promoting rhizobacterium (PGPR), *Bacillus cereus* strain RS87, has been demonstrated for it's efficacy of promoting plant growth in various crops both in greenhouse and field trials. However, application of strain RS87 is still based on using live cells which is routinely prepared in laboratory. The objective of this study was to investigate the feasibility of seed coating film containing spore of strain RS87 for plant growth promotion. Cucumber and pepper were tested. In greenhouse assays, the experiment consisted of 7 treatments including non-treated seed control (NTC), soaking seed with water control (WC), wet-seed coating film control (WFC), dry-seed coating film control (DFC), soaking seed with live cells RS87 (LC-RS87), wet-seed coating film with RS87 spore (WF-RS87spore), and dry-seed coating film with RS87 spore (DF-RS87spore). Three experiments including percentage of emergence (30 replications/treatment), root length (30 replications/treatment), and plant height (12 replications/treatment) were separately investigated. Each experiment was conducted twice. Results showed that DF-RS87spore provided the highest seed emergence of cucumber and pepper. LC-RS87 and WF-RS87spore gave better seed emergence than control treatments. All bacterized treatments (both live cells and spore of RS87) significantly promoted ($P \leq 0.05$) root length and plant height over control treatments. In conclusion, seed coating film with RS87 spore promoted plant growth of cucumber and pepper.

Keywords: Seed Coating Film, *Bacillus cereus* strain RS87, plant growth promotion

Outputs

1. Jetiyanon, K., Wittaya-areekul, S., Plianbangchang, P. Seed Coating Film of *Bacillus cereus* strain RS87 for Plant Growth Promotion. Canadian Journal of Microbiology (manuscript in preparation)

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ภาคผนวกที่ 8

Demographic Characteristics and Channels of Innovations Diffusion in Noen Maprang District, Phitsanulok: An Application for PGPR

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Abstract
Understanding of the community background and proper channel is essential for the success of innovations diffusion in a community. In this study, we investigated the demographic characteristics and acceptable channels of PGPR technology distribution in two villages of Noen Maprang district, Phitsanulok. The study was conducted between November 2006 and June 2007. Responses were gathered from 170 community members by means of a survey with structured questionnaires. Two focus group sessions were conducted with community leaders. The results indicated that the majority (72.9%) of community members in both villages were farmers, with primary school education (74.1%). Most of the respondents cultivated various crops in their fields. The main crop was rice (92.3%), followed by mango (56.1%) and corn (14.6%). Almost all farmers utilized agro-chemicals for plant production. Aproximately 50% used only chemicals, whereas 43.8% used a combination of chemicals and natural products. The majority of respondents (83.5%) reported affiliating with at least one group/organization. Economic was the most frequently mentioned reason (83.8%) for joining a group/organization. Most community members (74.1%) received agriculture-related information. The most frequently mentioned source of information was village head (66.7%), followed by district and subdistrict agricultural extensionists (56.3%), community broadcast (55.6%), and television (50.8%). A number of the respondents (78.2%) expressed intention to try alternative method for plant production. Results revealed that the most appropriate means to diffuse new technology was a field-trail demonstration, coupled with a workshop for interested members. Words of mouth from interested members to their social network, i.e., family members and neighbors, then, would be an effective communication channel. This study showed the problem of heavy chemical use that awaited resolution. In addition, community members exhibited interest in more environmental-friendly alternatives. The results from this study were utilized as a strategy to introduce and distribute PGPR knowledge and practice in these communities.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR), diffusion of innovations

Outputs
1. Jetiyanon, K. Plianbangchang P., Wittaya-areekul S. Demographic Characteristics and Channels of Innovations Diffusion in Noen Maprang District, Phitsanulok: An Application for PGPR, Agricultural System (manuscript in preparation).

