Department of Crop Sciences
Section Quality of Plant Products
Georg-August University of Göttingen

Characterization of a botanical fungicide from Thai origin and its efficiency in rice production

Pitipong Thobunluepop

Cuvillier Verlag Göttingen

Characterization of a botanical fungicide from Thai origin and its efficiency in rice production

Dissertation
to obtain the Ph. D. degree
in the Faculty of Agricultural Sciences,
Georg-August-University Göttingen, Germany

Presented by

Pitipong Thobunluepop
Born in Lampang, Thailand

Göttingen, January 2008

Bibliografische Information der Deutschen Nationalbibliothek
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2008
Zugl.: Göttingen, Univ., Diss., 2008

978-3-86727-521-7

D7

Referee : Prof. Dr. Elke Pawelzik
1st Co-referee : Prof. Dr. Dr. h. c. Udo ter Meulen
2nd Co-referee : Prof. Dr. Suchada Vearasilp
Examiner : Prof. Dr. Wolfgang Lücke

Date of disputation: 31 January 2008

Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

1. Auflage, 2008
Gedruckt auf säurefreiem Papier

978-3-86727-521-7

Dedicated to…

Prof. Dr. Suchada Vearasilp, and Prof. Dr. Therdchai Vearasilp

My parents, my elder brother and sisters, with lover

… Probable impossibilities are to be preferred to improbable possibilities …
(Aristotle)

… When you have eliminated the impossible, whatever remains, however improbable, must be true …
(Sir Arthur Conan Doyle)

Acknowledgements

First of all, I want to express sincere gratitude to my supervisor Prof. Dr. Elke Pawelzik and Prof. Dr. Suchada Vearasilp for their guidance through my time as a PhD-student, for enhancing my scientific knowledge and for their encouragement. Their guidance, wisdom and patience during the period of my study are a great unforgettable gift that enhanced my self-confidence to finish my studies. Their guidance in formulating the research work, careful reading and correction my dissertation and valuable suggestions are most gratefully acknowledged.

Similarly, I am also grateful to Prof. Dr. Araya Jatisatienr, Prof. Dr. Chaiwat Jatisatienr and Prof. Dr. Therdchai Vearasilp for their kindness, all means of support and for volunteering as my co-advisors. Moreover, my gratitude also goes to Prof. Dr. Dr. h. c. Udo ter Meulen and Prof. Dr. Wolfgang Lücke for willingness to be second supervisors and for the valuable comments and ideas on completing this dissertation, which they contributed.

I thankfully recognize that my PhD study was possible because of financial support by the Commission of Higher Education, Ministry of Education, and research support by the Office of the National Research Council of Thailand (NRCT), Ministry of Science and Technology of the Royal Thai Government. Scientific instruments were contributed supported by the Postharvest Technology Institute, Chiang Mai University, Thailand and Section of Quality Plant Products, Department of Crop Scienceat the Georg-August University of Goettingen, Germany. Special thanks for all supporters gives me a golden chance to pursuing this degree not only with financial support, but also a happy life.

I would like to express my deep thanks to Dr. Sa-nguansak Thanapornpoonpong for discussions regarding research. Also my thanks goes to Mr. Adul Jaiinphon, Ms. Kanokwan for all their help during experiments, Dr. Anna Keutgen, Dr. Silke Stracke and all the technical staff of the Section of Quality Plant Products for their assistance during laboratory work, valuable help, suggestions and interest during all the stages of this research, and also to all staff members of the Agronomy Research Station, Faculty of Agriculture, Chiang Mai University, for all their help during field experiments. I want to thank them for being kind and helpful during my year in our “house”.

Further, I want to thank all of my colleagues in the Section of Seeds Science and Technology, Faculty of Agriculture, Chiang Mai University and also in the Section of Quality Plant Products; Lak, Na, Fai, Nok, B, Frank, A, M, Aun, Nat, Antje, Britta, Le Le Win, and all the other members for their support and friendship. No words are needed to thank my friends for their never ending encouragement.

Last but not least, I do give my grateful thanks to my parents, my father, Jareoun Thobunluepop; my mother, Napaporn Thobunluepop; all of my elder brother and elder sisters; thank you for your encouragement, making my life special, filled with hope and love, and prayer strengthened me through many difficult times.

… Once again, thanks to you all …

Table of content

Table of content

List of tables

Page

List of tables

Page

List of figures

List of figures

Page

List of figures

Page

List of abbreviations, symbols and units

%(w/v)	percentage of weight by volume
µS mg^{-1}	micro ohm per milligram
%(w/w)	weight by weight percentage
%(v/v)	percentage volume by volume
AAT	accelerated aging test
AOS	active oxygen species
APX	ascorbate peroxidase
Aw	water activity
BD	breakdown viscosity
cfu	colony forming unit
CV	coefficient of variance
cv.	cultivar
DW	dry weight
EDTA	ethylene diamine tetraacetic acid
GI test	germination index test
GLC	gas liquid chromatograph
HPTLC	high performance thin layer chromatography
HV	hot viscosity
IC_{50}	inhibitory concentration for 50% inhibitory effect
LOX	lipoxygenase
LSD	least significant difference
MC	moisture content
MPa	micro Pascal
mM	mill mole
PDA	potato dextrose agar
PEG	polyethylene glycol
PT	pasting temperature
PV	peak viscosity
ROS	reactive oxygen species
RVU	rapid viscosity unit
SB	setback viscosity
SD	standard deviation
SDW	seedling dry weight
SGR	seedling growth rate
SOD	superoxide dismutase
TZ test	tetrazolium test

1. Introduction

1.1 Background

Rice (*Oryza sativa*) is a major staple food and a mainstay for the rural population and their food security in Asia, Latin America, Caribbean and Africa. It is central for the food security of over half the world population (Cheema *et al*., 1991). In Thailand, rice is mainly cultivated by small farmers in holdings of less than one hectare. Crop yields in the small scale or subsistence farming sector are relatively lower than in the commercial farming sector. This is due to lack of management capacity, unfamiliarity with appropriate technologies, and shortages of farming inputs such as irrigation, fertilizers, pesticides, and certified seeds (Reissig *et al*., 1986). The International Rice Research Institute (IRRI) has demonstrated that rice yield in Asia can be increased by applying scientific principles and new technologies. For examples, the use of modern varieties and improved cultural practices led to the increase of rice yield throughout tropical Asia (IRRI, 2007).

Plant diseases are most important factors, which challenge problems in commercial agriculture and pose real economics threats to both conventional and organic farming. Since the beginning of agriculture, seeds played a major role in agricultural production, because over 90% of the crop productions are propagated via seeds (Agrios, 1988). At the same time, seeds are the major carriers of plant pathogens, including bacteria, viruses and especially fungi. Thus, disease free seeds provide the front line of defense against the development and spread of plant diseases, which are responsible for large crop losses on worldwide basis (Islaml *et al*., 2000).

1.2 Literature review

1.2.1 Seed borne diseases

Seed qualities are seriously affected by many factors. The principle causes of seed quality losses are biological factors as fungi, insects, mites and rodents (Cheema *et al*., 1991). Fungi infection is one of the most important factors affecting storage life and is one of the major causes for seed quality deterioration as well as decreased crop yield. It may also degrade product quality (Farr *et al*., 1989). Rice crop is known to be attacked by many seed borne fungi, which cause major and minor diseases (Appendix 1). Fungicide seed treatment is a promising way to control the invasion of seed borne fungi. These treatments are used for three main reasons: (1) to control soil-borne fungi that cause seed rots, damping-off, seedling blights and root rot; (2) to control fungi that are surface-borne on the

seed, and (3) to control internally seed-borne fungi. The degree of control with fungicide seed treatment depends on: (1) active ingredients of the fungicide (2) rate of applied fungicide (3) presence of seed- and soil-borne pathogens (4) environmental conditions, and (5) application coverage (Smith *et al*., 1988).

Fungicide seed treatments do not control bacterial pathogens and most of them will not control all types of fungal diseases. Therefore, it is important to choose carefully the treatment that provides the best control of the disease organisms present on the seed or potentially present in the soil. The degree of control varies with product, rate of fungicide use, environmental conditions and disease organisms present. The rate of application prescribed by the label should be considered because over-treatment may injure the seed and under-treatment may not provide good disease control (Webster and Gunnell, 1992).

Fungicide seed treatments help to control soil-borne fungi that cause seed decay, seedling blight and root rot. Control of these diseases may result in better stands, more vigorous seedlings, and increased yields. The application of the fungicide captan (N (trichloromethylthio)-tetrahydrophthalimide) to rice seed has been found to be effective in enhancing seed germination and protecting rice seed borne fungi (Jiskani, 1999). However, in Europe, captan has been banned for use in agriculture because it is a known carcinogen. Additionally, chemicals application and their residues can generate tremendous negative side effects on soil, water and atmospheric environment, which may cause either direct or indirect impacts on human being or other living organisms (Baird, 1994).

The chemicals remain from fungicides in the environment occur either from the overdose application or their lengthy persistent effect and are present generally in soil and water with implications on all living beings in both direct and indirect ways. The WHO reported in 2005 that 5,500 Thai people, 50 % Philippines rice farmers, and 42,800 people in China have health problems because of pesticide poisoning (Jitsaguan, 2005). Those who are affected by chemical toxins are prone to high risk of cancer with possibility of genetic inheritance that can weaken the health status of future generations. The heavy and lengthy use of agrochemicals for pest control not only pollutes the environment, but can also affect negatively the ecosystem as well as the food chain and can lead to pesticide resistance problems. The whole process can generate the loss of biodiversity or even the extinction of some organisms. Additionally, agrochemical residues in food products may become

problems for international trade (Kamal and Moghal, 1968). The alternatives to chemical pesticides for pest control that receive attention are the use of mechanical methods, resistant plant varieties, improved cultural practices, and biological pesticides. Biological pesticides are pesticides whose active ingredients are plant-produced chemicals (Jiskani, 1999).

The growing environmental awareness and health concern worldwide at present time have led to consumer demand for safe and clean foods particularly those chemicals-free products. Therefore, nowadays there emerge many academic interests on bio-fungicides as an alternative to toxic synthetic fungicides to minimize undesirable side effects. Biological fungicides offer important advantages compared to chemical pesticides. The use of these agents in agriculture introduces fewer risks to human health and to the environment than chemical pesticides. Additionally, bio-fungicides are generally relatively inexpensive to produce. Therefore, the use of herbal extracts for plant protection could be a promising way in a chemical-free agricultural system (Horrigan *et al*., 2002).

Many researchers investigated the effect of extracts from *Eugenia caryophyllus* Bullock et. Harrison (Myrtaceae), *Acrorus caramus* Linn. (Araceae), *Mammea siamensis* (Miq.) T. (Guttiferae), *Eupatorium odoratum* L. (Compositae) and some species of Stemona (Stemonaceae) on different fungal diseases. Issakul *et al*. (2003) reported that extracts from *A. caramus*, *M. siamensis*, and *S. curtisii* have insecticidal properties. Chanthayot and Jatisatienr (2003) found in laboratory tests beneficial fungicidal properties with extracts from *E. caryophyllus* and *A. caramus*. On the other hand, *E. odoratum* had unclear biological activity. Wonggiratthiti and Jatisatienr (2003) reported that β-asarone, found in *A. caramus* and eugenol from *E. caryophyllus* showed very high fungicidal properties. Moreover, Mungkornasawakul *et al*. (2003 and 2004) decribed that stemocurtisine, found in *S. curtisii*, and surangin C from *M. siamensis*, showed high biological activity in *Atemia salina* Leach (Anostraca) and in some insects. Furthermore, the analysis of plants treated with extracts of the studied plants did not show any harmful residues. In field studies, farmers were very satisfied with the new botanical pesticides because they could use them instead of harmful synthetic pesticides.

One of the pest control methods that have received scant attention in developing countries and which have been extensively used in developed countries is seed coating technology. The coating of seeds by chemicals or alternative substances can reduce the percentage of plant damage by pests and the level of pesticide application significantly, which could decrease the amount of pesticide application by 85% compared to direct drench application methods (Embaby, 2006). Another advantage of seed coating technique is the preservation of the quality of seeds and the inhibition of seed deterioration (Whipp and Budge, 1993). At thus becomes an interest and a challenge to explore whether the western knowledge of seed coating technique and the available local bio-pesticides can be combined to produce efficient botanical fungicide coated seeds to respond the environmental awareness and meet consumer's demand for clean and safe food.

1.2.2 Effect of seed treatment on seed quality

Planting of high quality seeds is important for an efficient rice production system. In order to obtain optimum stands and yields, early planting, reduction of seeding rates, and the drill planting required high quality and vigorous seeds. Strong seedlings have faster growth rate, greater tolerance to fungi infection and stress than less vigorous ones.

Diseases affect seed quality, yield and may degrade product quality. A fungicide seed treatment protects the seeds and young seedlings from many seed borne and soil borne fungi infections. However, fungicides can produce phytotoxic effects that cause losses of seeds germinability and seedling vigor (Yildirim and Hoy, 2003). These phytotoxic effects may be amplified by the over-application of fungicides. Von Pine *et al*. (1995) reported that fungicide treatments with captan and pirimifos reduced maize seed qualities during storage and the posterior behavior under field conditions, which led to yield reduction. Silva *et al*. (1996) reported that clorpirifos and captan reduced the maize seeds germination and the vigor during the storage.

Therefore, the use of botanical fungicides for plant pathogens control could be a promising way in a chemical-free agricultural system (Bhatti and Soomro, 1996). However, there are also some disadvantages of botanical fungicides:

(1) They are more difficult to implement than the chemical fungicides,

(2) They have generally a narrow target range,

(3) They do not act as quick as chemical fungicides,

(4) They have a shorter shelf life than the chemical fungicides, and
(5) They are not compatible with chemical fungicides or herbicides.
Moreover, some essential oils from the botanical fungicides can cause phytotoxic effects on seed viability when used at high concentrations (5 to 10%, v/v) (Tworkoski, 2002).

Thus, the present study was conducted to address the following objectives:

1. To screen and select the best antifungal activity of plant crude extract or essential oil against pathogenic fungi on rice seeds,
2. To compare the effectiveness of conventional chemical seed treatment and botanical fungicide coated seed against rice seed borne fungi during storage,
3. To investigate the effects of various seed coating substances on rice seeds qualities, especially on chemical and biochemical properties,
4. To evaluate the effectiveness of various seed coating substances on seed practice and plant productivity under field conditions,
5. To evaluate the effect of seed coating substances on the quality of the harvested grain,
6. To evaluate the effect of various seed coating substances on environmental pollution

1.3 Thesis outline

In the Chapter 2, screening and selecting of the best antifungal activity of four different plant crude extracts or an essential oil against pathogenic fungi are described. These extracts will be used for seed coating technology in comparison to conventional chemical fungicide seed treatment to evaluate their effectiveness for seed borne fungi control before and during germination. These experiments are described in Chapter 3. Then, in Chapter 4, the effects of various seed coating substances on rice seed properties before and during storage are reported. The effect of various seed coating substances on rice seed properties before and during storage and the relationship between chemical and biochemical properties changes in rice seeds are discussed in Chapter 5. The results of field experiments to compare the effectiveness of the botanical fungicide and conventional chemical seed treatment on seed quality in practice are presented in Chapter 6. Chapter 7 characterizes the influence of various seed coating substances on the quality of harvested rice grain. Chapter 8 contains the conclusion and summary.

Predicted results: The tested plant extracts act as botanical fungicides and are an alternative source to replace chemicals for seed coating application in order to control

fungal diseases without physiological injuries in the seeds and to ensure high yields as well as a stable quality of the harvested grains. Moreover, fewer amounts of chemical residues contaminated in both soil and harvested product are expected.

1.4 References

Agrios, G.N. (1988). Plant Pathology, 3rd. ed. Academic Press, Inc.: New York. 803.

Baird, R.E. (1994). Evaluation of Seed Treatments on Shrunken-2 Sweet Corn. Plant Disease. 78(8), 817-821.

Bhatti, I.M., and Soomro, A.H. (1996). Agricultural inputs and field crop production in Sindh. Agricultural Research Sindh, Hyderabad.

Chanthayot, D., and Jatisatienr, A. (2003). Antibacterial Activity of *Eugenia* spp. Crude Extracts. International Congress and 51st Annual Meeting of the Society for Medicinal Plant Research. August 31- September 4, 2003 Kiel, Germany.

Cheema, S.S., Dhaliwal B.K., and Sahota, T.S. (1991). Theory and digest of agronomy. Kalyani Pub., New Delhi.

Embaby, E.M. (2006). Using a Biofungicide (*Coniothyrum minitans* Campbell.) In Controlling Some Soilborne Plant Pathogenic Fungi in Egypt. Research Journal of Agriculture and Biological Sciences, 2(6), 423-432.

Farr, D.F., Bills, G.F., Chamuris, G.P., and Rossman, A.Y. (1989). Fungi on plants and plant products in the United States. APS PRESS: St. Paul. 1252.

Horrigan, L., Lawrence, R.S., and Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environmental health perspective. 110, 445-456.

Islam1, M.S., Jahan, Q.S.A., Bunnarith, K., Viangkum, S., and Mercas, S.D. (2000).Evaluation of seed health of some rice varieties under different Conditions. Botanical Bulletin of Academia Sinica. 41, 293-297.

Issakul, K., Seangsawat, S., Chanthrayod, D., and Jatisatienr, A. (2003). The Insecticidal Properties of Some Plant Extracts. International Congress and 51st Annual Meeting of the Society for Medicinal Plant Research. August 31-September 4, 2003 Kiel, Germany.

Jiskani, M.M. (1999). A brief outline "THE FUNGI" (Cultivation of mushrooms). Izhar Pub. Tandojam. 94.

Jitsaguan, T. (2005). Sustainable agricultural system for small-scale farmers in Asia: Implication for the environment. Extension Bulletin of the Food&Fertilizers Technology Center. Retrieved 25 July 2007 from: http://fftc.agnet.org.

Kamal, M, and Moghal, S.M. (1968). Studies on plant diseases of South West Pakistan. Agricultural Research Institute, Tandojam.

Mungkornasawakul, P., Pyne, S.G., Jatisatienr, A., Supyen, D., Lie, W., Ung, A.T., Skelton, B.W. and White, A.H. (2003). Stemocurtisine, the First Pyridol [1,2-a] azaepine Stemona Alkaloid. J. Nat. Prod. 66, 980-982.

Mungkornasawakul, P., Pyne, S.G., Jatisatienr, A., Supyen, D., Jatisatienr C., Lie, W., Ung, A.T., Skelton, B.W., and White, A.H. (2004). Phytochemical and Larvacidal Studies on Stemona curtsii Hook. F.:Pyridol [1,2-a] azaepine Stemona Alkaloid. J. Nat. Prod. In Press.

Reissig, W.H., Heinrichs E.A., Litsinger J.A., Moody K, Fiedler L, Mew T.W., Barrion, A.T. (1986). Illustrated guide to integrated pest management in rice in tropical Asia. Manila (Philippines): International Rice Research Institute. 411.

Silva, F.M.A., Melo, P.C., and Oak, R.L. (1996). Effect of insecticide in the germination and vigor of maize seeds (*Zea mays* L.) at two times of storage. In National Congress of Maize and Sorgo. 21. Summaries. Native of London: IAPAR. 276.

Smith, I.M., Dunez, J., Phillips, D.H., Lelliott, R.A.,and Archer, S.A. (1988). European handbook of plant diseases. Blackwell Scientific Publications: Oxford. 583.

The International Rice Research Institute (IRRI). (2007). www.irri.org/BringingHope/ImprovingLives.pdf. visit at 12/08/2007.

Tworkoski, T. (2002). Herbicide effects of essential oils. Weed Science. Volume 50 (4), 425–431.

Von Pine, E.V.R., Silveira, J.F., and Vieira, M.G.G.C. (1995). Influence of the size and the treatment of maize seeds in the preservation of the quality during the storage and posterior behavior in the field. Cultivate and Practical science. 19(1), 30-36.

Webster, R.K., and Gunnell, P.S. (1992). Compendium of rice diseases. The American Phytopathological Society, St. Paul, MN.

Whipp, J.M. and Budge, S.P. (1993). Biological control of *S. sclerotiorum* in glasshouse crops. British Crop Protection Council (1992): 1 C.F. R. Pl. Pathol. Vol. 72(4), 27-132.

Wonggiratthiti, A., and Jatisatienr, C. (2003): Inhibitory effect of the combinations of eugenol plus *Mammea siamensis* Kost. and eugenol plus *Stemona curtisii* Hk. F. on some phytopathogenic molds. The 3 rd World Congress on Medicinal and Aromatic Plants for Human Welfare, February 3-7, 2003 Chiang Mai, Thailand.

Yildirim, E., and Hoy, C.W. (2003). Cyromazine seed treatments to control onion maggot, *Delia antiqua* on green onions J. Econ. Entomol. 96, 1494-1499.

2. *In vitro* screening of the antifungal activity of plant extracts as fungicides against seed borne fungi

2.1 Abstract

Plants containing active compounds are able to inhibit the microbial growth. These plant compounds have different structures and actions when compared with conventional fungicides. The antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism. Several chemical compounds of relatively composite structure with antimicrobial activity have been studied. The antifungal potential of crude extracts from *Acorus calamus* L., *Stemona curtisii* HK. f., *Stemona tuberose* L, *Memmea siamensis* Kost., *Eugenia caryophyllus*, and an eugenol essential oil were studied *in vitro*. Four pathogenic seed borne fungi, *Alternaria solani*, *Colletotrichum* sp., *Fusarium moniliforme*, and *Rhizoctonia solani* were selected as target organisms. The agar overlay technique and spore inhibition technique were applied for the determination of the antifungal activity. The antifungal activity was lined up into a series from strong to low, as follows: eugenol > *Eugenia caryophyllus* > *Acorus calamus Linn.* > *Stemona tuberosa L.*>*Mammea siamensis Kost.*= *Stemona curtisii Hk.f.* Eugenol showed strongly antifungal activity against pathogenic fungi. Moreover, after eugenol application, lysis of spore and inhibition of mycelium growth were detected. Microscopic analysis exhibited complete lysis of spores after 24 hrs at a concentration of 1.00 %v/v. Moreover, at the same concentration and 96 hrs incubation the mycelia growth was completely inhibited.

2.2 Introduction

Rice production is known to be attacked by many pathogenic fungi, are *Alternaria sp.*, *Colletotrichum sp.*, *Fusarium sp*., and *Rhizoctonia sp*. These microorganisms cause a serious deterioration when they occur in/on rice grain. Moreover, they can colonize on diverse substrates, because of their powerful arsenal of hydrolytic enzymes, and they can be responsible for considerable economic losses (Zhu, 1998). Furthermore, some of the above-mentioned fungi can act as potential producer of mycotoxins, which potentially damage consumers' health (Evandro *et al*., 2005).

Chemical treatment is one practice of plant protection. Traditionally, chemical treatments are used widely to protect the germinating seedling, during vegetative and reproductive growth, and after harvest from pathogenic fungi infection (Aleieri *et al*., 1984). Currently, the use and expectations of chemical treatments are greatly concerned due to the impact of

the chemical to the environment, which can be of primary or secondary influence on human or other living organisms (Badei *et al*., 1996). It also has negative effects on the food chain and increase fungicide resistance problems (Ester *et al*., 2003). To avoid these disadvantages, new strategies for fungicide use and disease management must be developed and identified. The alternatives of synthetic fungicides could be the development of effective phytochemicals from plant origin, which are expected to be more advantageous than synthetic fungicides (Davidson and Parish, 1989). The increased importance of the development and application of biological fungicides is recognized under the concept of Integrated Pest Management (IPM) (Del Campo *et al*., 2002). Under this concept, all possible modes of plant disease control methods should be integrated to minimize the excessive use of synthetic fungicides (Bishop and Thornton, 1997).

It is well known that some plants contain active compounds which are able to inhibit the microbial growth (Naqui *et al*., 1994). These plant compounds have different structures and antimicrobial activities when compared with conventional fungicides (Nascimento *et al*., 2000). The potential antimicrobial properties of a plant is related to their ability to synthesize several chemical compounds of relatively complex structure with antimicrobial activity, including alkaloids, flavanoids, isoflavanoids, tannins, cumanins, glycosides, terpenes, and organic acids (Nychas, 1996). For examples, a solvent extract from clove flower buds that contains eugenol as main active compound was antifungal active against *Alternaria* sp., *Fusarium* sp., *Botrytis* sp., and *Rhizoctonia* sp. (Cowan, 1999; Soatthiamroong *et al*., 2003). Sage oil was active against *Botrytis* sp. (Carta *et al*., 1996) and thyme inhibited post harvest diseases of tomato (Plotto *et al*., 2003).

Thus, the aim of this study was the screening for the best *in vitro* antifungal activity of *Acorus calamus*, *Stemona curtisii* HK.f., *Stemona tuberosa* L., *M*emmea *siamensis* Kost., and *Eugenia caryophyllus* crude extracts and eugenol oil against pathogenic fungi as a possible alternative for synthetic chemical antifungal compounds.

2.3 Materials and methods

The experiment was conducted at Seed Science and Technology Laboratory, Section of Seed Science and Technology, Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand.

2.3.1 Antifungal agents

Plant extracts and eugenol as an essential oil were used as natural antifungal agents. Plant extracts from *Acorus calamus* Linn., *Stemona curtisii* Hk.f., *Stemona tuberosa* L., *Memmea siamensis* Kost., and *Eugenia caryophyllus* were obtained from Prof. Dr. Araya Jatisatienr, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. Eugenol was purchased from Fluka (Steinheim, Germany).

2.3.2 Evaluation of the antifungal activity

Alternaria solani, *Aspergillus flavus, Aspergillus niger, Bipolaris oryzae, Colletotrichum sp*., *Fusarium moniliforme*, *Nigrospora sp*., and *Rhizoctonia solani* were used as target fungi which provided from the collection of the Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand.

Potato Dextrose Agar (PDA) was used as the main medium in the experiments. PDA medium containing 2%(w/v) dextrose, 1%(w/v) sucrose, 0.1%(w/v) agar and 20 g potato were boiled in 100 ml distilled water by using an apparatus for re-cooling for 1 h, and were filtered, cooled and sterilized by autoclaving at 121^{o}C for 30 min, while PDA aqueous medium were prepared without agar.

2.3.2.1 Mycelium growth inhibition analysis

The experiments were conducted by agar overlay technique (Morris *et al*., 1979). The plant extract and eugenol at different concentrations (0.10, 0.25, 0.50, and 1.00 %v/v) on PDA medium was *in vitro* tested against the fungi mycelium growth. Medium (20 ml) was dispensed into Petri dish and 5 mm diameter of the test fungi cut from the middle of 7-days-old cultures were incubated upside down separately to each assay plate and incubated for 96 hrs at 25±2°C. The colony diameter was measured and the mycelium inhibition percentage was calculated by following Deans and Svoboda (1990). Four replicates of each treatment were tested and the average was calculated. Control sets were simultaneously run without using the plant extract and eugenol.

$$\textbf{Inhibition (\%)} = [(C - T) / C] \times 100$$

C is the colony diameter of the mycelium on the control plate (mm), and T is the colony diameter of the mycelium on the treatment plate (mm).

Concentration response curves were obtained whereby the percentage of fungal inhibition was plotted against concentration. The concentration required to give 50% inhibition of fungal growth (IC_{50}) was calculated from the regression equation.

2.3.2.2 Spores inhibition analysis

Spores of *Alternaria solani*, *Fusarium moniliforme*, and *Rhizoctonia solani* were produced by using a modification method of Mitchell and Yang (1966). The fungus was grown for 3 days at 25±2°C in PDA aqueous medium. To induce spores formation, fungal mycelium was rinsed 3 times with distilled water, for approximately 30 s each time, and after the final rinsed, cultures were kept at 25±2°C for 2 days. Spore concentration was adjusted to approximately to 10^6cfu ml^{-1} by the hemacytometer. Sterile microscope slides were dropped 100 µL of PDA aqueous medium to obtain a thin agar layer on the slide, then 100 µL of spores suspension sample was gently spread on each slide. An uncovered watch glass containing either 100 µL of sterile water as control, or 100 µL of plant extract and an essential oil at different concentration (0.10, 0.25, 0.50, and 1.00 %v/v) were dropped into slide. After that, slides were place in glass Petri dishes lined with moistened filter paper, covered and sealed with parafilm, incubated for 24 hrs at 25±2°C, all of the encysted spores on each slide were counted with compound microscope at magnification of x100, and spores inhibition was calculated.

2.4 Results

Figure 2.1 demonstrated that most of the studied fungi were at 1% v/v of *A. calamus* Linn., *A. solani*, *Colletotrichum* sp., and *R. solani* were completely inhibited (100%). Nevertheless, *F. moniliforme* was inhibited about 95.91%. *R. solani* was slightly sensitive to *S. curtisii* HK.f. extract, and was inhibited to 49.54% at 1.00 %v/v concentration. The growth of *A. solani*, *Colletotrichum* sp., and *F. moniliforme* were uninhibited by *S. curtisii* HK.f. extract (Figure 2.2). Figure 2.3 summarized the antifungal activity of *S. tuberosa* L. which had no inhibition effect on *A. solani*, *Colletotrichum* sp., and *F. moniliforme*. However, *S. tuberosa* L. extract inhibited *R. solani* for 70% at 1.00%v/v concentration. As shown in Figure 2.4, the *M. siamensis* Kost. crude extract was unable to control all fungi species. Figure 2.5 showed that *E. caryophyllus* extract at 0.25 %v/v completely inhibited *A. solani*, *Colletotrichum* sp., and *F. moniliforme*. Moreover, when the concentration increased to 0.50%v/v, this extract inhibited *Rhizoctonia solani* completely.

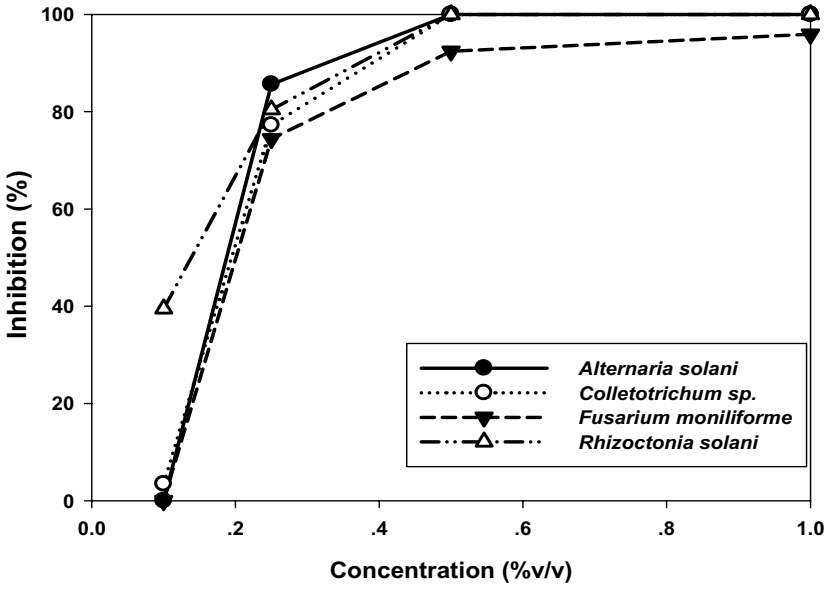


Figure 2.1: Inhibition of pathogenic fungi by extract of *Acorus calamus* Linn.

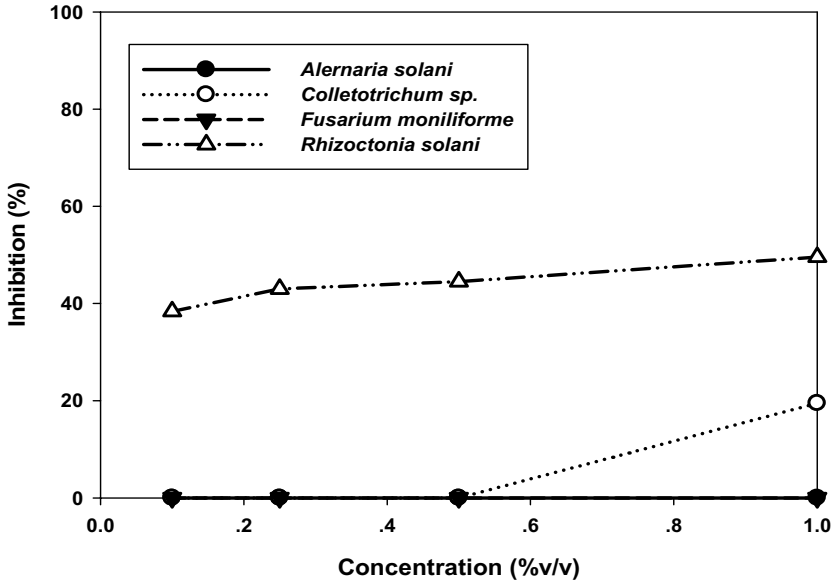


Figure 2.2: Inhibition of pathogenic fungi by extract of *Stemona curtisii* Hk.f.

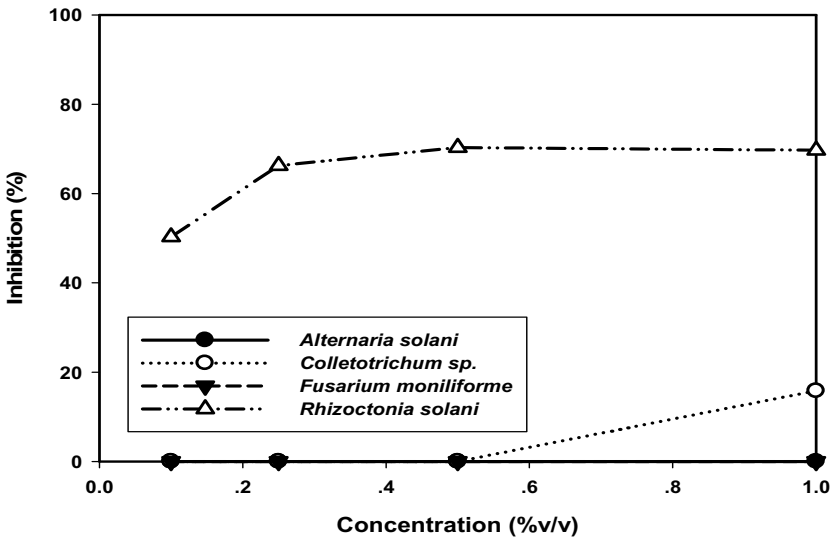


Figure 2.3: Inhibition of pathogenic fungi by extract of *Stemona tuberosa* L.

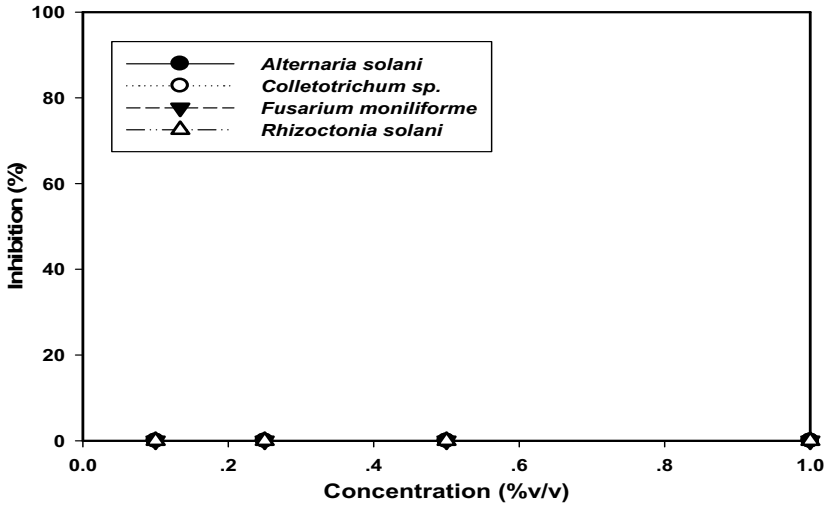


Figure 2.4: Inhibition of pathogenic fungi by extract of *Memmea siamensis* Kost.

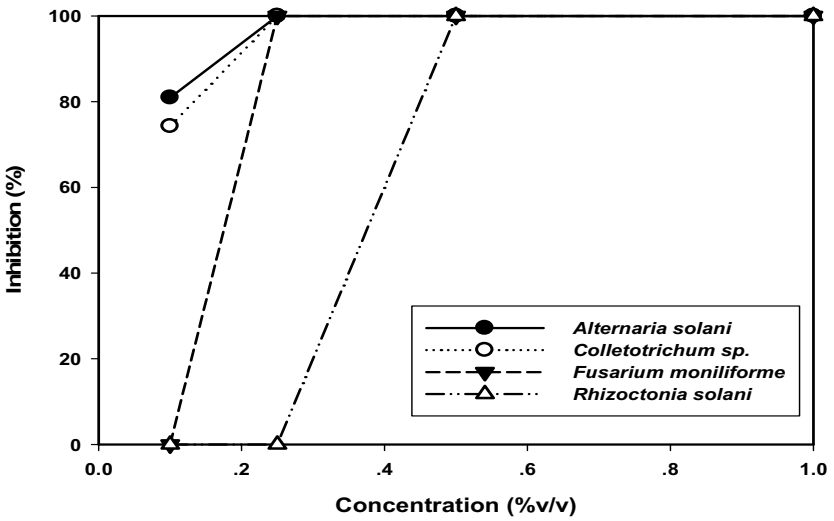


Figure 2.5: Inhibition of pathogenic fungi by extract of *Eugenia caryophyllus*

The IC_{50} values indicated that *E. caryophyllus* crude extract as well as *A. calamus* Lin showed strongly antifungal activity against pathogenic fungi. However, *S. curtisii* Hk.f, *S. tuberosa* L., and *M. siamensis* Kost. were unable to inhibit all fungi species. *R. solani* seemed to be less susceptible to the plant extracts than the other fungi (Table 2.1).

Table 2.1: The IC_{50} of plant extracts against pathogenic fungi

Botanical fungicides	IC_{50} (%v/v)			
	*Alter.**	*Collet.***	*Fus.****	*Rhi.*****
Eugenia caryophyllus	**0.0087**	**0.0219**	**0.1968**	**0.1894**
***Acorus calamus* Linn.**	**0.2076**	**0.2097**	**0.2220**	**0.1445**
***Stemona tuberosa* L.**	**-**	**-**	**-**	**0.0846**
***Stemona curtisii* Hk.f.**	**-**	**-**	**-**	**-**
***Mammea siamensis* Kost.**	**-**	**-**	**-**	**-**

* *Alternaria solani,* ** *Colletotrichum sp.,* *** *Fusarium moniliforme,* **** *Rhizoctonia solani*
IC_{50}: The concentration required to give 50% inhibition of fungal growth, - : Not inhibited

Table 2.2 indicated that, eugenol showed the strongest antifungal activity at 1.00 %v/v and had the strongest antifungal activity (showed 100% inhibition zone) against the each fungal species as well as *E. caryophyllus* did (Table 2.3).

Table 2.2: Effect of the concentration of eugenol against pathogenic fungi

Fungi	Inhibition (%)	
	0.25%(v/v) Eugenol	1%(v/v) Eugenol
Alternaria solani	**100.00**	**100.00**
Aspergillus flavus	**44.22**	**100.00**
Aspergillus niger	**70.58**	**100.00**
Bipolaris oryzae	**86.98**	**100.00**
Curvilaria sp.	**50.04**	**97.04**
Fusarium moniliforme	**100.00**	**100.00**
Nigrospora sp.	**50.04**	**98.23**
Rhizoctonia solani	**100.00**	**100.00**

Table 2.3: The antifungal activity of *Eugenia caryophyllus* crude extract and eugenol (purified) against pathogenic fungi

Treatment (at 1%v/v)	Pathogenic fungi inhibition (%)			
	*Alter.**	*Collet.***	*Fus.****	*Rhi.*****
***Eugenia caryophyllus* crude extract**	**85.66**	**77.24**	**74.42**	**80.44**
Eugenol	**100.00**	**100.00**	**100.00**	**100.00**

** Alternaria solani, ** Colletotrichum sp., *** Fusarium moniliforme, **** Rhizoctonia solani.*

Eugenol was similar effective in inhibiting the mycelium growth of each fungus as captan, especially on *A. solani*, *A. flavus*, *A. niger*, *B. oryzae*, and *F. moniliforme* (Table 2.4., Figure 2.6). Nevertheless, captan was effective on inhibition of spore germination completely at 0.10 %v/v but eugenol significantly inhibited fungal spore germination only at 0.50 or 1.00 %v/v (Table 2.5, Figure 2.7).