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ภาคผนวกที่ 9

“การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร”

26 มิถุนายน 2551

ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

I. ความนำ

คณะผู้วิจัย อันประกอบด้วย รองศาสตราจารย์ ดร.กัญชลี เจติยานนท์ สังกัดคณะเกษตรศาสตร์ทรัพยากรธรรมชาติและสิ่งแวดล้อม รองศาสตราจารย์ ดร.ศักดิ์ชัย วิทยาอารีย์กุล และรองศาสตราจารย์ ดร.ภิญญุภา เปลี่ยนบางช้าง สังกัดคณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร ได้รับทุนสนับสนุนการวิจัยจากสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) เพื่อดำเนินโครงการวิจัยแบบมุ่งเป้า ในหัวข้อ “การพัฒนาสูตรตำรับ ทดสอบประสิทธิภาพ และถ่ายทอดเทคโนโลยีของผลิตภัณฑ์ส่งเสริมการเจริญเติบโตของพืชจากไรโซแบคทีเรีย” ระยะเวลา 2 ปี ตั้งแต่ พ.ศ. 2549-2551

การดำเนินงานที่ผ่านมาเป็นไปตามแผนการวิจัย โดยผู้วิจัยได้พัฒนาสูตรตำรับปุ๋ยที่มีส่วนผสมของเชื้อไรโซแบคทีเรียที่มีความคงตัว มีประสิทธิภาพในการส่งเสริมการเจริญเติบโตของพืช ทั้งในภาวะควบคุมและภาคสนาม นอกจากนี้ ยังได้เผยแพร่นวัตกรรม “ปุ๋ยไรโซ” ให้กับเกษตรกรผู้สนใจในอำเภอเนินมะปราง จ.พิษณุโลก ด้วยกระบวนการแพร่กระจายนวัตกรรมที่เหมาะสมกับพื้นที่ นั่นคือ ใช้ผู้นำชุมชน (ผู้ใหญ่บ้าน) ซึ่งเป็นผู้นำทางความคิดของชาวบ้านเป็นจุดเริ่มต้น เพื่อระบุกลุ่มผู้รับนวัตกรรมเร็ว จากนั้นใช้เทคนิคการทดลองเพื่อให้เห็นผลเชิงประจักษ์และการบอกต่อเป็นช่องทางการแพร่กระจายนวัตกรรมในชุมชนต่อไป เกษตรกรอาสาสมัครได้ทดลองใช้ “ปุ๋ยไรโซ” ทดแทนปุ๋ยเคมีบางส่วนโดยได้ผลผลิตไม่แตกต่างกัน แต่มีความพึงพอใจกับ “ปุ๋ยไรโซ” มากกว่า เนื่องจากเห็นว่า เป็นมิตรกับสิ่งแวดล้อมและผลผลิตที่ได้มีคุณภาพดีกว่าการใช้ปุ๋ยเคมีเต็มสูตรอย่างเดียว เช่น แตงกวามีรสหวานกว่า เก็บผลผลิตได้นานกว่า เป็นต้น นอกจากนี้ ตลอดระยะเวลาการทดลอง มีเพื่อนบ้านและเกษตรกรจากชุมชนใกล้เคียงซึ่งได้เห็นผลเชิงประจักษ์ของ “ปุ๋ยไรโซ” ให้ความสนใจอีกจำนวนหนึ่ง

ขั้นตอนสุดท้ายของโครงการ คือ การถ่ายทอดนวัตกรรมไรโซแบคทีเรียหรือ “ปุ๋ยไรโซ” สู่เกษตรกร ด้วยเหตุนี้ คณะผู้วิจัยจึงได้จัดโครงการอบรมเชิงปฏิบัติการ “การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร” ขึ้น เพื่อเปิดโอกาสให้เกษตรกรผู้สนใจทั่วประเทศ ได้รับความรู้เกี่ยวกับหลักการของไรโซแบคทีเรียในการเสริมสร้างความเจริญเติบโตให้กับพืช ได้รับชมการสาธิตการผลิต “ปุ๋ยไรโซ” และแลกเปลี่ยนประสบการณ์กับเกษตรกรอาสาสมัครที่เคยทดลองใช้ผลิตภัณฑ์แล้ว

การอบรมเชิงปฏิบัติการนี้จัดขึ้นในวันพฤหัสบดีที่ 26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก โครงการแบ่งเป็น 2 ช่วง ช่วงเช้าเป็นการสาธิตการผลิต “ปุ๋ยไรโซ” ณ แปลงทดลอง ช่วง