Table 2.4: The effect of captan and eugenol treatment on mycelia inhibition

Pathogenic fungi	Treatment	Mycelia growth (cm)	Inhibition (%)
A. solani	**Captan**	**7.25**	**4.6**
	1% Eugenol	**7.4**	**2.63**
A. flavus	**Captan**	**7.9**	**8.13**
	1% Eugenol	**7.05**	**18.02**
A. niger	**Captan**	**8.25**	**4.06**
	1% Eugenol	**7.4**	**13.95**
Bipolaris oryzae	**Captan**	**8.9**	**1.11**
	1% Eugenol	**7.95**	**21.1**
Curvilaria sp.	**Captan**	**6.5**	**8.45**
	1% Eugenol	**6.45**	**9.15**
F. moniliforme	**Captan**	**7.5**	**12.28**
	1% Eugenol	**6.55**	**23.39**
Nigrospora sp.	**Captan**	**6.95**	**2.82**
	1% Eugenol	**6.8**	**6.97**
Rhizoctonia solani	**Captan**	**2.6**	**13.78**
	1% Eugenol	**2.5**	**17.06**

Table 2.5: Effect of captan, *Eugenia caryophyllus*, and eugenol treatment on inhibition of spore germination

Treatment	Conc. (%v/v)	Spore inhibition (%)					
		A. flavus	*A. niger*	*A. solani*	*F. moniliforme*	*Curvularia sp.*	*R. solani*
Captan	**0.10**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
	0.25	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
	0.50	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
	1.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Eugenol	**0.10**	**54.00**	**37.59**	**14.41**	**21.88**	**44.94**	**93.53**
	0.25	**65.60**	**65.60**	**24.16**	**74.11**	**85.39**	**100.00**
	0.50	**100.00**	**100.00**	**65.68**	**100.00**	**100.00**	**100.00**
	1.00	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

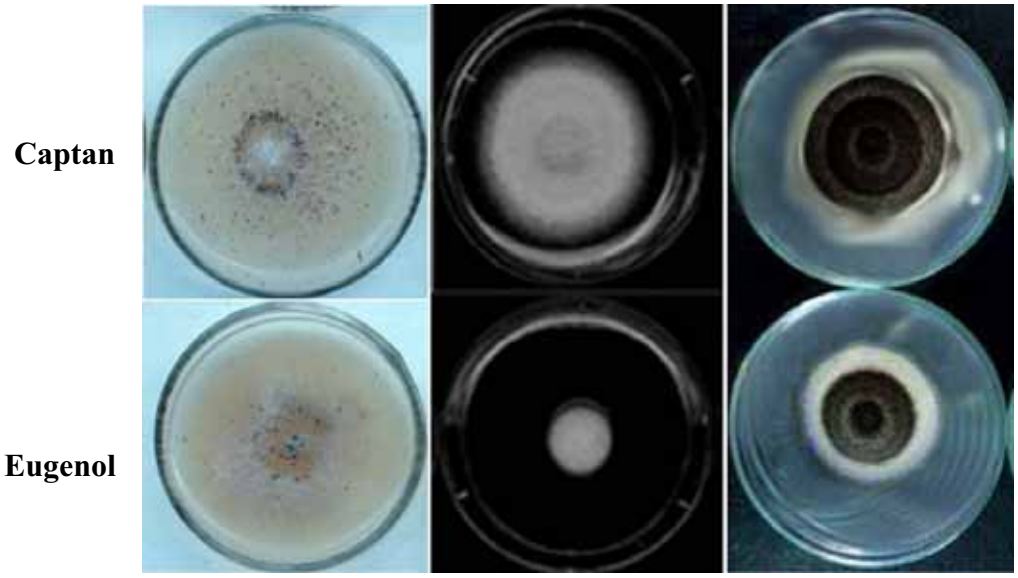


Figure 2.6: Effect of captan and eugenol treatment at 1.00 %v/v on mycelium growth inhibition. Both, eugenol and captan treatment inhibited fungal mycelium growth

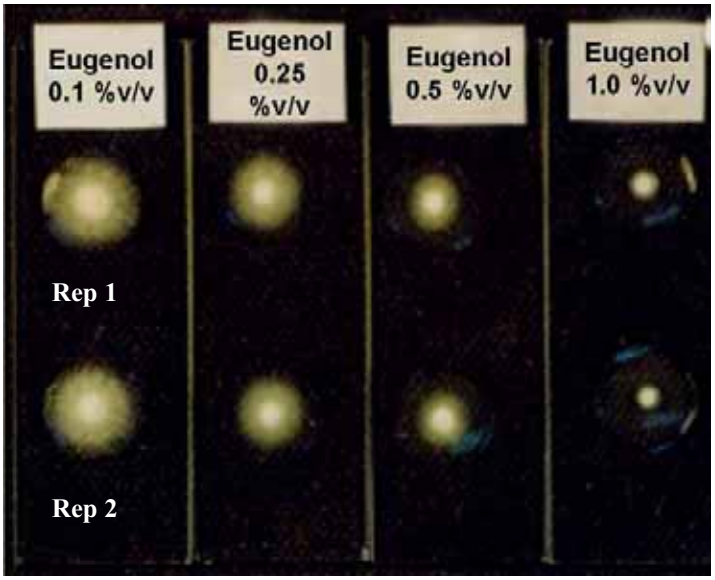


Figure 2.7: Effect of various concentrations of eugenol on inhibition of *Fusarium moniliforme* spores germination. Two replication studies showed that eugenol at concentration higher than 0.5%v/v completely inhibited spore germination

2.5 Discussion

The antifungal activity of plant crude extracts, or essential oil against pathogenic fungi can be lined up in order from strongest to lowest effectiveness as follows: eugenol>*Eugenia caryophyllus*>*Acorus calamus Linn.*>*Stemona tuberosa L.*> *Mammea siamensis Kost.*= *Stemona curtisii Hk.f.*

At low concentration, *S. tuberose* L., and *S. curtisii* Hk.f. crude extracts were unable to control all species of fungi. Although, at the highest assayed concentration (1.0%v/v), *S. tuberose* L. extract inhibited only *Rhizoctonia sp.*. Previous studies reported that *S. curtisii* Hk.f., and *S. tuberose* L. crude extracts had much stronger insecticidal than fungicidal activity (Kaltenegger *et al.*, 2003; Chantawannakul *et al*., 2005). Furthermore, Jiyavorranant *et al.* (2001) found that *A. calamus* Linn. extract showed strong antifungal activity but the *M. siamensis* Kost. crude extract has no antifungal activity. However, *M. siamensis* Kost. was previous found to have a strong insecticidal activity (Issakul *et al*., 2004; Plotto *et al*., 2003).

The present study found that *A. calamus* Linn. (Sweet flag) has an effective antifungal activity. Beta-asarone, and α-asarone are the major constituents in the rhizomes and leaf oils of *A. calamus* Linn (Raina *et al.*, 2003; Cowan, 1999). These compounds are antifungal active against *Curvularia* sp., and *Alternaria* sp (Janssen *et al*., 1988). Ghosh (2006) reported that the *A. calamus* Linn. crude extract inhibited hyphal growth of *F.*

moniliforme, whereas Lee (2006) found that methanolic extract of *A. calamus* L. had strong fungicidal activity against *R. solani*. The present experiments found that the *A. calamus* Linn. extract showed a strong antifungal activity on pathogenic fungi when the concentration was higher than 0.50 %v/v. These results are in agreement with Knobloch *et al*. (1986). Nevertheless, the results suggested that *E. caryophyllus* crude extract had the strongest antifungal activity at its lowest concentration.

The concentration and type of active compound in plant products are the most important factors in their antifungal activity. Obviously, the results indicated that at high concentration (1.00% v/v) of *E. caryophyllus* crude extract could inhibit the pathogenic fungi. However, in term of spore germination and mycelial growth inhibition, eugenol was found to have better inhibition ability than *E. caryophyllus* crude extract, which was in agreement with Viollon and Chaunomt (1994). Eugenol is the major phenolic compound of *E. caryophyllus* and have a strong antibacterial and antifungal activity, e.g. against *Aspergillus* sp. and *Fusarium* sp. (Pauli and Knobloch,1987), as well as against *Alternaria* sp., *Fusarium* sp., *Curvularia* sp., and *Rhizoctonia* sp. (Zafor Beg and Ahmed, 2002).

The present study established the high effectiveness of eugenol at the minimum fungistatic concentration (MFC) of 0.50 %v/v. At concentrations higher than 0.50 %v/v, lysis of spore and inhibition of mycel growth were detected and confirm the results of Neni *et al*. (2006). However, the MFC and toxicity concentration varied from study to study. This is probably due to the different extraction methods of essential oils, and different sensitivity of the test strains used (Saikia *et al*., 2001). The plant extracts showed clear antimicrobial properties, although the mechanistic of action are poorly understood. However, it must be pointed out that the intrinsic activity of a compound is very important for its effectiveness. In this context, the essential oil containing phenolic compound was reported to exhibit a high inhibitory effect (Bennis *et al.*, 2004).

The mode of action of antifungal agents depends also on the type of target microorganisms and is mainly related to their cell wall structure and the outer membrane arrangement (Dorman and Deans, 2000). These observations suggested that the physical and chemical properties (solubility and volatility) might have considerably effects on the *in vitro* antimicrobial activity (Inouye *et al*., 2000). In this study, eugenol was found to have a strong activity due to its relatively low capacity to dissolve in water, which is in agreement

with Hili *et al*. (1997). The effectiveness of eugenol depends on the structure of the phenolics which where in previous studies identified as active compounds, e.g. the hydroxyl group and its relative position within the molecule (Tullio *et al*., 2006). High hydrophobic compounds are generally reported to be very effective on the primary site at the cytoplasmic membrane (Sikkema *el al*., 1995). The effect of eugenol when separated from the fungi membranes suggests that its activity is based on the lipophilic properties. The interactions between antimicrobial compounds and cell membranes affect both the lipid ordering and the bilayer stability (Ben Arfa *et al*., 2006). Their mode of action appeared to be at the phospholipid bilayer, caused by biochemical mechanisms, catalyzed by the phospholipid bilayers of the cell, and related to the cell membrane disruption. These processes include the inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions (Knobloch *et al*., 1988). Eugenol is able to inhibit the respiration and ion transport processes, increase membrane permeability and the release of cellular content (Andrews *et al*., 1980; Uribe *et al*., 1985). Moreover, it is able to inhibit the respiration of cell suspensions and to disrupt the permeability barrier of microbial membrane structure (Cox *et al*., 2000). Morris *et al*., (1979) reported that the fungi after treated with eugenol, decreased its size, appeared in irregular shape with cell wall modifications and the cell surface depressions. Such modifications may be related to the interference of the oil components with enzymatic reactions of cell wall synthesis, which affects fungal morphogenesis and inhibited growth.

According to Helal *et al*. (2006), the antifungal activity of eugenol may take place via two steps. The first step involves the passive entry of the oil into the plasma membrane in order to initiate cell membrane disruption. The second step, the accumulation of oil in the plasma membrane results in the inhibition of cell growth. This can be ascribed as combination of membrane effects such as increased bi-layer disorder and ion leakage. These effects disturb the osmotic balance of the cell through the loss of ions, making its membrane associated proteins inefficient due to increased membrane disorder eventually leading to inhibition of cell growth. The cytoplasmic, plasma and mitochondrial membrane of fungal provide a barrier to the passage of small ions such as H^+, K^+, Na^+ and Ca^{2+} and allow cells and organelles to control the entry and exit of different compounds (Suhr and Nieken, 2003). This permeability barrier of cell membranes is integral to many cellular functions, including the maintenance of the energy status of the cell, other membrane-coupled energy-transducing process, solute transportation, regulation of metabolism and control of

turgor pressure (Trumpower and Gennis, 1994). Cox *et al*. (2000) observed the leakage of the K^+, Ca^{2+} and Mg^{2+} from exposed fungal cells with eugenol. Some changes in the cell membrane may occur in spite of the damage to the plasma membrane. These changes are accompanied with the loss of chemiosmotic control disrupted the permeability barrier of cell membrane structure. Ultee *et al*. (2002) hypothesized that the hydroxyl group and the presence of a system of delocalized electrons are important for the antimicrobial activity of the eugenol. Such a particular structure would allow compounds to act as proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. Eventually, the reduction of the proton motive force and the depletion of the ATP pool lead to the cell death. Hence, these findings supported our results that eugenol was identified as the best antifungal effect of all studied plant extracts.

2.6 Conclusion

The crude plant extracts from *Stemona curtisii* Hk.f., *Stemona tuberose* L., and *Mammea siamensis* Kost. did not have an antifungal activity. However, high concentrations of *Acorus calamus* Linn., and the *Eugenia caryophyllus* crude extracts can inhibit the studied pathogenic fungi. Eugenol, an active compound of *Eugenia caryophyllus,* showed the strongest antifungal activity in inhibiting the growth of pathogenic fungi. The mode of action based on the deterioration of the fungal cellular structure leading to complete cell death, even at lower concentration of eugenol. Therefore, based on the antifungal activity against pathogenic fungi, the following order from strongest to lowest effectiveness was found: eugenol > *Eugenia caryophyllus* > *Acorus calamus Linn.* > *Stemona tuberosa L.* > *Mammea siamensis Kost.* = *Stemona curtisii Hk.f.* This finding increases the possibility of exploiting eugenol as a promising candidate for safe natural antifungal agent.

2.7 References

Aleieri, A.A., Jr., Landdon, K.R., Wehlburg, C., and Kimbrough, J.W. (1984). Index of plant disease in Florida. Fla. Dep. Agric. Consumer Serv. Div. Plant Ind. Bull. 11.

Andrews, R.E., Parks, L.W., and Spence, K.D. (1980). Some effects of Douglas fir terpenes on certain microorganisms. Appl. Eviron. Microbiol., 40. 301-304.

Badei, A.Z.M., El-Akel, A.T.M., Morsi, H.H., Baruah, P., Sharma, R.K., Singh, R.S., and Ghosh, A. (1996). Fungicidal activity of some naturally occurring essential oils against *Fusarium moniliforme*. Journal of Essential Oil Research. 8, 411-412.

Ben Arfa, A., Combes, S., Preziosi-Belloy L., Gontard, N., and Chalier, P. (2006). Antimicrobial activity of carvacrol related to its chemical structure. Letters in Applied Microbiology. 43, 149-154.

Bennis, S., Chami, F., Chami, W., Bouchikhi, T., and Remmal, A. (2004). Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eubenol. Appl. Microbiol. 38, 454-458.

Bishop, C.D., and Thronton, I.B. (1997). Evaluation of the antifungal activity of the essential oils of *Monarda citriodora* var. *citriodora* and *Melaleuca alternifolia* on post harvest pathogens. Journal of Essential Oils Research. 9, 77-82.

Carta, C.M., Moretti, D.L., and Alessandra, T. (1996). Activity of the oil of *Salivia officianialis* against *Botrytis cinerea*. Journal of Essential Oil Research. 8, 399-404.

Chantawannakul, P., Puchanichanthranon, T., and Wongsiri, S. (2005). Inhibitory effect of some medicinal plant extracts on the growth of *Ascosphaera apis*. ActaHort. No. 678, 183-189.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Review. 12, 564-582.

Cox, S.D., Mann, C.M., Harkham, J.L., Bell, H.C., Gustafson, J.E., Warmingtio, J.R., and Wyllie, S.G. (2000). The mode of antimicrobial action of essential oil of *Melaleuca alternifolia* (tea tree oil). J. Appl. Microbiol., 88, 170-175.

Davidson, P.M., and Parish, M.E. (1989). Methods for testing the efficacy of food antimicrobials. Food Technology. 43, 148-155.

Deans, S.G., and Svoboda, K.P. (1990). The antifungal properties of margoram (*Origanum majorana* L.) volatile oil. Flavour and Fragrance Journal. 5, 187-190.

Del Campo, J., Amiot, M.J., and Nguyen T.C. (2002). Antimicrobial effect of plant extract. Journal of Food Protection. 63, 1359-1368.

Dorman, H.J.D., and Deans, S.G. (2000). Antimicrobial agents from plants: Antimicrobial activity of plant volatile oils. J. Appl. Microbiol. 88, 308-316.

Ester, A., De Putter, H., and van Bilsen, J.G.P.M. (2003). Film coating the seed of cabbage (*Brassica oleracea* L. convar. Capitata L.) and Cualifower (*Brassica oleracea* L. var. Botryti L.) with inidacloprid and spinosad to control insect pests. Crop production 22, 761-768.

Evandro, L.S., Edeltrudes, O.L., Kristerson, R.L., Freire, and Cristina, P.S. (2005). Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Brazilian Achieves of Biology and Technology. 48(2), 245-250.

Ghosh, M. (2006). Antifungal properties of Haem peroxidase from *Acorus calamus*. Annals of Botany. 98, 1145-1153.

Helal, G.A., Sarhan, M.M., Abu Shahla A.N.K., and Abou El-Khair, E.K. (2006). Effect of *Cymbopogon citratus* L. essential oil on growth and morphogenesis of *Saccharomyces cerevisiae* ML2-strain. J. Basic Microbiol, 46(5), 375-386.

Hili, P., Evans, C.S., and Veness, R.G. (1997). Antimicrobial action of essential oils : The effect of dimethylsulphoxide on the activity of cinnamon oil. Applied Microbiology. 24, 269-275.

Inouye, S., Tsuroka, T., Watanabe, M., Takeo, K., Akao, M., Nishiyama, Y., and Yamaguchi, H. (2000). Inhibitory effect of essential oils on apical growth of *Aspergillus fumigatus* by vapour contact. Mycoses. 43, 17-23.

Issakul, K., Kongtrakoon, W., Dheeranupatan, S., Jangsutthivoranat, S., and Jatisatienr, A. (2004). Insecticidal effect of compounds from *Mammea siamensis* Kost. against *Musca domestica* Linn. ActaHort. 629, 103-107.

Janssen, A.M., Scheffer, J.J.C., Parhan-Van Atte, A.W., and Svendsen, A.B. (1988). Screening of some essential oil for their activities on dermatophytes. Pharm. Weekbl [Sci]. 10, 277-280.

Jiyavorranant, T., Chanbang, Y., Supyen, D., Sonthichai, S., and Jatisatienr, A. (2001). The effect of *Acorus calamus* Linn. and *Stemona tuberose* Lour. Extracts on the insect pest, *Plutella xylostella* (Linnaeus). ActaHort. 597:International Conference on Medicinal and Aromatic Plants.

Kaltenegger, E., Bram, B., Merciter, K., Kalchhauser, H., Kahlig, H., Hofer, O., Vajrodaya, S., and Geger, H. (2003). Insecticidal pyrido [1,2-a] azepine alkaloids and related derivatives from Stemona species. Phytochemistry. 63, 803-816.

Knobloch, K., Weigand, N., Weis, H.M., and Vigenchow, H. (1986). *Progress in Essential Oil Research ed.* Brunke E. J. pp 429. Berlin : Walter de Gruyther.

Knobloch, K., Pauli, A., Iberl, N., Weis, H.M., and Weigand, N. (1988). Mode of action of essential oil components on whole cell of bacteria and fungi in plate test. *In Bioflavour* ed. Schreier : 287-299. Berlin. Walter de Gruyther.

Lee, H.S. (2006). Fungicidal property of active component derived from Acorus plants against phytopathogenic fungi. Agric. Chem. Biotechnol. 14(5), 685-688.

Mitchell, J.E., and Yang, C.Y. (1966). Factors affecting growth and development of *Aphanomyces euteiches*. Phytophatology. 56, 917-922.

Morris, J.A., Khettry, A., and Seitz, E.W. (1979). Antimicrobial activity of aroma chemicals and essential oils. J. Am Oil Chem Soc. 56, 595-603.

Naqui, S.H.A., Khan, M.S.Y., and Vohora, S.B. (1994). Antibacterial, antifungal and anthelmintic investigation on Indian medical plants. Fitoterapia. 62, 221-228.

Nascimento, G.G., Locatelli, J., and Freitas, P.C. (2000). Antimicrobial activity of plants extracts and phytochemical on antibiotic resistant bacteria. Brazilian Journal of Microbiology. 31, 247-256.

Neni, F., Mari, M., and Brigati, S. (2006). Control of *Penicillium expansum* by plant volatile compounds. Plant Pathology. 55, 100-105.

Nychas, G.J.E. (1996). Natural antimicrobial from plants. In : Gould, G. W. New methods of food preservation. Londres : CRC Press. 235-258.

Pauli, A., and Knobloch, K. (1987). Inhibitory effect of essential oils components on growth of food-contaminating fungi. Z Lebensm Unters Forsch. 185, 10-13.