บ่ายเป็นการบรรยายความรู้และแลกเปลี่ยนประสบการณ์ ที่อาคารคณะเกษตรศาสตร์ฯ ผู้เข้าร่วมโครงการจะได้รับตัวอย่าง “ปุ๋ยไรโซ” เพื่อทดลองใช้คนละ 1 กิโลกรัม

II. ผลการดำเนินงาน

ผลการดำเนินงานเชิงปริมาณ:

จากใบลงทะเบียน (ภาคผนวก 9ก) มีผู้ลงทะเบียน 59 คน แบ่งเป็น ผู้ทรงคุณวุฒิจาก สกว. 2 คน คณะผู้วิจัย 2 คน วิทยากร 3 คน ผู้สังเกตการณ์จาก สกว. 1 คน ผู้สังเกตการณ์จากมหาวิทยาลัยนเรศวร 1 คน และเกษตกรผู้สนใจ 50 คน

ผลการดำเนินงานเชิงคุณภาพ:

ผลการดำเนินงานเชิงคุณภาพประเมินจากแบบประเมินความพึงพอใจ (ภาคผนวก 9ข) ที่แจกให้ผู้เข้าร่วมโครงการในช่วงพักรับประทานอาหารกลางวัน จากแบบประเมินที่แจก 50 ฉบับ ได้รับกลับคืนมา 48 ฉบับ คิดเป็นอัตราการตอบกลับร้อยละ 96

ผู้ตอบแบบประเมินส่วนใหญ่ (ร้อยละ 70.8) เป็นเพศชาย อายุกระจายกันตั้งแต่ ต่ำกว่า 25 ปีไปจนถึงสูงกว่า 55 ปี โดยเกือบครึ่ง (ร้อยละ 46.8) มีอายุ 51 ปีขึ้นไป ผู้เข้าร่วมโครงการ 14 รายมาจากจังหวัดพิษณุโลก นอกนั้นมาจากจังหวัดราชบุรี (8 ราย) กรุงเทพฯ (7 ราย) สุโขทัย (5 ราย) กำแพงเพชร (4 ราย) พิจิตร (2 ราย) และจันทบุรี ชัยนาท ชัยภูมิ นครปฐม ปทุมธานี ประจวบคีรีขันธ์ สมุทรปราการ และอุตรดิตถ์ จังหวัดละ 1 ราย

ผู้เข้าร่วมโครงการเกินครึ่ง (ร้อยละ 62.5) ประกอบอาชีพเกษตรกรรมเป็นหลัก ที่เหลือมีอาชีพค้าขาย/ประกอบธุรกิจ หรือรับราชการ (หรือเป็นข้าราชการบำนาญ) โดยประกอบอาชีพเกษตรกรรมเป็นอาชีพเสริมหรืองานอดิเรก

ช่องทางสื่อสารที่เข้าถึงผู้เข้าร่วมโครงการครั้งนี้มากที่สุด คือ การประชาสัมพันธ์ของผู้ให้ทุนผ่านหนังสือพิมพ์เดลินิวส์ (เข้าถึงผู้เข้าร่วมโครงการร้อยละ 74.5) รองลงมาเป็นการสื่อสารจากปากต่อปาก คือ รับทราบจากผู้วิจัยโดยตรง (ร้อยละ 12.8) และวิทยุคลื่น สวท.พิษณุโลก (ร้อยละ 6.4)

การประเมินความพึงพอใจต่อโครงการแบ่งเป็น 2 ส่วน คือ การบริหารจัดการและวิชาการ ตัวเลือกแบบประเมินเป็นแบบ dichotomous (พอใจ/ไม่พอใจ) พร้อมให้ระบุข้อเสนอแนะหากผู้ประเมินไม่พึงพอใจต่อประเด็นนั้นๆ

ในภาพรวม ผู้เข้าร่วมโครงการมีความพึงพอใจต่อการบริหารจัดการเกินร้อยละ 80 ทุกด้าน (ตารางที่ 1) โดยเฉพาะด้านอาหารและเครื่องดื่มและสถานที่บรรยายช่วงบ่าย (ห้อง AG 2303 คณะ