Plotto, A., Roberts, D.D., and Roberts, R.G. (2003). Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycoperscion esculentum*). ActaHort. 628, 737-745.

Raina, V.K., Srivastava, S.K., and Syamasunder, K.V. (2003). Essential oil composition of *Acorus calamus* L. from the lower region of the Himalayas. Flavour Frag. J. 18, 18-20.

Saikia, D., Khanuja, S.P.S., Kahol, A.P., Gupta, C.S., and Kumar, S. (2001). Comparative antifungal activity of essential oils and constituents from three distinct genotypes of *Cympogon* sp. Current Science. 86, 1264-1266.

Sikkema, J., De Bont, J., and Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59, 201-222.

Soatthiamroong, T., Jatisatienr, C., and Supyen, D. (2003). Antifungal activity of extract of *Eugenia aromatica* (L.) Baill. (Myrtaccae) against some pathogenic molds. ActaHort. 597, 209-214.

Suhr, K.I., and Nieken, P.V. (2003). Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. Journal of Applied Microbiology. 94, 665-674.

Trumpower, B.L., and Gennis, R.B. (1994). Energy transduction by cytochrome complexes in mitochondrial and bacterial respiration: the enzymology of coupling electron transfer reactions to trans-membrane proton translocation. Ann. Rev. Biochem. 36, 675-716.

Tullio, V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, A.M., Cuffini, A.M., Alonzo, V., and Carlone, N.A. (2006). Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. Journal of Applied Microbiology. 1-7.

Ultee, A., Bennik, M.H.J., and Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food borne pathogen *Bacillus cereus*. Applied and Environmental Microbiology. 68, 1561-1568.

Uribe, S., Samirez, T., and Pena, A. (1985). Effect of ß-pinene on yeast membrane functions. J. Bacteriol. 161, 1195-1200.

Viollon, C., and Chaumont, J.P. (1994). Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans*. Mycopathologia. 128, 151-153.

Zafor Beg, A., and Ahmed, I. (2002). *In vitro* fungitoxicity of the essential oil of *Syzygium aromaticum*. World Journal of Microbiology & Biotechnology. 18, 313-315.

Zhu, Y.P. (1998). Chinese materia medica-chemistry, pharmacology and applications. Harwood Academic Publisher. ISBN No. 90-5702-285-0.

3. Comparison of the inhibitory effect of various seed coating substances against rice seed borne fungi during storage

3.1 Abstract

Presently, chemical seed treatments are in discussion due to their directly or indirectly impacts on human health or other living organisms. They may also negatively affect the ecosystem and the food chain. In rice seeds, chemicals may cause phytotoxic effects including seed degradation. Eugenol is the main component of clove (*Eugenia caryophillis*) oil and it acts simultaneously as bactericide, fungicide and virocide. The present study aim to compare the inhibitory effect of the following seed treatment substances to protect the seeds against seed borne fungi during 12 months of storage: captan (CA), chitosan-lignosulphonate polymer (CL), eugenol incorporated into chitosan-lignosulphonate polymer (E+CL) and control (CO). The obtained results of fungi inhibition showed at first that CA treatment led to a better, i.e. longer, inhibitory effect on *Alternaria padwickii*, *Rhizoctonia solani*, *Curvularia sp.*, *Aspergillus flavus*, and *Aspergillus niger* than E+CL coating polymer. Secondly, E+CL coating polymer showed the longest inhibitory effect against *Bipolaris oryzae* and *Nigrospora oryzae* compared to CA and CL coating polymer. Finally, both CA and E+CL coating polymer had non-significant different inhibitory effect on *Fusarium moniliforme*. The CL variant was only during the first 6 months of storage able to inhibit all species of the observed seed borne fungi, whereas CA and E+CL were capable to inhibit most of the fungi until 9 months of storage.

3.2 Introduction

According to Agrawal *et al*. (1989), more than 90% of the field crops grown in the world are propagated through seeds, and all of them are attacked by devastating seed born pathogens. The rice crop (*Oryza sativa*) is known to be attacked by many pathogenic fungi, e.g. *Bipolaris* sp. (brown spot), *Alternaria* sp. (stackburn), *Fusarium* sp. (bakanae), *Rhizoctonia* sp. (sheath blight), *Nigrospora* sp. (kernel smut), *Curvularia* sp (blast). Important storage fungi are *Aspergillus flavus* and *Aspergillus niger*. Fungi contamination is a major cause leading to seed deterioration and finally to the degradation of rice grain qualities (Hewett, 1987; Kaiser, 1987; Siddiqui *et al*., 1983).

Today, fungicide seed treatments are the most used traditional application to protect the seeds and young-seedlings from many seed- and soil-borne pathogens. However, the uses

and expectations of chemical seed treatment have raised the concern due to the impact on environment, which cause either direct or indirect impacts on human or other living organisms. It can also negatively affect the food chain, the ecosystem at all and may lead to fungicide resistance problems (Ester *et al*., 2003). Moreover, the fungal pathogen normally attached to the seed, so it is difficult to find chemical substances that will destroy the fungus without harming the seeds, because many fungicides were developed from bromine, iodine, sulfur, copper, and chloride compounds. Furthermore, the toxicity of fungicide has been ascribed to produce phytotoxic compounds induced seeds deterioration (Han, 2000).

The use of medical plant extracts in plant pathogenic fungi control is a key application in chemical-free agricultural system (Anonymus, 2000). The use of biodegradable, low-toxicity, natural products for biological control of fungi is the today trend (Burt and Reinders, 2003). In order to minimize undesirable side effects, many academic interests have focused on natural bio-fungicides as alternatives for toxic synthetic fungicides (Anonymus, 2004). Several antifungal compounds of plant origin are known to control the seed borne infection (El-Ghaouth, 1997). Phenolic compounds, as eugenol and eugenol acetate, are the main components of clove (*Eugenia caryophillis*) (Velluti *et al*., 2004). They were proved to have bactericide (Pawar and Thaker, 2006), virocide (Tullio *et al*., 2006) and fungicide effects and act against many seed borne fungi (Chami *et al*., 2005a). Additionally, they can easily be obtained from readily available plant (Mungkornasawakul *et al*., 2003) and possess no residues and phytotoxic effects (Chami *el al*., 2004).

The use of coated seeds with chemicals or alternative bioactive substances may reduce significantly the percentage of plant damage by pest and the level of pesticide application (Badei *et al*., 1996). In the modern era of seed treatment, compared with direct drench application methods, the seed coating technology can decrease the pesticide utilization about 85% (Chami *et al*., 2005b). Moreover, the conventional seed coating substances can be mixed with natural products, which may have protective effects on seeds. Another advantage of seed coating is the preservation of the quality of seeds and the seed components (Ester *et al*., 2003). These new uses often require improved application systems for better establish dosages and coverage of materials (Chami *et al*., 2005a). However, there is a paucity of information on the comparison of inhibitory effect of eugenol as bioactive compound and the synthetic chemical seed treatment to eradicate seed

born fungi (Chao and Young, 2000). Thus, this study aims to compare the inhibitory effect of traditional chemical fungicide (captan) and botanical fungicide (eugenol) seed coating substances against rice seed borne fungi. In practice, rice seeds will be also stored before sowing; therefore, the effectiveness of the different seed coating substances during storage was also studied.

3.3 Materials and methods

3.3.1 Seed materials preparation

The study was conducted at the Seed Science and Technology Laboratory, Faculty of Agriculture, Department of Agronomy, Chiang Mai University, Thailand. Dry graded rice seeds (*Oryza sativa* L. cv. KDML 105) from one seed lot were supplied from the Bureau of Seed Multiplication of Thailand. The initial seed moisture content and germination percentage were 10.65% and 96.00%, respectively. The split-plot design with four replications was applied. The main plot was seed coating substances, which were captan (CA), only chitosan lignosulphonate polymer (CL) eugenol incorporated into chitosan lignosulphonate polymer (E+CL) and control (CO) (Figure 3.1). The sub-plot was the 12-month storage period, seeds were randomly stored in a plastic bag sealed and then kept in an incubation chamber, (KPB6395FL, Termaks, S/N 2-858 Germany). The storage was carried out at controlled temperature of 30±2°C and relative humidity of 40±5% (Appendix 2). Seeds were sampled immediately, and then, every month for seed borne fungi determination collected.

Traditional captan treatment preparation (CA)

Captan fungicide (1, 2, 3 and 6-tetrahydro-N-(trichloromethyl thio) phthalimide was applied as slurry dust in a solution of polyethylene glycol (PEG 8000 at -2 MPa) (4 g of captan per 1 kg of the seeds). After that, seeds were dried to 10 ± 2 % of moisture content (MC) at 35°C to obtain a similar MC as the control (untreated) seeds.

Chitosan-lignosulphonate coating polymer preparation (CL)

Three percentage of chitosan-lignosulphonate coating polymer was prepared by adding 3 g chitosan (Fluka, Germany) into 100 ml of 1% v/v acetic acid combined with 1% w/v sodium lignosulphonate (Fluka, Germany) in distilled water. Then, the chitosan-lignosulphonate polymer was sprayed and mixed well into 500 g seeds. The seed MC of 10 ± 2 % was obtained after drieing at 35°C.

Eugenol incorporated into chitosan-lignosulphonate coating polymer preparation (E+CL)

One percentage of eugenol incorporated into chitosan-lignosulphonate coating polymer was prepared by adding 0.5 ml eugenol solution (Fluka, Germany) into 50 ml of 3% chitosan-lignosulphonate coating polymer binder. Then, it was sprayed onto mixed-well 500 g seeds samples. The seeds were dried at 35°C to obtain MC of 10 ± 2 %.

Figure 3.1: Rice seed coated with various seed coating substances; A: CO, B: CA, C: CL, D: E+CL

3.3.2 Seed health testing

According to ISTA (2006), the Blotter method is the testing method recommended for seed borne fungi detection. The procedure started with the test of a 400 seeds working sample in four replications, and 25 seeds per dish were placed on three filter papers (blotters) which were soaked well in sterilized water. The seeds were later incubated at 20-25°C in 12 hours light for 14 days. Seed borne fungi infection was recorded under a stereoscopic microscope (Olympia-SZ61). Then, the inhibition percentage of each seed born species was calculated, based on control seed, with the following equation:

$$\textbf{Inhibition percentage} = \frac{(\%\ \textbf{Infection}_{\text{control}} - \%\ \textbf{Infection}_{\text{sample}})}{\%\ \textbf{Infection}_{\text{control}}} \times 100$$

3.3.3 Calculation and statistical analysis

The data are presented as mean ± standard deviation. The analysis of variance was performed for data analysis and differentiated with LSD test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

3.4 Results

The ANOVA results indicated that the effectiveness of seed borne fungi control was significantly affected by seed treatment variants, storage duration as well as the interaction between them (Table 3.1). As shown in Figure 3.2, E+CL showed a strong inhibitory effect on seed borne fungi depending on storage period, especially the early storage period. Within 5 months storage there was no significant difference of the inhibitory effects in E+CL and CA. The inhibitory effects differences of both treatments became significant during storage longer than 6 months ($P \leq 0.05$). CL showed slightly inhibitory effect, prior to 3 months storage.

Table 3.1: The analysis of variance (ANOVA) for seed treatments and storage time on seed borne fungi control

Source of variation	DF	Statistic significant (P)							
		A. solani	*R. solani*	*Curvularia sp.*	*A. flavus*	*A. niger*	*B. oryzae*	*N. oryzae*	*Fusarium sp.*
Treatment (T)	**2**	*	*	*	*	*	*	*	*
Storage time (S)	**12**	*	*	*	*	*	*	*	*
T x S	**24**	*	*	*	*	*	*	*	*

*: significant different at the 0.05 level of probability

The results of 50% inhibitory effect (IE_{50}) could be classified into three groups. Firstly, CA had more capability to inhibit *Alternaria solani*, *Rhizoctonia solani*, *Curvularia* sp., *Aspergillus flavus* and *Aspergillus niger* than CL and E+CL (Table 3.2). For *A. solani*, CA could maintain the 50% inhibitory effect for 9 months. The E+CL kept the inhibitory effect for 7 months, whereas CL inhibited this fungus only for 4 months (Figure 3.3, Table 3.2).

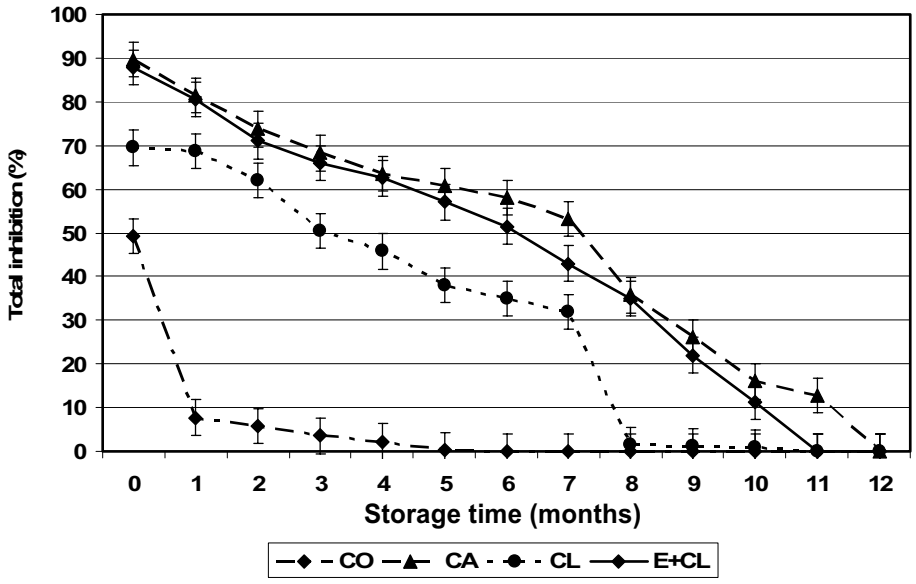


Figure 3.2: Effect of various seed coating substances on the total fungi inhibition percentage during 12 months of storage (The results are expressed as mean ± SD)

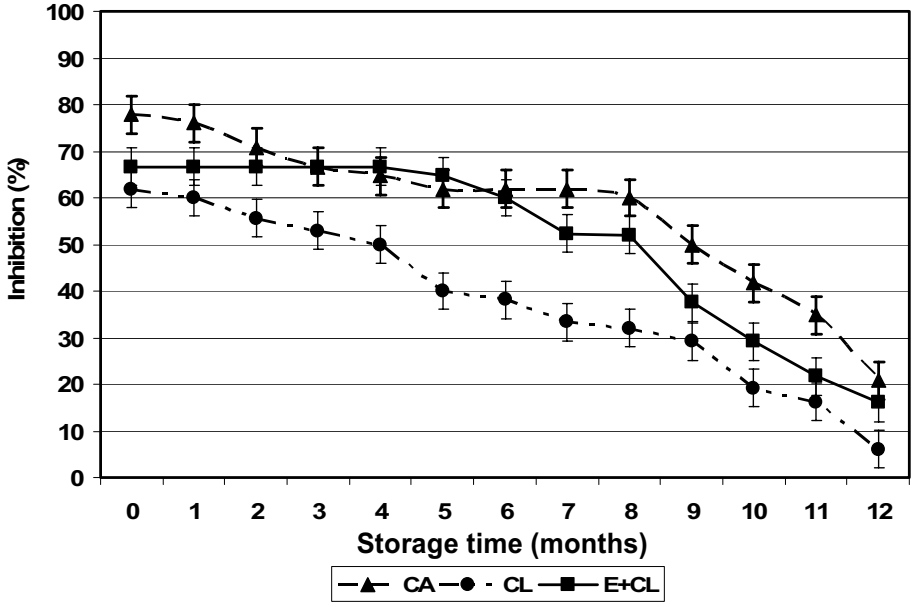


Figure 3.3: Inhibitory effect of various seed coating substances on *Alternaria solani* during 12 months of storage (The results are expressed as mean ± SD)

Comparing the inhibitory effects on *R. solani*, the CA and E+CL variants showed the 50% inhibitory effect at 8 and 7 months respectively, but the CL treatment inhibited *R. solani* for only 3 months (Figure 3.4, Table 3.2). CA and E+CL could remain inhibitory effect on *Curvularia* sp. for at least 6 and 5 months, respectively (Figure 3.5, Table 3.2).

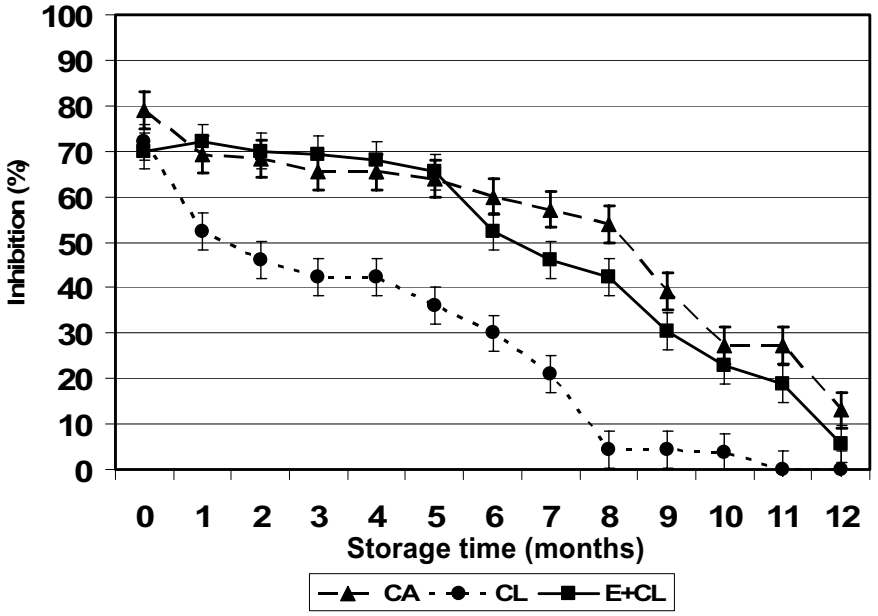


Figure 3.4: Inhibitory effect of various seed coating substances on *Rhizoctonia solani* during 12 months of storage (The results are expressed as mean ± SD)

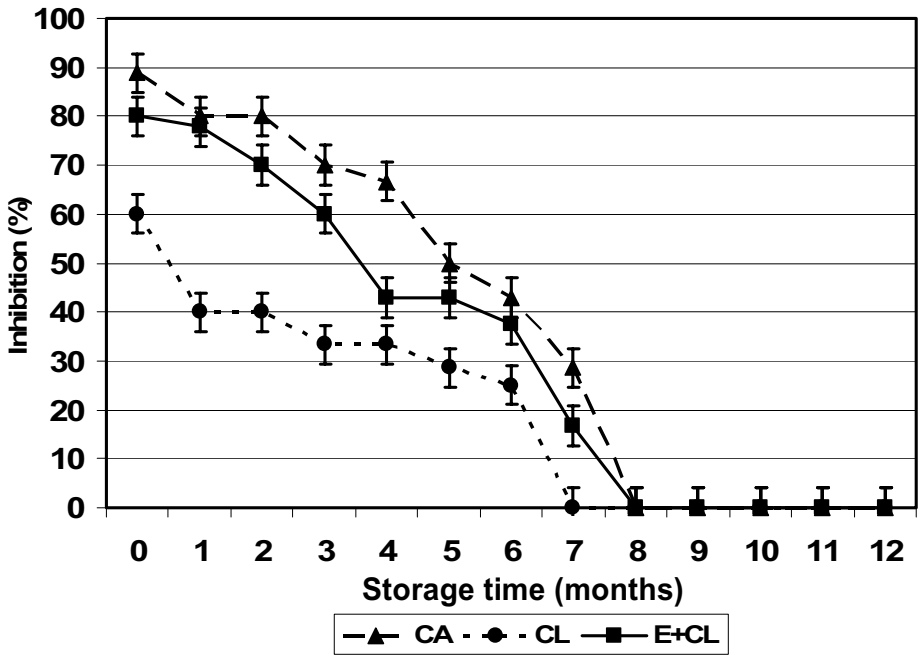


Figure 3.5: Inhibitory effect of various seed coating substances on *Curvularia sp.* during 12 months of storage (The results indicated in term of mean ± SD)

Figure 3.6 and Figure 3.7 demonstrate the antifungal activity of various seed coating substances on *A. flavus* and *A. niger*. CA could enhance 50% inhibitory effect up to 6 months for *A. flavus* and 5 months for *A. niger*. E+CL could maintain inhibitory effect to 3 months for *A. flavus* and 4 months for *A. niger*. CL showed the highest susceptibility and could inhibit both fungi for only 3 months.