เกษตรศาสตร์ฯ) ที่ผู้เข้าร่วมโครงการทุกคนมีความพึงพอใจ ด้านที่ได้รับความพึงพอใจจากผู้เข้าร่วมโครงการน้อยที่สุด คือ การประชาสัมพันธ์โครงการ โดยมีข้อเสนอแนะว่า ควรประชาสัมพันธ์ให้ทั่วถึงมากกว่านี้ เช่น การติดป้ายประชาสัมพันธ์ตามสำนักงานเกษตรจังหวัด เกษตรตำบล ประชาสัมพันธ์ผ่าน อบต. การใช้สื่อที่เข้าถึงเกษตรกร เช่น หนังสือพิมพ์ไทยรัฐและเดลินิวส์ วิทยุชุมชน หรือรายการโทรทัศน์สำหรับเกษตรกร เป็นต้น

นอกจากนี้ ผู้เข้าร่วมโครงการบางรายยังมีข้อเสนอแนะให้จัดอบรมในระยะเวลานานขึ้น โดยมีการเสนอเป็น 2 วัน (5 ราย) จัดในวันหยุดสุดสัปดาห์ (3 ราย) และควรจัดที่กรุงเทพฯ เพื่อให้ผู้สนใจจากต่างจังหวัดสามารถเข้าร่วมประชุมได้สะดวกขึ้น (5 ราย)

ตารางที่ 1 ความพึงพอใจต่อการบริหารจัดการโครงการฯ

ด้าน...	**จำนวนผู้ที่พึงพอใจ (ร้อยละ)**
1. วันที่จัดโครงการ (26 มิ.ย. 51) (n=46)	42 (91.3)
2. จำนวนวันที่จัดโครงการ (1 วัน) (n=47)	43 (91.5)
3. การประชาสัมพันธ์ (n=46)	39 (84.8)
4. การเดินทาง (n=46)	40 (87.0)
5. การลงทะเบียนและการต้อนรับ (n=47)	44(93.6)
6. อาหารและเครื่องดื่ม (n=46)	46 (100.0)
7. สถานที่ช่วงเช้า (แปลงสาธิต) (n=45)	39 (86.7)
8. สถานที่ช่วงบ่าย (คณะเกษตรฯ) (n=45)	45 (100.0)

ในด้านวิชาการ ผู้เข้าร่วมโครงการมีความพึงพอใจต่อการบริหารจัดการเกินร้อยละ 80 ทุกด้านเช่นกัน (ตารางที่ 2) โดยการบรรยายในช่วงบ่ายได้รับความพึงพอใจจากผู้เข้าร่วมโครงการทุกคน ด้านที่ได้รับการประเมินต่ำที่สุดในกลุ่มนี้ คือ ประโยชน์ที่ได้รับจากโครงการในภาพรวม ทั้งนี้เนื่องจากผู้วิจัยยังไม่สามารถถ่ายทอดเทคโนโลยีทั้งหมดในการผลิตปุ๋ยไรโซให้กับเกษตรกรได้ด้วยข้อจำกัดด้านเครื่องมือวิทยาศาสตร์และเทคนิคการเลี้ยงเชื้อขั้นสูง โดยเกษตรกรยังจำเป็นต้องขอรับเชื้อไรโซแบคทีเรียจากห้องปฏิบัติการทุกครั้ง เพื่อนำไปผลิตปุ๋ยไรโซ จึงทำให้เกษตรกรจำนวนหนึ่งรู้สึกว่า การอบรมครั้งนี้ยังไม่เกิดประโยชน์สูงสุดกับตนเอง

นอกจากนี้ ผู้เข้าร่วมโครงการ 5 รายยังเห็นว่า การสาธิตในช่วงเช้ามีรายละเอียดน้อยเกินไปจนไม่เข้าใจ มี 2 รายเสนอให้จัดสถานที่ชมการสาธิตที่เป็นอาคาร กันแดดได้ และมี 1 รายที่เสนอแนะว่าควรแบ่งกลุ่มเข้าชมการสาธิตให้ชัดเจน เพื่อความเป็นระเบียบ