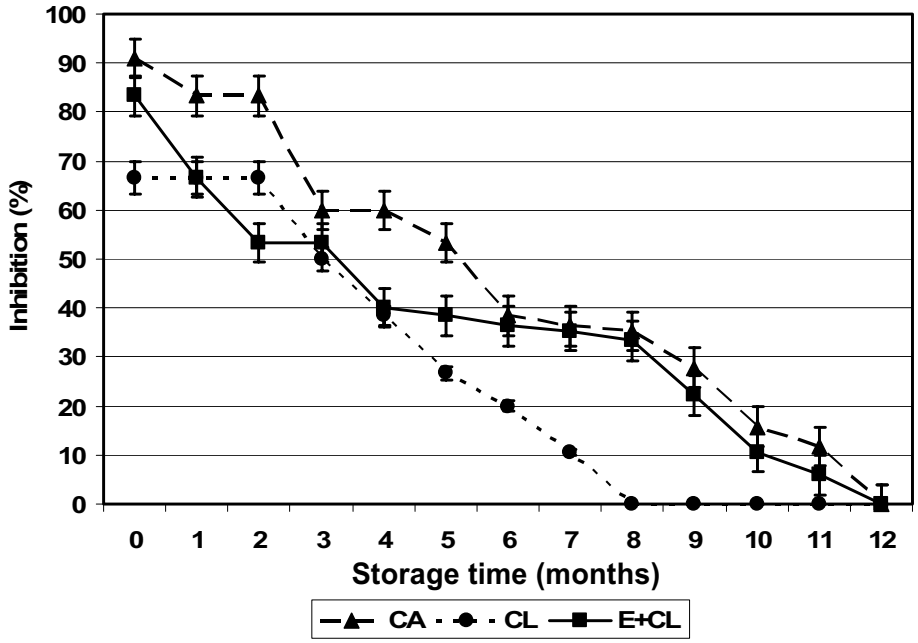


Figure 3.6: Inhibitory effect of various seed coating substances on *Aspergillus flavus* during 12 months of storage (The results are expressed as mean ± SD)

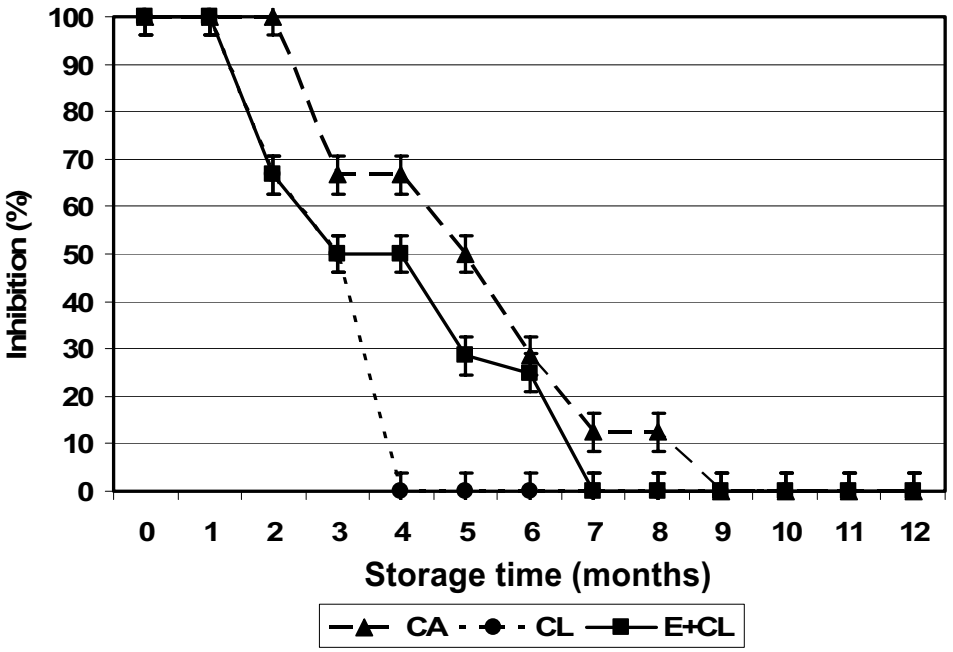


Figure 3.7: Inhibitory effect of various seed coating substances on *Aspergillus niger* during 12 months of storage (The results are expressed as mean ± SD)

In the second group, E+CL showed stronger inhibitory effect on *Bipolaris oryzae* (11 months) than CA (7 months) and CL (6 months) (Figure 3.8, Table 3.2). This treatment had 4 months an inhibitory effect on *N. oryzae*. CA inhibited *N. oryzae* for 3 only months and CL for less than 2 months (Figure 3.9, Table 3.2).

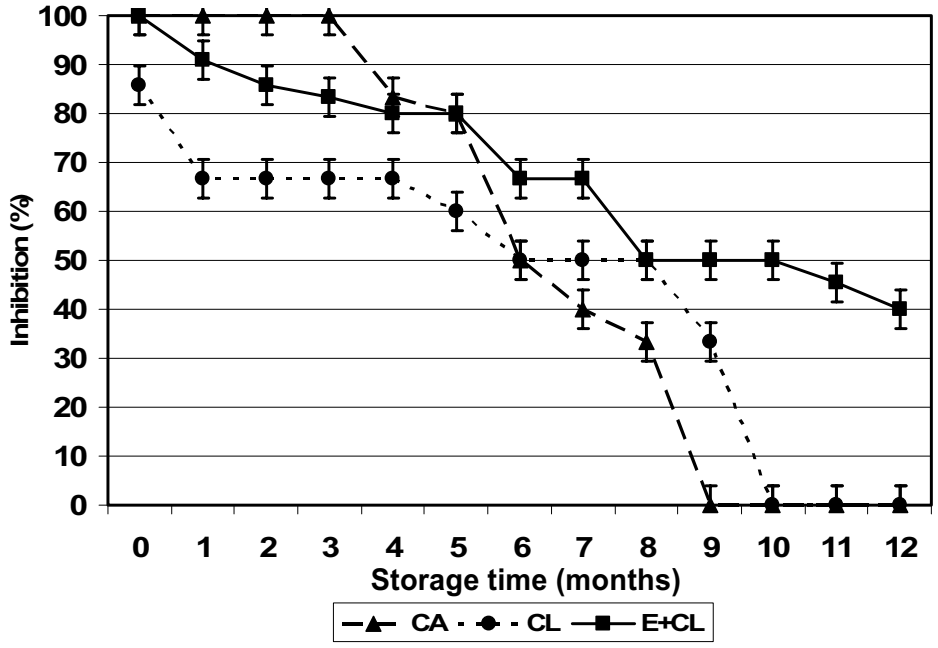


Figure 3.8: Inhibitory effect of various seed coating substances on *Bipolaris oryzae* during 12 months of storage (The results are expressed as mean ± SD)

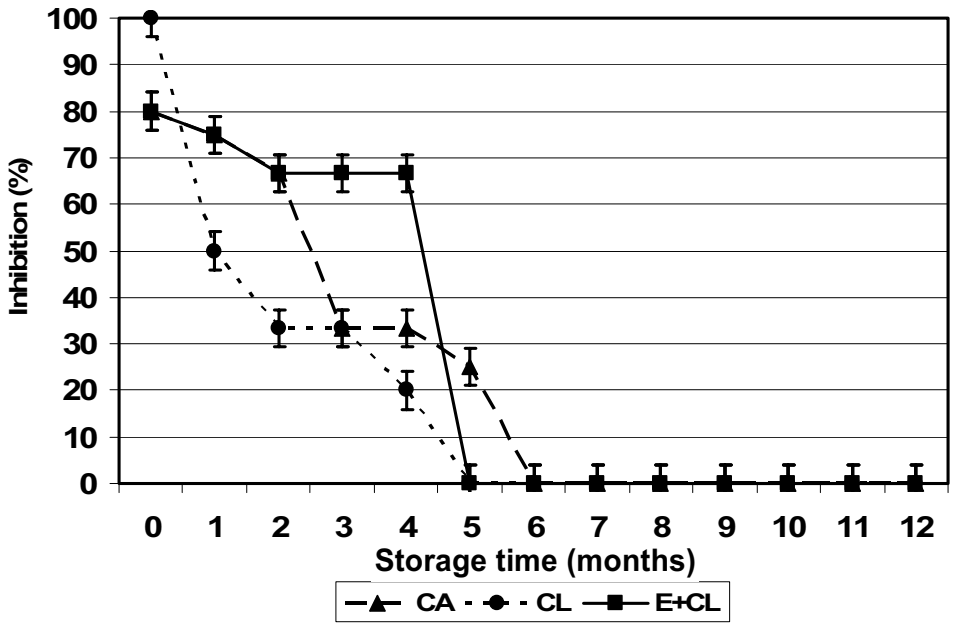


Figure 3.9: Inhibitory effect of various seed coating substances on *Nigrospora oryzae* during 12 months of storage (The results are expressed as mean ± SD)

For the last group, as showed in Figure 3.10, both CA and E+CL variants showed no significant difference of inhibitory effect on *F. moniliforme*. The E+CL coating polymer and CA could control *F. moniliforme* within 7 months of storage. With CL treatment, *F. moniliforme* could be generated after 3 months (Table 3.2).

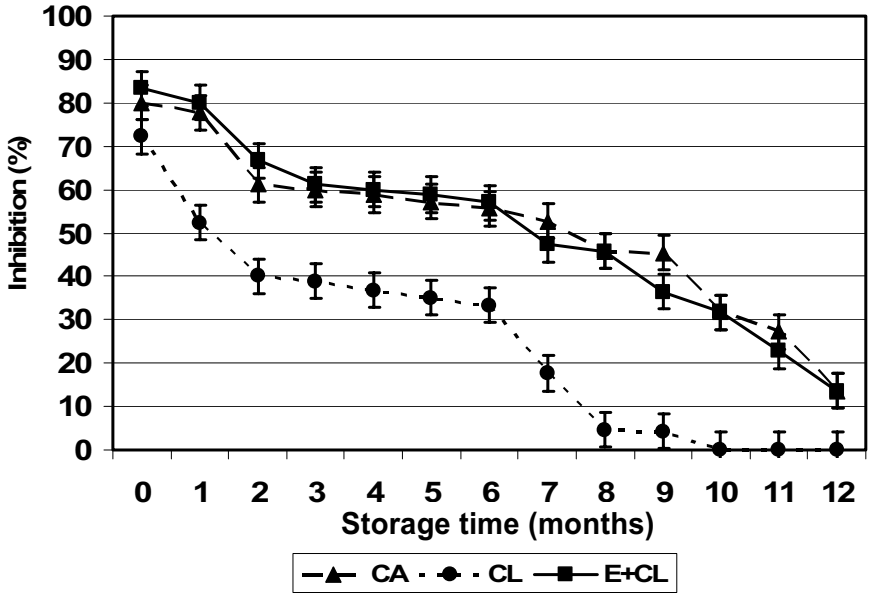


Figure 3.10: Inhibitory effect of various seed coating substances on *Fusarium moniliforme* during 12 months of storage (The results are expressed as mean ± SD)

Table 3.2: The 50% inhibitory effect of various seed coating substances against rice seed borne fungi during storage

Storage (Months)	Treatment		
	CA	**CL**	**E+CL**
0	-	-	-
1	-	*Curvularia sp.*	-
2	-	*Nigrospora oryzae.*	-
3	*Nigrospora oryzae.*	*Fusarium moniliforme, Rhizoctonia solani*	-
4	-	*Alternaria solani, Aspergillus flavus, Aspergillus niger*	*Nigrospora oryzae.*
5	-	-	*Aspergillus flavus, Aspergillus niger, Curvularia sp.*
6	*Aspergillus flavus, Aspergillus niger, Curvularia sp.*	*Bipolaris oryzae*	-
7	*Bipolaris oryzae, Fusarium moniliforme*	-	*Alternaria solani, Fusarium moniliforme, Rhizoctonia solani*
8	*Rhizoctonia solani*	-	-
9	*Alternaria solani*	-	-
10	-	-	-
11	-	-	*Bipolaris oryzae*
12	-	-	-

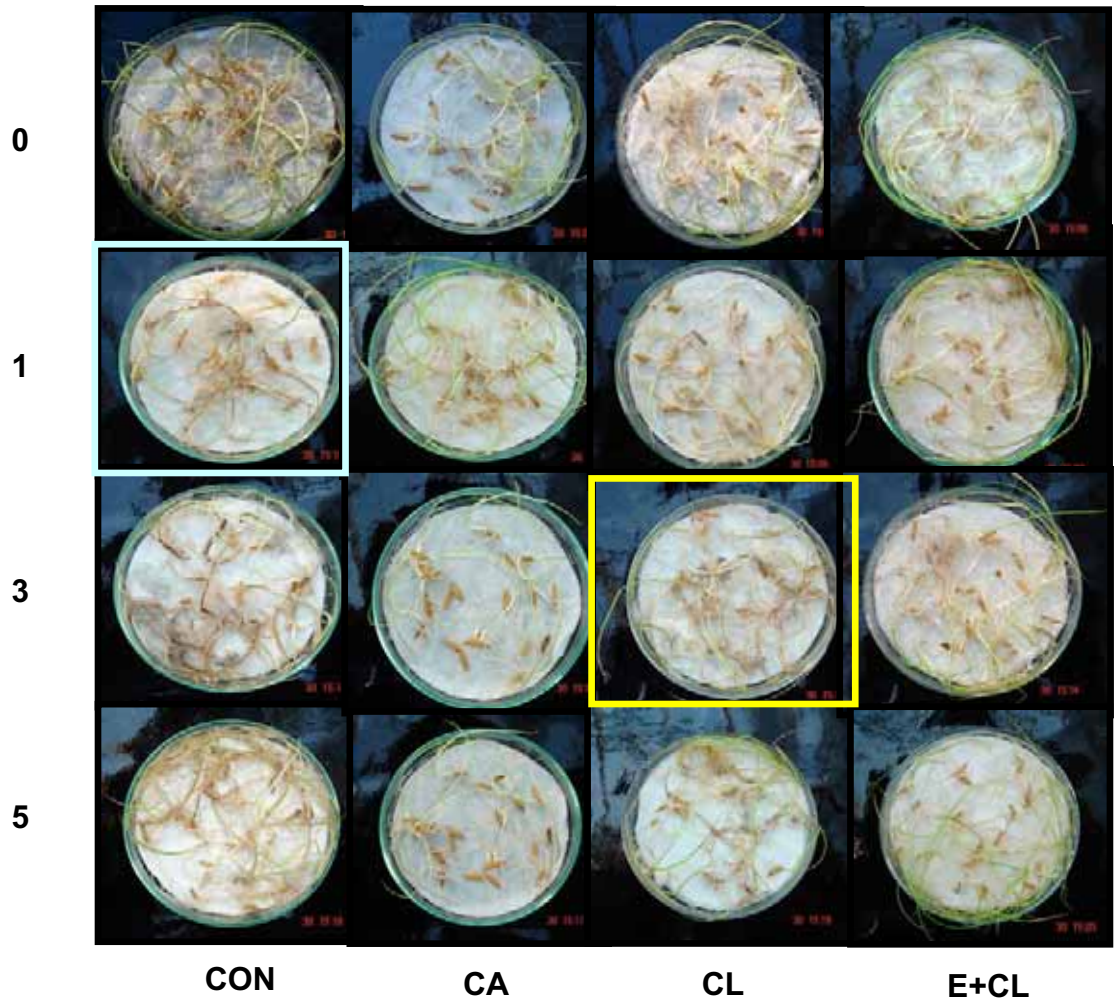


Figure 3.11: Inhibitory effect of CA, CL and E+CL against rice seed borne fungi during 0, 1, 3 and 5 months of storage

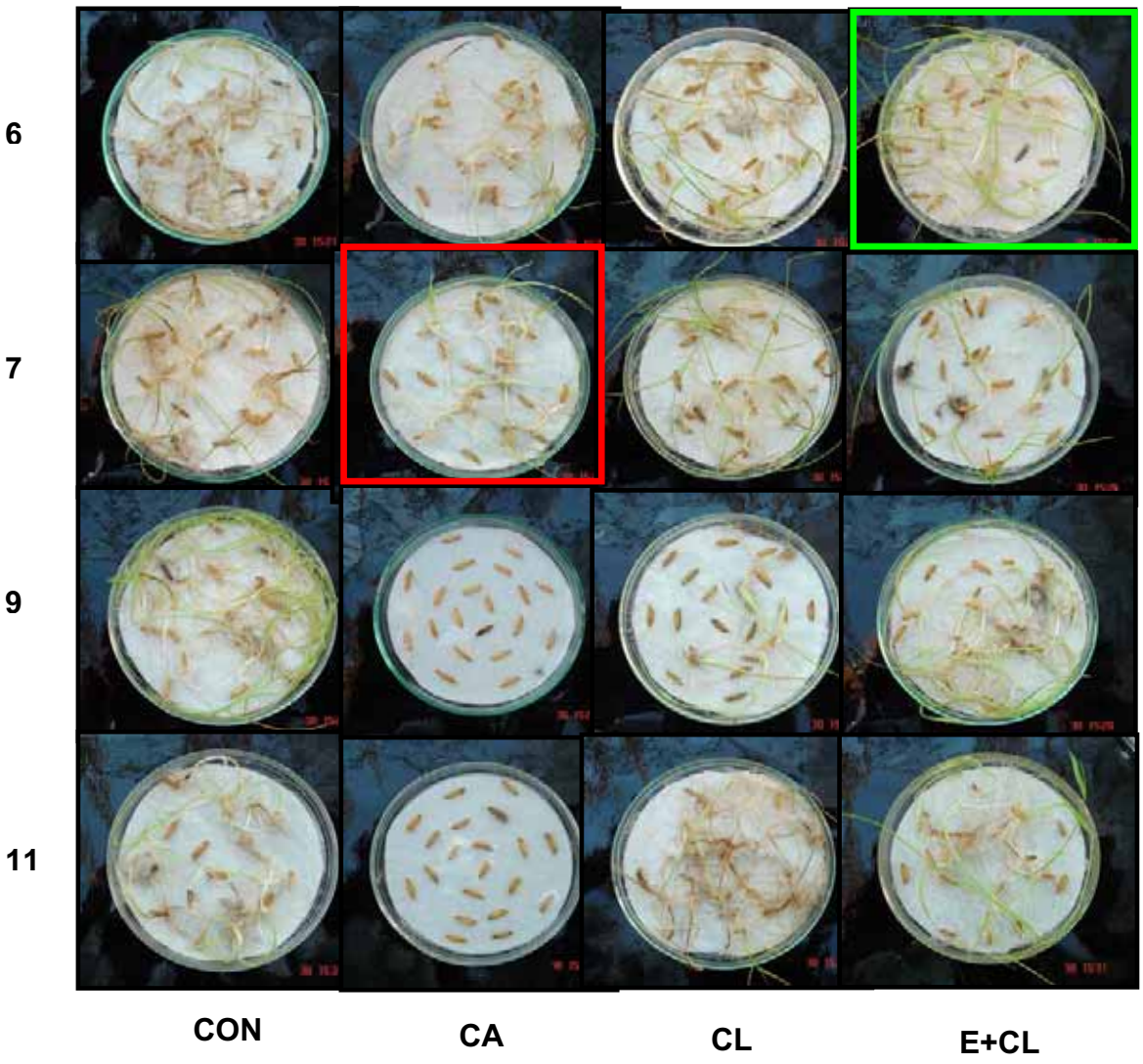


Figure 3.12: Inhibitory effect of CA, CL and E+CL against rice seed borne fungi during 6, 7, 9 and 11 months of storage

3.5 Discussion

The present study demonstrated the antifungal activity of E+CL treatment. Moreover, the combination of E+CL might be useful as part of a strategy to reduce losses caused by fungi infection, declined resistant of current use of chemical fungicide, but offered equivalent inhibition of seed borne fungi to the conventional fungicide captan.

This study has indicated that a bioactive coating polymer consisting of a combination of eugenol with chitosan polymer, which combines both antifungal and eliciting properties of chitosan polymer, as well as the biological activity of eugenol.