ตารางที่ 2 ความพึงพอใจต่อเนื้อหาวิชาการของโครงการฯ

ด้าน...	จำนวนผู้ที่พึงพอใจ (ร้อยละ)
1. ความสามารถของวิทยากร (n=46)	45 (97.8)
2. เวลาการสาธิตผลิตปุ๋ยช่วงเช้า (n=45)	42 (93.3)
3. เวลาการบรรยายช่วงบ่าย (n=44)	44 (100.0)
4. การเปิดโอกาสให้ซักถามแลกเปลี่ยน (n=45)	43 (95.6)
5. ประโยชน์ที่ได้รับจากโครงการในภาพรวม (n=44)	37 (84.1)

เมื่อสอบถามถึงความสนใจในการเข้ารับการอบรมในลักษณะเดียวกันในครั้งต่อไป ผู้เข้าร่วมโครงการทุกคนแสดงความจำนงเข้าร่วมโครงการ พร้อมทั้งให้ชื่อ-ที่อยู่และหมายเลขโทรศัพท์ติดต่อไว้กับผู้วิจัย

III. บทสรุป

โครงการอบรมเชิงปฏิบัติการ “การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร” เป็นส่วนหนึ่งของโครงการวิจัยเรื่อง “การพัฒนาสูตรตำรับ ทดสอบประสิทธิภาพ และถ่ายทอดเทคโนโลยีของผลิตภัณฑ์ส่งเสริมการเจริญเติบโตของพืชจากไรโซแบคทีเรีย” ที่ได้รับการสนับสนุนทุนวิจัยแบบมุ่งเป้าจาก สกว. ผลการดำเนินงานพบว่า ประสบผลสำเร็จทั้งเชิงปริมาณและคุณภาพ โดยมีผู้เข้าร่วมโครงการ 59 คนจากจังหวัดต่างๆ ทั้งภาคเหนือตอนล่าง ภาคกลาง ภาคตะวันตก ภาคตะวันออกเฉียงเหนือ ภาคตะวันออก และภาคใต้ตอนบนของประเทศ ผู้เข้าร่วมโครงการมีความพึงพอใจต่อประเด็นต่างๆ ของการจัดโครงการในระดับดีมาก

ผลจากการจัดโครงการครั้งนี้แสดงให้เห็นถึงความสนใจของเกษตรกรในการแสวงหาทางเลือกในการเกษตร โดยเฉพาะในยุคที่ปุ๋ยเคมีมีราคาแพง ดังนั้น จึงมีความเป็นไปได้สูงในด้านการตลาดที่จะพัฒนานวัตกรรม “ปุ๋ยไรโซ” สู่เกษตรกรต่อไป อย่างไรก็ตาม อุปสรรคสำคัญที่ต้องข้ามผ่านให้ได้เพื่อถ่ายทอดเทคโนโลยีนี้สู่เกษตรกรอย่างแท้จริง คือ การเพาะเลี้ยงเชื้อไรโซแบคทีเรีย ซึ่งในปัจจุบันต้องการเครื่องมือวิทยาศาสตร์และเทคนิคการเลี้ยงเชื้อขั้นสูง เกษตรกรไม่สามารถนำไปประยุกต์ใช้ได้เอง จึงควรมีการศึกษาเพิ่มเติมเพื่อแก้ปัญหานี้

ปัญหาและอุปสรรคที่พบจากการจัดโครงการ ซึ่งจะเป็นประโยชน์ในการพัฒนาการจัดโครงการครั้งต่อไป คือ การประชาสัมพันธ์ โดยผู้เข้าร่วมโครงการเสนอแนะให้ใช้สื่อที่เข้าถึงเกษตรกรมากขึ้น เช่น หนังสือพิมพ์ไทยรัฐหรือเดลินิวส์ การติดป้ายประชาสัมพันธ์ตามสำนักงานเกษตรจังหวัดหรือเกษตรตำบล เป็นต้น

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ภาคผนวก 9ก

ใบลงทะเบียน

ใบลงทะเบียน

โครงการอบรมเชิงปฏิบัติการ
"การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร"

26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

ลำดับที่	ชื่อ-สกุล	ลายเซ็น
1	ศาสตราจารย์ ดร.วิชัย บุญแสง	
2	ศาสตราจารย์ ดร.ประเสริฐ โสภณ	
3	รองศาสตราจารย์ ดร.กัญชลี เจดียานนท์	
4	รองศาสตราจารย์ ดร.ภิญญภา เปลี่ยนบางช้าง	
~~—~~	~~รองศาสตราจารย์ ดร.ศักดิ์ชัย วิทยาอารีย์กุล~~	
5	นางสาวสุทิศา ด้วงบ้านยาง	
6	นางเยี้ยม ฉุยฉาย	
7	นายสว่าง สมพิมเสน	
8		
9		