The results suggested that the effectiveness of E+CL coating polymer might be related to the interaction of the antifungal activity of eugenol as active compound and chitosan polymer. Consistent with other scientific data, Don *et al.* (2001) reported that chitosan polymer had inhibitory effect against 16 different fungi, especially on *Fusarium* sp., *R. solani* and *Phomopsis* sp. Han (2000) supported that chitosan as bio-polymer provided antifungal activity against *A. solani* and *F. moniliforme*. Moreover, the result of the previous experiment (Chapter 2) indicated that eugenol had a strong antifungal activity. Furthermore, a number of studies supported that eugenol from clove oils was effective against seed borne fungi (Chami *et al.,* 2005b; Evandro *et al*., 2005; Guynot *et al*., 2003).

The HPTLC results indicated that eugenol essential oil was persisted well on the E+CL coated seeds (Appendix 3). From the literature it is known that chitosan polymer h provide carrier, barrier and protective functions of eugenol, which could be incorporated into the coating material to improve its general functions. Labuza and Breene (1988) found that when antifungal agents are incorporated into the chitosan polymer, the coating polymer could either inhibit or prevent microbial, especially fungal growth, because chitosan polymer can be prolong the shelf life of antifungal agent and maintain its effectiveness (Ouattara *et al.*, 1999).

A major consideration for the study is how to achieve and maintain the antifungal activity of active compounds. The present results suggested that CL polymer could control and enhance the releasing rate of antifungal agent for a storage period over some months. Smith *et al*. (1990) also reported that antifungal agents once incorporated into CL polymer could provide antifungal activity by releasing the active compound at a controlled rate. Ngah and Liang (1999) found that the use of chitosan coating polymer is better than the

direct drench application, because it can control and enhance the releasing rate of antifungal agent for a long time. Normally, when an antifungal agent is added into the coating polymer, it affects general physical and chemical properties of coating polymer substances such as elongation, oxygen and water permeability and water absorptiveness (Han, 1996). The adequate solubility of eugenol is an important point for incorporation into chitosan polymer (Padgett *et al*., 1998).

According to Jun *et al*. (2005), antifungal agent can initially be incorporated into the chitosan coating polymer and migrate to the seed surface through diffusion and partitioning. The controlling of release rate and migration of antifungal agent from chitosan coating polymer to contact target microorganism is a very important factor in establishing the effectiveness of antifungal agent (Roller and Covill, 1999). However, the present results showed the reduction of antifungal activity due to the deterioration of antifungal agents during long-term storage. Horita and Kodama (1996) reported that the deterioration of the antifungal activity is among others related to high temperatures and high relative humidity, which affect the chemical stability of incorporated antifungal agent and may reduce so their activity (Lee *et al*., 2004). Besides chemical degradation, loss of volatile property of essential oil is a reason for the loss of antifungal activity during the storage (Han and Floros, 1999). Moreover, their efficacy is often limited by poor retention at the site of action due to the self-cleansing action of eugenol (Don *et al.,* 2001).

In addition, further reasons for the loss of antifungal activity of eugenol during storage are the differences of the chemical and biological characteristics between seed and coating substances such as pH, water activity, temperature, oxygen availability, carbon dioxide levels and the presence of antimicrobial substances as well as nutrient availability. Nah and Jang (2002) found that the seed lipids might affect the activity of essential oils because of the hydrophobic properties of their active compounds. This minor activity *in vivo* could be because of interactions of essential oils active compounds (known to be lipophilic) with chemical components, such as proteins and lipids, decreasing their effective level (Nugraha *et al*., 2004). Water activity, storage temperature and their interactions alter the antimicrobial activity and chemical stability of the incorporated active substance and so the antifungal activity may decrease (Han, 2000).

However, the interactions of rice seed, essential oil, coating substances and pathogen are very complex and that may be a reason why the result *in vivo* was different from that *in vitro*. According to the present study, the antifungal mechanisms of essential oils combined with chitosan coating polymer remain still not clear. More work on the synergistic action of essential oils and coating material *in vitro* and *in vivo* conditions is required.

The eugenol (the main component of clove oil) is a phenolic compound. The antimicrobial activity of this essential oil can be attributed to the presence of an aromatic nucleus and a phenolic -OH group that is known to be reactive and to form hydrogen bonds with active sites of target enzymes (Farag *et al*., 1989). It was described that the hydroxyl group (bound to a benzene ring) is important for the activities of antifungal compounds that these activities are enhanced by the presence of a–b double bonds (Ultee *et al.*, 2002). The experiment suggested that the acidic pH conditions from CL polymer could alter the ionization (dissociation/association) of hydroxyl group, which can change the antimicrobial activity of eugenol (Wang *et al*., 2006). Moreover, this condition affects the growth rate of fungi which grow generally well under acidic conditions.

The results of our experiments showed that during storage, the antifungal activities of captan fungicide were not long lasting because captan has low antifungal level. Moreover, the results suggested that the resistance of certain insolated fungus to captan could be caused by its intensive exposure to the chemical control, which leads to pre-resistance to the fungicide application.

3.6 Conclusion

This study demonstrated that eugenol is very effective to inhibit rice seed borne fungi and could be an alternative replacement for the synthetic chemical seed treatment. E+CL coating polymer had a potential antifungal activity against seed borne fungi as *F. moniliforme*, *A. solani*, *B. oryzae*, *R. solani*, *Curvularia spp*., *A. flavus* and *A. niger* which are often resistant to available antifungal agents. However, the inhibitory effects *in vivo* were not as strong as those *in vitro*. Further studies are required to emphasize the effects of E+CL coating polymer in rice seed and examining its safety for use as antifungal agent against rice seed borne fungi.

3.7 References

Agrawal, P.C., Mortensen, C.N., and Mathur, S.B. (1989). Blast: Seed-borne diseases and seed health testing. CAB Int. Mycol. Inst., Kew, England. 7-14.

Anonymus. (2000). Natural Chemical Product Research and Technology Institute in Agriculture. Pli Bai Newsletter, Nov. vol. 10. Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

Anonymus. (2004). http://home.t-online.de/home/hydrobio.hw/bterms.htm, visited at 01.11.2004

Badei, A.Z.M., El-Akel, A.T.M., Morsi, H.H., Baruah, P., Sharma, R.K., Singh, R.S., and Ghosh, A. (1996). Fungicidal activity of some naturally occurring essential oils against *Fusarium moniliforme*. Journal of Essential Oil Research. 8, 411-412.

Burt, S.A., and Reinders, R.D. (2003). Antimicrobial activity of selected plant essential oils against *Escherichia coli* 0157:H7. Applied Microbiology. 36, 162-167.

Chami, N., Chami, F., Bennis, S., Trouillas, J., and Remmal, A. (2004). Antifungal treatment with carvacrol and eugenol of oral candidasis in immunosuppressed rats. Braz J Infect Dis. 8, 217-226.

Chami, N., Chami, F., Bennis, S., Bouchikhi, T. and Remmal, A. (2005a) Oregano and clove essential oil induce surface alteration of Saccharomyces cerevisiae, Phytother Res. 19, 405-408.

Chami, N., Bennis, S., Chami, F., Aboussekhra, A. and Remmal, A. (2005b). Study of anticandidal activity of carvacrol and eugenol *in vitro* and *in vivo*. Oral Microbiol. Immunol. 20, 106-111.

Chao, S.C., and Young, D.G. (2000). Screening for inhibitory activity of essential oils on selected bacterial, fungi and viruses. J. Essent. Oil Res. 12, 630-649.

Dix N.J., and Webster J. (1995). Fungal ecology. London: Chapman& Hall. 549.

Don, T.M., Hsu, S.C., Chiu, W.Y. (2001). Structure and Thermal properties of chitosan-modified poly (methyl methacrylate). Journal of Polymer Science. Part A. 39, 1646-1655.

El-Ghaouth, A. (1997). Biologically based alternatives to synthetic fungicides for the control of post harvest diseases. Journal of Industrial Microbiology&Biotechnology. 19, 160-162.

Enokibara S, Suzuki A, Fujita C, Kashiwagi M, Mori N, and Kitamoto Y. (1993). Diversity of pH spectra of cellulolytic enzymes in Basidiomycetes. Trans Mycol Soc Japan 34, 221–228.

Ester, A., De Putter, H., and van Bilsen, J.G.P.M. (2003). Film coating the seed of cabbage (*Brassica oleracea* L. convar. Capitata L.) and Cualifower (*Brassica oleracea* L. var. Botryti L.) with inidacloprid and spinosad to control insect pests. Crop production 22:761-768.

Evandro, L.S., Lima, E.L., Reinaldo, K., Freire, and Christina, P. (2005). Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Brazilian Archives of Biology and Technology. 48(2), 245-250.

Farag, R.S., Daw, Z.Y. and Abo-Raya, S.H. (1989) Influence of some spice essential oils on Aspergillus parasiticus growth and production of aflatoxins in a synthetic medium. Journal of Food Science. 54, 74–76.

Guynot, M.E., Ramos, A.J., Setio, L., Purroy, P., Sanchis, V., and Marin, S. (2003). Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. Journal of Applied Microbiology. 94, 893-899.

Han, J.H. (1996). Modeling the inhibition kinetics and the mass transfer of controlled releasing potassium sorbate to develop an antimicrobial polymer for food packaging. J. Plastic Film Sheeting. 13, 287-298.

Han, J.H., and Floros, J. (1999). Modeling antimicrobial activity loss of potassium sorbate against Baker's yeast after heat process to develop antimicrobial food packaging materials. Food Sci. Biotechnology. 8(1), 11-14.

Han, J.H. (2000). Antimicrobial food packaging. Food Technology. 54(3), 56-65.

Hewett, P.D. (1987). Pathogen viability on seed in deep freeze storage. Seed Science and Technology. 15, 73-77.

Horita, H. and Kodama, F. (1996). Bud rot of chrysanthemum caused by *Fusarium roseum* in commercial production of cutting of carnation, *Dianthus caryophyllus*. Plant Dis. Rep. 11, 840-844.

International Seed Testing Association. (2006). International Rules for seed testing. Seed Science and Technology, 21 (Supplement).

Jun, Q., Wang, R., Yan, J., and Hu, J. (2005). Seed film coating with unconazole improves rape seedling growth in relation to physiological changes under water logging stress. Plant Growth Regulator. 47, 75-81.

Kaiser, W.J. (1987). Testing and production of healthy plant germplasm. Tech. Bull. 2 Dan. Gov. Inst. Seed Pathol. Dev. Countries, Copenhagen, Denmark. 30.

Labuza, T.P., and Breene, W.M. (1988). Application of active packaging for improvement of shelf life and nutritional quality of fresh and extended shelf-life foods. J. Food Proc. Reserv. 13, 1-69.

Lee, C.F., Wen, C.F., Lin, C.L., and Chiu, W.Y. (2004). Morphology and temperature responsiveness-swelling relationship of poly (*N*-isopropylamide-chitosan) copolymers and their application to drug release. Journal of Polymer Science. Part: A. 42, 3029-3037.

Mungkornasawakul, P., Pyne, S.G., Jatisatienr, A., Supyen, D., Lie, W., Ung, A.T., Skelton, B.W. and White, A.H. (2003). Stemocurtisine, the First Pyridol [1, 2-a] azaepine Stemona Alkaloid. J. Nat. Prod. 66, 980-982.

Nah, J.W., and Jang, M.K. (2002). Spectroscopic characterization and preparation of low molecule, water-soluble chitosan with free-amine group by novel method. Journal of Polymer Science. Part A. 40, 3796-3803.

Ngah, W.S., and Liang, K.H. (1999). Adsorption of Gold (III) ions onto chitosan and *N*-Carboxymethyl chitosan: Equilibrium studies. Ind. Eng. Chem. Res. 38, 1411-1414.

Nugraha, E.S., Alain, C., Lin, T., and Veronique, C. (2004). Mechanical and barrier properties of biodegradable films made from chitosan and poly (Lactic acid) blends. Journal of Polymers and the Environment. 12, 1-6.

Ouattara, B., Simard, S., Piette, G.J.P., Holley, R.A., and Begin, A. (1999). Diffusion of acetic and propionic acids from chitosan films immerged in water. Ann. Mig. of Inst. of Food Technologist, Chicago, III., 24-28.

Padgett, T., Han, I.Y., and Dawson, P.L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. J. Food Protect. 61, 1330-1335.

Paster, N., Menasherov, M., Ravid, U. and Juven, B.J. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. Journal of Food Protection 58, 81–85.

Pawar, V.C., and Thaker, V.S. (2006). *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. Mycoses. 49, 316-323.

Roller, S., and Covil, N. (1999). The antifungal properties of chitosan in laboratory media and apple juice. Int. J. Food Microbiol. 47, 67-77.

Smith, J.P., Ramaswamy, H.S., and Simpson, B.K. (1990). Developments in food packaging technology. Part II: Storage aspects. Trends Food Sci. Technol. 111-118.

Siddiqui, M.R., Mathury, S.B. and Neergaard, P. (1983). Longevity and pathogenicity of *Colletotrichum* spp. In seed stored at 5°C. Seed Science and Technology. 11, 353-361.

Tullio V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, M.A., Cuttini, A.M., Alonzo, V., and Carlone, N.A. (2006). Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapor contact methods. Journal of Applied Microbiology. 1-7.

Ultee, A. Bennik, M.H.J. and Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogen Bacillus cereus. Applied and Environmental Microbiology. 68, 1561–1568.

Velluti, A., Sanchis, V., Ramos, J.A., and Marin, S. (2004). Effect of essential oils of cinnamon, clove, lemon grass, oregano and palmaross on growth of and fumonisin B_1 production by *Fusarium verticillioides* in maize. J. Sci. Food Agric. 84, 1141-1146.

Wang, S., Chen, L., and Tong, Y. (2006). Structure-property relationship in chitosan-based biopolymer/montmorillonite nanocomposits. Journal of Polymer Science. Part: A. 44, 68

Zambonelli, A., Zechini D'Aulerio, A., Bianchi, A. and Albasin, A. (1996). Effects of essential oils on phytopathogenic fungi in vitro. J Phytopathol Berlin. 144, 491–494.

4. The effects of the seed coating substances on rice seed quality

4.1 Abstract

Seed coating is the application of pesticides or other substances on seed, which is rapidly becoming a standard agricultural practice. Nevertheless, the application of fungicides may produce phytotoxic effects on seed, which may cause losses of seeds germinability and seedling vigor. Botanical fungicides seed coating technology is an application to preserve the quality of seeds and the constituents inside them may not easily be deteriorated. This study evaluated the changes of seed qualities and several physiological performances of rice seeds (cv. KDML 105) which were coated with various seed coating substances, i.e., CA, CL and E+CL and stored for 12 months. The results revealed that CA significantly increased seed moisture content and seed water activity throughout the storage period. The qualities and viability of the seeds were seriously declined by this treatment. Moreover, CA inhibited the shoot and root development, seedling dry weight accumulation, delayed the seed germination and seedling growth rate. CA treated seeds were susceptible to stress conditions that declined the seed germination potential under cold, high moisture and temperature stress conditions. Nevertheless, CL and E+CL coating polymer could maintain seed storability. Bio-compounds significantly improved seed germination and seedling performances. These improvements were attributed to maintain the nutritive reserve and dehydrogenase activity in seeds. Moreover, the biological seed treatment stimulated the embryo growth and so speeding up the seedling emergence.

4.2 Introduction

Today, the most widely used seed treatment to protect the seed- and soil-borne fungi in the period immediately after planting is the application of synthetic fungicides (Neergaard, 1997). However, they may produce phytotoxic effects in the seeds. The impact of fungicides on seeds can cause losses of germinability and seedling vigor. Von Pine *et al*. (1995) found that the fungicide treatments with captan and pirimifos reduced the qualities of maize seed during storage and influenced the following behavior under field conditions. Moreover, Silva *et al*. (1996) reported that the application of clorpirifos and captan reduced the germination and vigor of maize seeds during storage. Therefore, the alternatives to chemical fungicides that receive increasingly attention are mechanical methods, resistant plant varieties, improved cultural practices, and natural or botanical fungicides. Currently, increasing academic interest has emerged on the botanical

fungicides as an alternative to synthetic fungicides, which might have the potential to minimize undesirable side effects. The use of herbal extracts for plant pathogens control is a promising way in a chemical-free agricultural system (Anonymus, 2000). However, the disadvantages of botanical fungicides are more difficult to implement. Generally, they have a narrow target range, are not acting as quick as chemical fungicides, they have a shorter shelf life and are not compatible with chemical fungicides or herbicides. Moreover, some essential oils are able to produce phytotoxic effects on seed viability when used at high concentrations. Tworkoski (2002) reported that essential oils (5-10%v/v) from red thyme, summer savory, cinnamon, and clove were at most phytotoxic and caused electrolyte leakage resulting in cell death of rice seed. Eugenol and eugenol acetate are active compounds contained in clove. They act simultaneously as bactericide, fungicide and virucide. Additionally, these extracts are affordable and can easily be obtained from readily available plant (Mungkornasawakul *et al*., 2003).

Although the pest control application has been extensively used as a seed coating technology in developing countries, they have received less attention in Thailand. According to Yildirim and Hoy (2003), seed coating with chemicals or alternative substances can reduce the percentage of plant damage by pest. They found that when comparing with direct drench application methods, the seed coating technology significantly decreased the percentage of plant damage at 85% of the level of pesticide utilization. In the past decade, the seed coating technology has been continuously improved, particularly in European countries and North America. The new techniques often require improving application systems for better establish dosages and coverage of materials. The coating material is thin and water-permeable. It can be mixed with natural products or other compounds, which have protective effects on seeds. Another advantage of seed coating material is the preservation of the quality of the seeds and the constituents inside the seeds may not easily be deteriorated (Ester *et al*., 2003). Seed coating techniques have been used to reduce the seedling emergence time, produced synchronized emergence, improved emergence rate and increased seedling stand production in many field crops like wheat (Basra *et al*., 2005) and rice (Farooq *et al*., 2004).

Seed quality has pronounced effects of progeny survival and fitness, especially under storage conditions and stressful conditions in general (Agrawal, 2002). The morphological and chemical attributes of these seed components are critical for successful seedling

establishment under various storage or stress conditions. Moreover, seedlings development depends on seed reserves in the early stages of development. This is particularly relevant under storage conditions. For example, cotyledons that emerge before the canopy closes in spring are exposed to fungicides levels that are capable of damaging plant DNA and photosynthetic machinery, which is the cause of abnormal seedling (Berjak and Villiers, 1972). The present study aimed to investigate the effects of various seed coating substances on rice seed (cv. KDML 105) quality and seedling performances during storage.

4.3 Materials and methods

Dry graded rice seeds (*Oryza sativa* L. cv. KDML 105) from one seed lot were supplied by Bureau of Seed Multiplication of Thailand. The initial seed moisture content and germination percentage were 10.65% and 96.00%. The split-plot design with four replications was applied. The main plot was seed treatment: captan (CA), only chitosan lignosulphonate polymer (CL), eugenol incorporated into chitosan lignosulphonate polymer (E+CL) and control seeds (CO). The sub-plot was the storage duration (12 months). The seeds of each treatment were randomly stored in plastic bag sealed in an incubation chamber (KPB6395FL, Termaks, S/N 2-858 Germany), at controlled temperature of 30±2°C, and relative humidity at 40±5% (Appendix 2). Seeds were sampled immediately and then collected each month for the determination of seed qualities and the seedling performances.

4.3.1 Seeds quality assessment

The seeds moisture content (MC) was determined by hot air oven method and calculated on a wet mass basis ISTA (2006). Testo 650 apparatus was used for analyzing the seed water activity (Aw).

Seed viability was measured as percentage of normal seedlings by standard germination test (Germ) in rolled-towel paper at 28°C for 14 days (ISTA, 2006). Dehydrogenase activity of seeds was analyzed by tetrazolium test (TZ test) according to ISTA (2006): fifty seeds were cut longitudinally through the embryo and soaked in 0.20% (w/v) of 2,3,5-triphenyltetrazolium chloride solution for 24 h at 20°C in the darkness, then scored according to intensity and location of red stain on seed (Figure 4.1).

Figure 4.1: Different categories of rice viability characteristic determined by Tetrazolium test A: Non-viable, injured, or death seed, B: Viable seeds, which can be observed from the red color strain by formazan in viable tissue.

Seed vigor was estimated by assessing the following parameters:

Shoot (Sh) and root (Ro) lengths of 20 normal seedlings grown in moist towel paper kept at optimum temperature (28°C) were measured in centimeter on the day of the final count (14 days). Seed samples showing maximum shoot and root length were considered vigorous seedling (ISTA, 2006).

Germination index (GI) as high speed of germination is an indication of vigorous seed lots. The number of germinating seeds was counted every day from the first day (14 days). The cumulative index was calculated by the following formula:

$$\mathbf{GI = (n1/1 + n2/2 + n3/3 + \ldots + nx/x)}$$

Where, n1…nx: are the number of germinated seed on day 1 to day x

High value of GI indicates high seed vigor. Seeds were considered germinating when the radical had appeared (ISTA, 2006).

Seedlings dry weight (SDW) determining by the dry weights (milligram) of seedlings was determined on the 14^{th} day after germination. After that, seedlings were oven dried at 80°C for 24 h. The lot exhibiting the maximum seedling dry weight was considered as vigor (ISTA, 2006).

Seedling growth rate (SGR) to assess the amount of dry seed weight that was respired for producing 1 g of dry root and shoot was metabolic efficiency of the seed. Thus, higher the value of SGR, which meant the efficiency of seed as more seed reserve are mobilized for producing root and shoot (ISTA, 2006). The amount of SGR was calculated as:

$$SGR = SDW – (SHW + RTW + RSW)$$

Where, SDW: dry weight of seed before germination, SHW: dry weight of shoot, RTW: dry weight of root, RSW: dry weight of seed after germination

Accelerated aging test (AAT) was evaluated by keeping un-imbibed seeds at high temperature (45°C) and relative humidity (around 100%) for 3 days. The seeds were then removed from the stress conditions and placed under optimum germination conditions (28°C) for 14 days (ISTA, 2006). After germinating, the number of normal seedling was counted as mean the potential of the seed could produce normal seedlings after exposed to stress conditions.

The cold emergence test (Cold) is an index for identifying the seed vigor which is identical to the standard germination test. However, the seeds were exposed to 10°C for 7 days prior to the warm greenhouse bench environment. The normal seedlings were counted after 14 days (ISTA, 2006).

The electrical conductivity test (Cond) was used to determine seed membrane integrity by measuring the seed leakage. This test followed procedures described by ISTA (2006). First, 100 seeds were weighted and soaked into de-ionized water. Then, they were placed at 25°C for 24 h. Finally, the solution was determined for electrical conductivity by the conductivity meter (Sartorius, model PP-20).

The percentage of field emergence (Field) and uniformity of seedling under field condition were determined by Brix test (ISTA, 2006).

4.3.2 Calculation and statistical analysis

The data are presented as mean ± standard deviation. The analysis of variance was performed for data analysis and differentiated with LSD test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

4.4 Results

The ANOVA results indicated that seed treatment variants, storage duration as well as the interaction between them had significant affects on all analyzed parameters. However, SDW, SGR, and Cond were not significantly affected by the interaction between seed treatment variants and storage duration (Table 5.1).

Table 4.1: Analysis of variance for seed treatments and storage time on seed properties

Source of variation	DF	Statistic significant (P)												
		MC	Aw	Germ	TZ	Sh	Ro	SDW	GI	SGR	Cold	AAT	Cond	Field
Treatment (T)	3	*	*	*	*	*	*	*	*	*	*	*	*	*
Storage time (S)	12	*	*	*	*	*	*	*	*	*	*	*	*	*
T x S	36	*	*	*	*	*	*	NS	*	NS	*	*	NS	*

*: significant different at the 0.05 level of probability, NS: Not significant different

The MC at the beginning of storage (0 month) ranged from 11.18-11.50 g $100g^{-1}$ DM in each treatment. However, the MC increased after 12 months storage especially the CA treatment. CL and E+CL were no significantly different from CO (Figure 4.2).

Aw ranged from 0.628 – 0.642 % in each treatment at the beginning of storage (0 month). During the storage period, Aw was significantly affected by the seed treatment variants (Figure 4.3). After 12-months storage, in the seeds of CA treatment, the Aw significantly increased at 0.824. However, CL and E+CL treatments were non-significantly different when compared with CO.

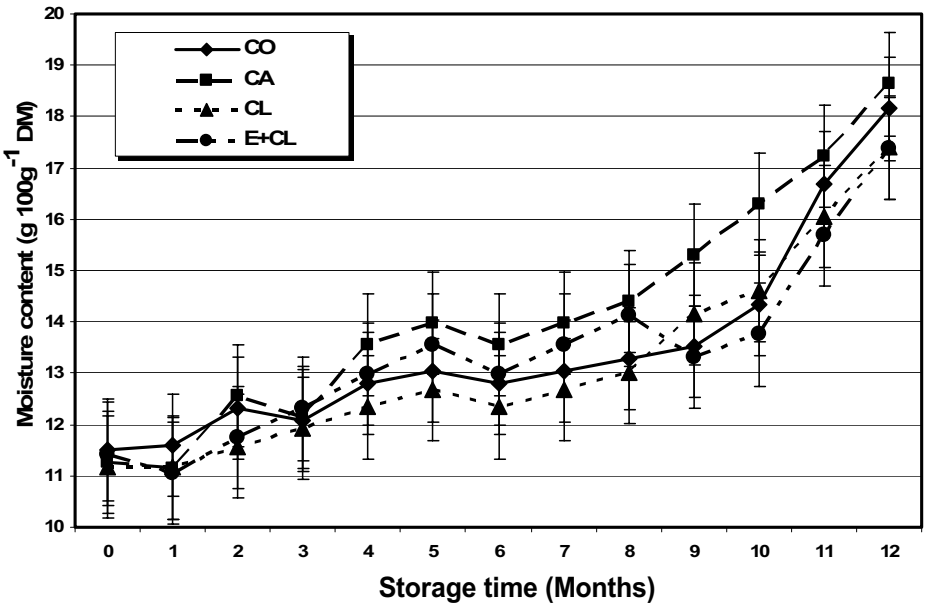


Figure 4.2: The effect of seed coating substances and storage time on seeds moisture content

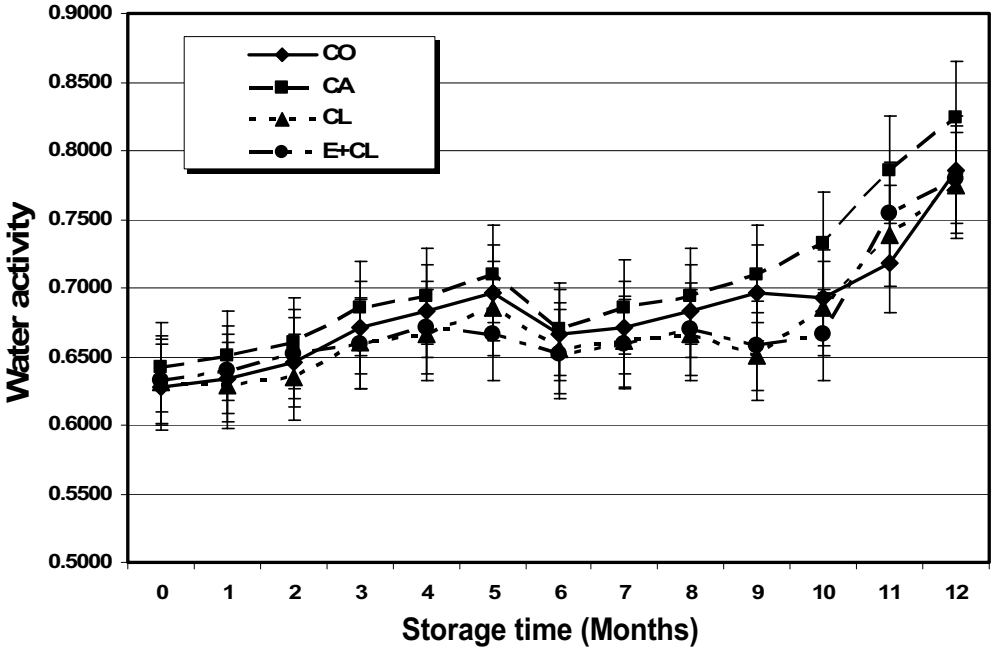


Figure 4.3: The effect of seed coating substances and storage time on seeds water activity

As presented in Figure 4.4, the potential of the seed to germinate before storage ranged from 94–98% in each treatment. After 12-months storage, in the CA treatment, the germination rate was decreased to 60% and the number of abnormal seedlings was increased. Figure 4.5 shows abnormalities in seedlings appearing in roots and coleoptiles. These varied significantly between CA, CL and E+CL treatments, thick and short roots showed higher incidence followed by thin, weak and long roots. In most cases, coleoptiles were either disproportionately short or missing. However, the biological coated seed could maintain the high level of germination percentage, which were non-significantly different from CO.

At the beginning of storage (0 month), seed treatment variants did not affected seed viability. However, the seed viability of CA declined rapidly to 85% after storage for 8 months and reduced further to 62% after 12months of storage. In the case of biological treatments, seed viability did not changed appreciably during storage although slightly changes were observed. This result was not significantly different when compared with CO (Figure 4.6).

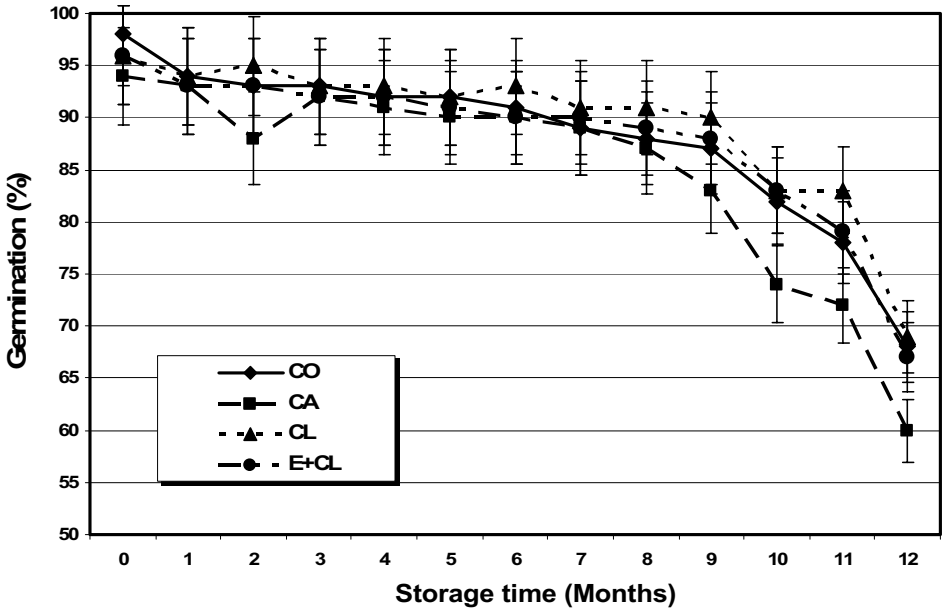


Figure 4.4: The effect of seed coating substances and storage time on seeds germination

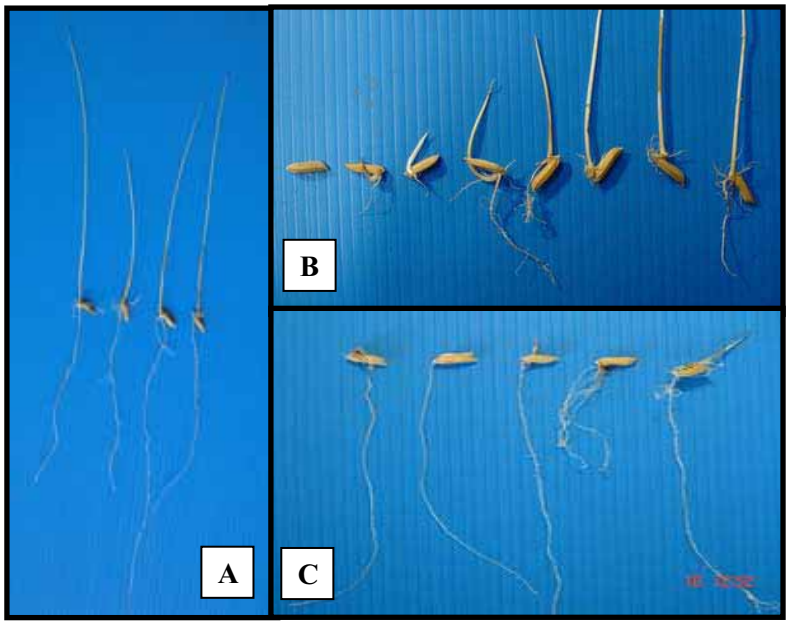


Figure 4.5: Seedling damage caused by CA impact to the coleoptiles and shoot compared with normal seedlings A: Normal seedlings; B: Decay and no root; C: Decay in coleoptiles and shoots.

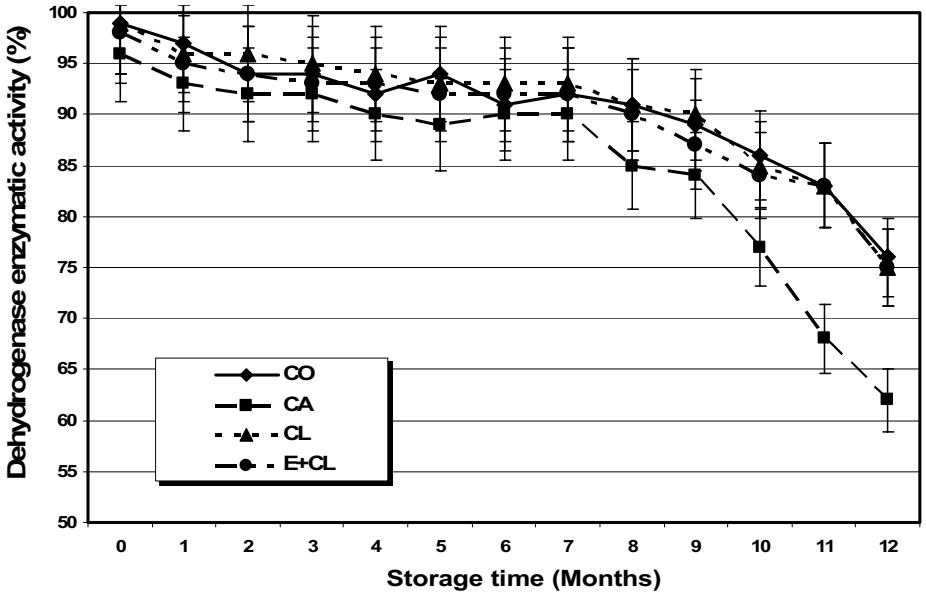


Figure 4.6: The effect of seed coating substances and storage time on seeds viability

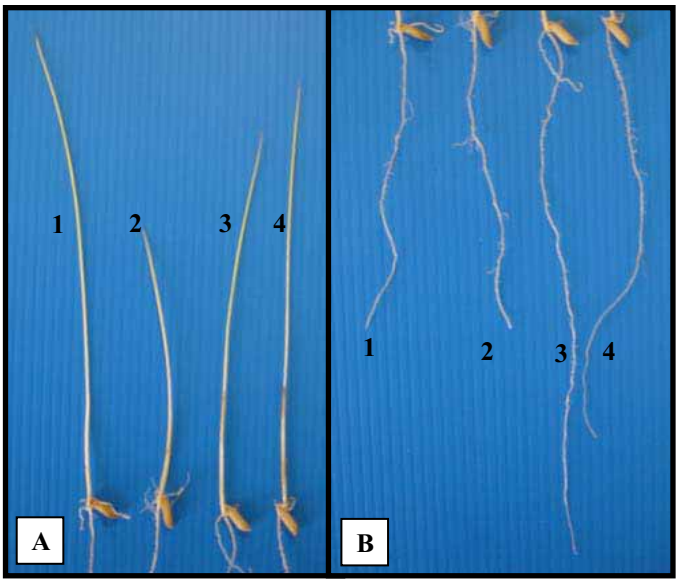


Figure 4.7: The effect of seed coating substances on shoots (A) and root (B) lengths
1: CO; 2: CA; 3: CL; 4: E+CL

The rice seedling vigor was reflected by the changes of shoot and root length. A reduction of both shoot and root length was affected by CA treatment. Dynamic changes in shoot and root length occurred during storage period. Biological treatments did not significantly affected on the reduction of both shoot and root length (Figure 4.8). The difference of shoot and root length between seed treatment variants was shown in Figure 4.7.

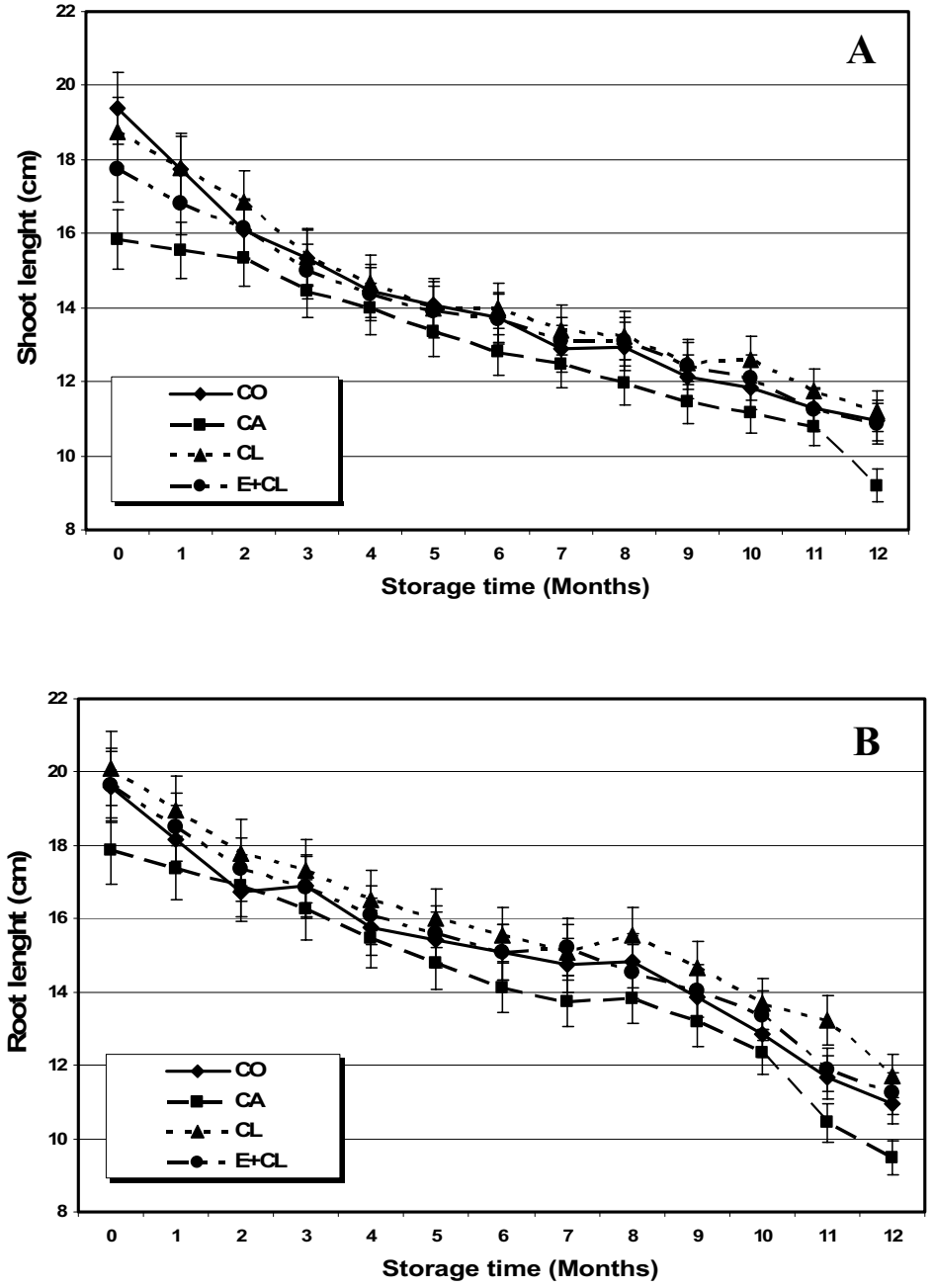


Figure 4.8: The effect of seed coating substances and storage time on seedling shoot (A) and root length (B)

CA did not affect only shoot and root length, but it led to the reduction of seedling dry weight. The results demonstrated a strong correlation between cell elongation and seedling dry weight, which was affected by CA. However, seedling dry weight was reduced more drastically than root and shoot lengths (Figure 4.9). This result indicated that CA affected the reduction of seedling vigor greater than the biological coated seeds.