ใบลงทะเบียน

โครงการอบรมเชิงปฏิบัติการ
"การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร"

26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

ลำดับที่	ชื่อ-สกุล	ลายเซ็น
10		081-6665979
11		0849106463
12		089-9432140
13		08-9402-9443
14		0870250260
15		084-3085059
16		0846489004
17		0816047446
18		
19		089-4144458
20		081-7052169
21		086 0073577
22		083 6035539
23		089-9882702
24		
25		055611448
26		0849394
27		081-7073216
28		089-5682077
29		089-704-7334

ใบลงทะเบียน

โครงการอบรมเชิงปฏิบัติการ
"การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร"

26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

ลำดับที่	ชื่อ-สกุล	ลายเซ็น
30	นาย ปรีชา	
31	นาย ปรีชา	
32	นาย	
33	พ.ท.	พ.ท.
34	นาย	08A-A133470
35		
36		087-1959939
37	นาย	0844516942

ใบลงทะเบียน

โครงการอบรมเชิงปฏิบัติการ
"การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร"

26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

ลำดับที่	ชื่อ-สกุล	ลายเซ็น
38	นางจารุณี เพชรพุก	จารุณี เพชรพุก 081-2844947
39	นาง มัลลอย เหลี่ยมจุ้ย	มัลลอย เหลี่ยมจุ้ย 089-7027776
40	นางสมบูรณ์ เรืองคำ	สมบูรณ์ เรือง 086-1658568
41	น.ส. ธนุกร ธรรรัตน์	ธนุกร ธรรรัตน์ 0833381982
42	นาย สมปอง แสงสกุลลิ้ม	[illegible]
43	นางมณีวรรณ แสงสิทธิ์	[illegible]
44	นายปริญญา ศรีเลิศชัย	[illegible] 0860946423
45	นาย ธนัญชัย หาญลำเลิศ	[illegible] 0819293998
46	นางดารี ชาริ	[illegible] 0862032161
47	นาง สมหวง รองคำภีร์	สมหวง 087-3392505
48	นาย สำราญ ปิ่นหมวก	สำราญ 0852736357
49	ศรายุทธ ศิริสุบรรณ	[illegible] 081-5334587.

ใบลงทะเบียน

โครงการอบรมเชิงปฏิบัติการ
"การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร"

26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

ลำดับที่	ชื่อ-สกุล	ลายเซ็น
50	นาง เยี่ยม คุณดาว	เยี่ยม
51	นาง สมหวัง ตาเดชิ	0816338043
52	นาง ดิด จันจันทร์	084-1954939
53	นายลำยงค์ ชะเกด	
54	นายทวีสิทธิ์ หอมแพ่ง	
55	เสย ริ้มหอม	
56	สาวิตรี บุญนาค	
57	นาย ณัชพล บุญมี	084-7292969
58	นาย ธีระ เอี่ยมพิบูลย์สวัสดิ์	ธีระ 081-9625213
59	ร.ต. สิทธิ์ เนียมทรัพย์	081-9625213

ภาคผนวก 9ข

แบบประเมินความพึงพอใจ

แบบประเมินความพึงพอใจ

โครงการอบรมเชิงปฏิบัติการ "การถ่ายทอดเทคโนโลยีการผลิตปุ๋ยไรโซแบคทีเรียสู่เกษตรกร"

26 มิถุนายน 2551 ณ มหาวิทยาลัยนเรศวร จ.พิษณุโลก

คำชี้แจง:

แบบประเมินนี้จัดทำขึ้นเพื่อรับทราบระดับความพึงพอใจที่ท่านมีต่อแง่มุมต่างๆ ของโครงการ ผลการประเมินจะนำไปใช้ปรับปรุงการจัดโครงการครั้งต่อไป

โปรดทำเครื่องหมายถูก (✓) ในช่องสี่เหลี่ยม (❑) ด้านหน้า/ด้านล่างของข้อความ หรือตอบคำถามในช่องว่างตามความเป็นจริง

ส่วนที่ 1: ข้อมูลทั่วไปของท่าน

1. เพศ ❑ ชาย ❑ หญิง

2. อายุ ❑ ต่ำกว่า 25 ปี ❑ 25-30 ปี ❑ 31-35 ปี ❑ 36-40 ปี
❑ 41-45 ปี ❑ 46-50 ปี ❑ 51-55 ปี ❑ มากกว่า 55 ปี

3. อาชีพหลัก ❑ เกษตรกรรม ❑ อื่นๆ คือ .....

4. ภูมิลำเนา ❑ จังหวัดพิษณุโลก ❑ อื่นๆ คือ จังหวัด .....

5. ท่านได้ข้อมูลเกี่ยวกับโครงการนี้จาก
❑ อาจารย์กัญชลี ❑ หนังสือพิมพ์ ❑ วิทยุ คลื่น ..... ❑ โทรทัศน์ ช่อง .....
❑ หอกระจายข่าวหมู่บ้าน ❑ อื่นๆ คือ .....

ส่วนที่ 2: ความพึงพอใจต่อโครงการ

2.1 ส่วนการบริหารจัดการ

ด้าน...	ระดับความพึงพอใจ		ข้อเสนอแนะ
	พอใจ	ไม่พอใจ	
1. วันที่จัดโครงการ (26 มิ.ย. 51)			ควรเป็น .....
2. จำนวนวันที่จัดโครงการ (1 วัน)			ควรเป็น .....
3. การประชาสัมพันธ์			ควรเป็น .....
4. การเดินทาง			ควรเป็น .....
5. การลงทะเบียนและการต้อนรับ			ควรเป็น .....
6. อาหารและเครื่องดื่ม			ควรเป็น .....
7. สถานที่ช่วงเช้า (แปลงสาธิต)			ควรเป็น .....
8. สถานที่ช่วงบ่าย (คณะเกษตรฯ)			ควรเป็น .....

2.2 ส่วนเนื้อหาการอบรม

ด้าน...	ระดับความพึงพอใจ		ข้อเสนอแนะ
	พอใจ	ไม่พอใจ	
1. ความสามารถของวิทยากร			ควรเป็น .....
2. เวลาการสาธิตผลิตปุ๋ยช่วงเช้า			ควรเป็น .....
3. เวลาการบรรยายช่วงบ่าย			ควรเป็น .....
4. การเปิดโอกาสให้ซักถามแลกเปลี่ยน			ควรเป็น .....
5. ประโยชน์ที่ได้รับจากโครงการในภาพรวม			ควรเป็น .....

ถ้ามีการจัดโครงการเช่นนี้อีก ท่านสนใจเข้าร่วมหรือไม่

❑ ไม่สนใจ

❑ สนใจ กรุณาให้ชื่อ-ที่อยู่สำหรับติดต่อ

ชื่อ – สกุล .....

ที่อยู่ .....

.....

.....

หมายเลขโทรศัพท์ .....

☺ ขอบคุณที่กรุณาสละเวลาตอบแบบประเมินชุดนี้ ☺

ภาพกิจกรรม

แผ่นป้ายต้อนรับผู้เข้าร่วมโครงการบริเวณแปลงสาธิต

ผู้เข้าร่วมโครงการรับฟังการอธิบายส่วนผสมของปุ๋ยไรโซ

รศ.ดร.กัญชลี เจติยานนท์ วิทยากร

ชมการสาธิตที่หน่วยผลิตบริเวณแปลงทดลอง

รับฟังการบรรยายกรรมวิธีการผลิตเชื้อไรโซแบคทีเรียและการเตรียมผลิตภัณฑ์
ที่คณะเกษตรศาสตร์ฯ

นางเยี้ยม ฉุยฉาย หนึ่งในเกษตรกรอาสาสมัคร แลกเปลี่ยนประสบการณ์กับผู้เข้าร่วมโครงการ

บรรยากาศการซักถามและแลกเปลี่ยนความคิดเห็น

ถ่ายภาพร่วมกันเป็นที่ระลึก