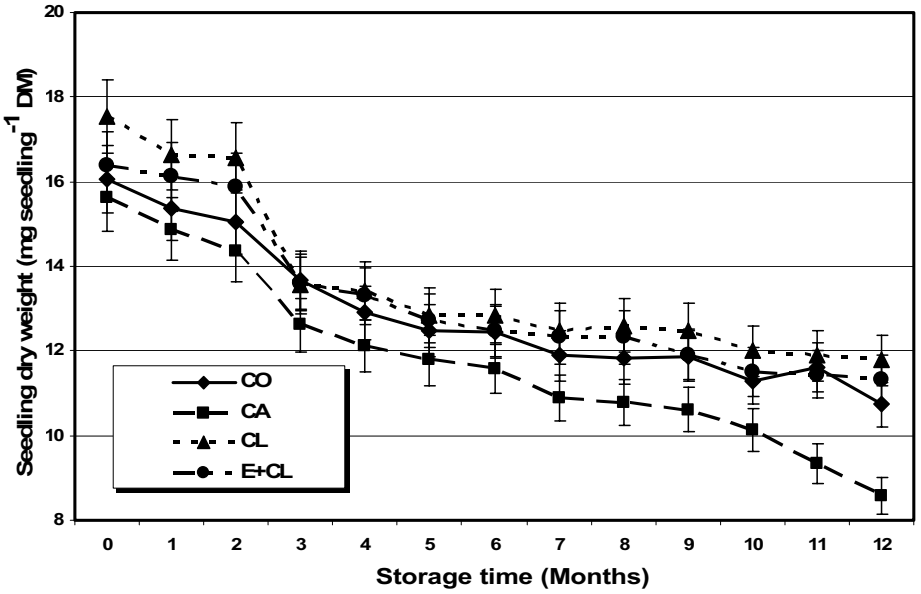


Figure 4.9: The effect of seed coating substances and storage time on seedling dry weight

The effect of seed treatment variants on GI is shown in Figure 4.10. At the beginning of storage (0 month), CA treatment showed the lowest GI (16.1). However, the biological coating substances did not adversely affected GI. This result is non-significant different with CO. After 12-months storage, both CL and E+CL treatment maintain the high level of GI, but the CA treatment inhibited seedling development as it is shown by the significant lower GI level compared with the control.

CA also reduced the potential of the seed for the efficient usage of the reserve to produce a vigorous seedling. Figure 4.11 indicates significant differences in SGR between the seed treatment variants. Both CL and E+CL treatments showed constantly the highest level of SGR throughout the storage duration. These treatments provided significantly higher SGR than control seeds. On the contrary, CA treatment showed the lowest of SGR. Moreover, SGR was also affected by the storage duration. The reduction of SGR was observed throughout the storage duration.

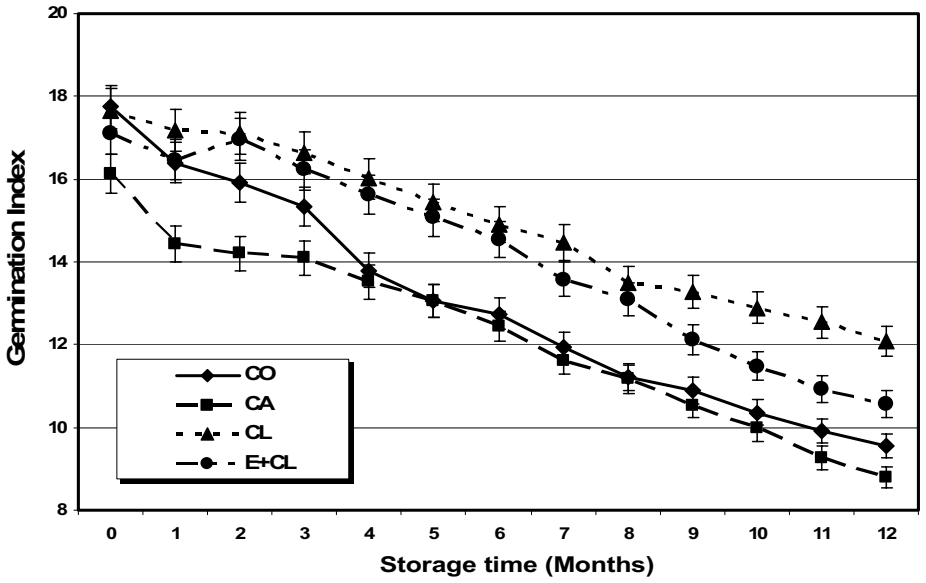


Figure 4.10: The effect of seed coating substances and storage time on germination index

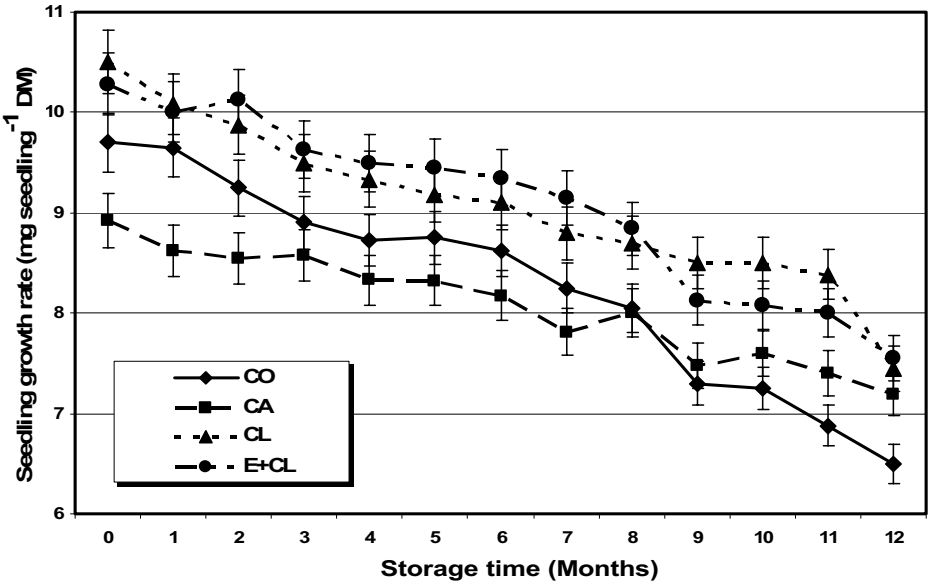


Figure 4.11: The effect of seed coating substances and storage time on seedling growth rate

At the beginning of storage time (0 month), CA treatment showed the lowest germination percentage after being exposed to cold stress condition but the germination of CO and biological coated seeds were similar (Figure 4.12). Eventually, the results indicated that CA caused losses of the viability under cold stress conditions. After stored for 12 months, CA apparently decreased the germination percentage. This result was significantly

different from CO. CL and E+CL could maintain the germination percentage, which was significantly different from CO.

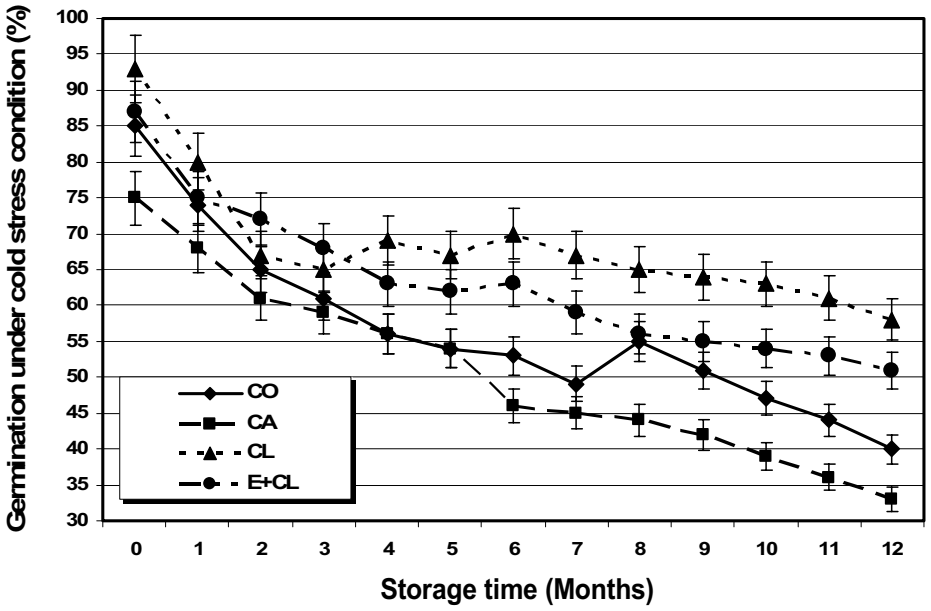


Figure 4.12: The effect of seed coating substances and storage time on seed germination after exposed to cold stress condition

Figure 4.13 indicates, at the beginning of storage (0 month), that AAT results were not affected by seed coating variants. However, the germination declined after 9-months of storage, which was similar to the trends of other vigor indices. The tests were highly correlated across all treatments, especially CA treatment. However, CL and E+CL remained a high germination rate of the aged seed. Furthermore, after 12-month storage, CA seriously reduced the potential of seeds that could germinate under stress conditions. On the contrary, CL and E+CL kept the high level of germination of the aged seeds, which were non-significant different with CO.

The effect of seed treatment variants and storage time on the conductivity value is showed in Figure 4.14. At the beginning of storage (0 month), conductivity values of the CA, CL, and E+CL were 69.61 µS mg^{-1}, 65.42 µS mg^{-1}, and 66.22 µS mg^{-1}, respectively. The level of conductivity of CO was the lowest (62.37 µS mg^{-1}). The conductivity increased throughout the storage time. After 12-months storage, the highest value of conductivity was reached in CA treatment. However, the difference of conductivity between CL, E+CL and CO was found to be non-significant.

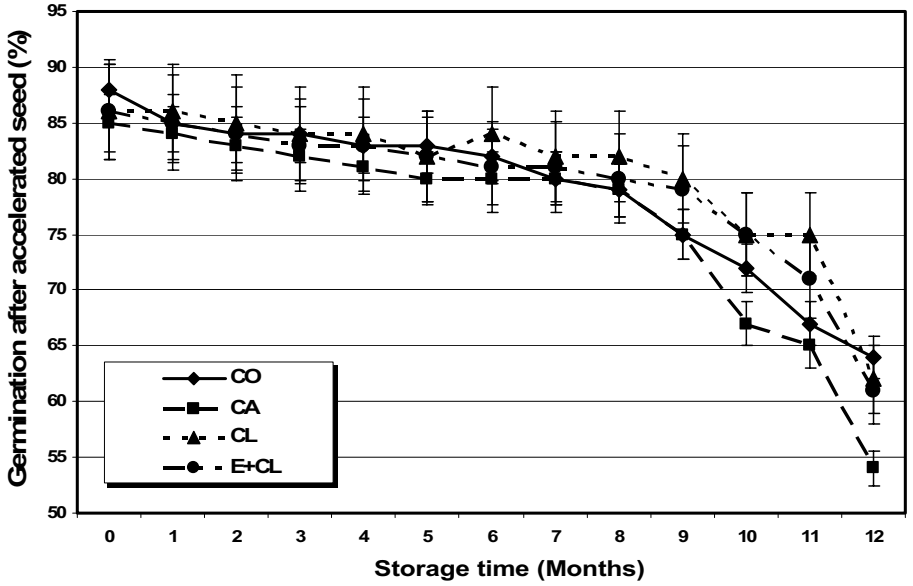


Figure 4.13: The effect of seed coating substances and storage time on seed germination after exposed to high temperature and moisture stress condition (aged seed)

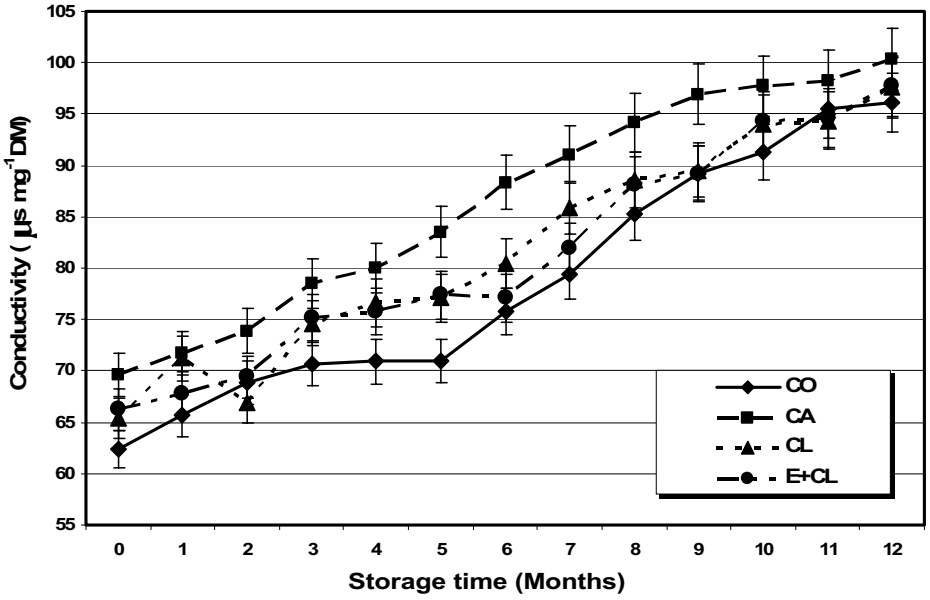


Figure 4.14: The effect of seed coating substances and storage time on seed electrolyte leakage

Both seed treatment variants and storage time decreased the number of germinating seedling under field conditions. At the beginning of storage, CA gave a lower number of germinating seedlings than CO, CL and E+CL did (Figure 4.15). After 7-months of storage, CA conclusively inhibits seeds that could germinate under field conditions. These

results were significantly different with biological treatment. After 12-months storage, CA inhibited the potential of seeds to germinate under field condition (Figure 4.16). The observed results were significantly different in biological coated seeds. Those variants indicated the highest potential to germinate under field condition. Figure 4.17 showed that CA not only affected the field emergence, but it also reduced seedling establishment under field condition. On the other hand, the biological coated seeds showed the best seedling establishment.

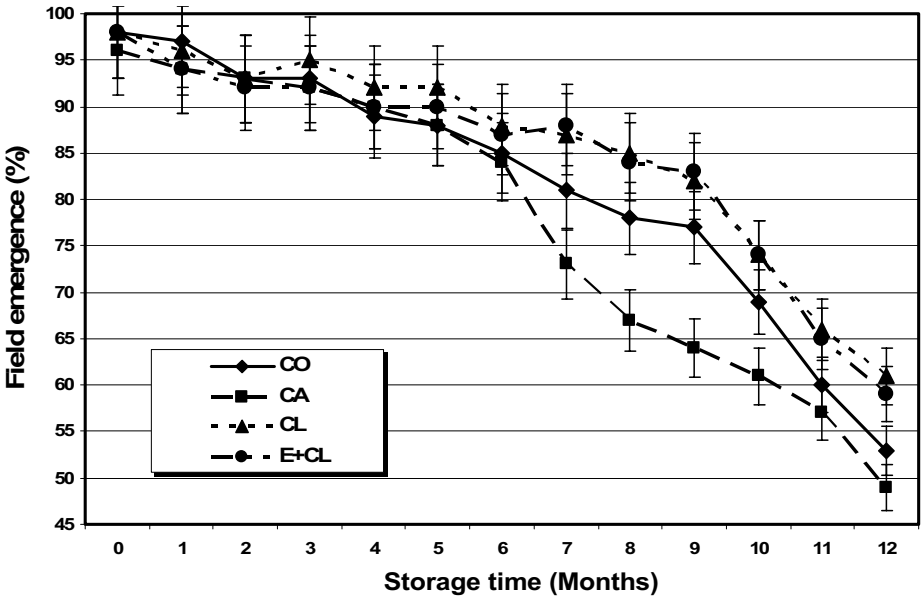


Figure 4.15: The effect of seed coating substances and storage time on seedling establishment in the field condition

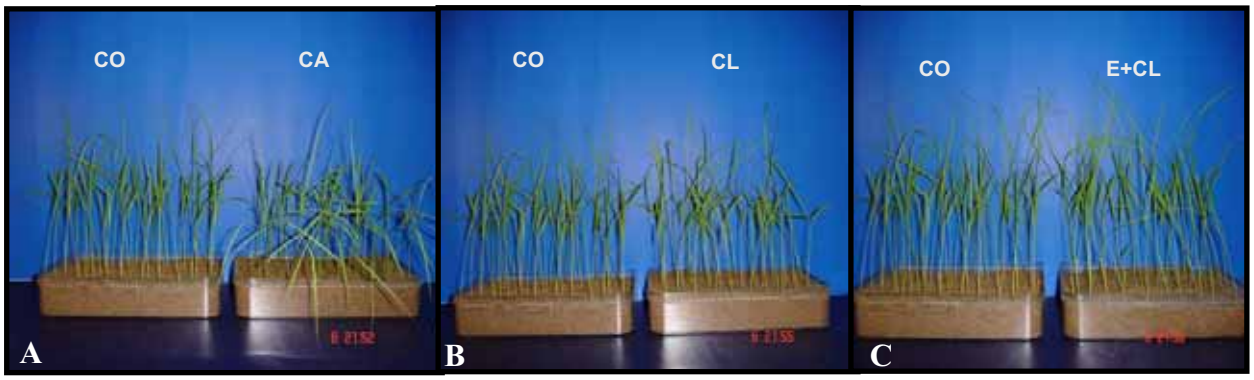


Figure 4.16: The effect of various seed coating substances on seedling germination under field condition after stored for 12 months A: Difference of CO and CA, B: Difference of CO and CL, C: Difference of CO and E+CL

Figure 4.17: The effect of various seed coating substances on seedling performance after germinating under field condition A: CO, B: CA, C: CL, D: E+CL

4.5 Discussion

In rice seed, as well as in other crops, seed properties are the most commonly used criteria for measuring seed deterioration. The results of this study have enhanced the understanding of the effect of various seed coating substances and storage time on seed quality. This experiments reported here indicated that seed lots with equal germination but different vigor result in different plant stand establishment. This finding is in agreement with Sinkkonen (2004) who found that when two wheat seed lots were exposed to fungicide (captan), the seeds with lower vigor showed reduction of emergence and seedling establishment and lower yield.

The resent study found that CA increased MC and Aw. MC is known to be influenced by two factors. Firstly, the physical process of water uptake leads to the activation of metabolic processes and the dormancy of seeds which is broken after the hydration. The first phase of water uptake by seeds involves the movement of water into the free space (apoplast) and does not depend on the osmotic potential of the surrounding solution. Secondly, the slower linear phase of water uptake involves the movement of water across cell membranes into the cells of the seed (Simon, 1984). This phase is determined by the difference between the osmotic potential of seeds and that of the medium. The increasing rate of water uptake by the CA is probably caused by the decrease of water potential

gradient between seeds and their surrounding media. This is the primary effect of CA, which induces the seed moisture content (US patent, 48121159). Moreover, the present study showed that CA treatment decreased seed germination and seedling vigor greater than the CL and E+CL treatments did. According to Bugbee (1994) captan produced Cl^- which is translocated across the cell membrane into the cytoplasm of the cells unless an active metabolic pump prevents accumulation of the ions. Cl^- in the cytoplasm can result in toxic accumulation of a particular ion or decreased availability of some essential nutrients (Bewley and Black, 1994). Additionally, the accumulation of Cl^- in the cell affected the potential of seeds to germinate and the seedling performances (Bradford, 1995). Moreover, captan contain active ingredients from the triazole family and both tetrahydrophthalimide and tetrahydrophthalic acid (Paulsen *et al*., 1981). These compounds delay or even reduce seedling performances (Lin and Sung, 2001).

In the present study, the seeds coated with CL and E+CL polymers showed long stem and the extensive root system. The results suggested that chitosan treatment might induce the activity of phytohormones inside the seeds. Normally, after seeds imbibitions, phytohormones are activated to induce seed germination and seedling development. Under chemical stress conditions it is possible thatthe activation of plant hormones in seeds is declined (Hutton *et al*., 1982). Exogenous application of phytohormones could be ameliorate the inhibitory effect of chemical stress and induce seed to germinate (Chandrkrachang, 2002). Several studies reported that chitosan polymer acting like phytohormones, i.e. auxin (Khairullin *et al*., 2002), kinetin (Bozcuk, 1981), and cytokinin (Kurosaki *et al*., 1987), which alleviated fungicide stress conditions. Furthermore, because of the increased cell division and elongation, chitosan strengthen shoot and root systems (Petrukhina *et al*., 1994). In contrast, after CA treatment, the shoot and root systems were weak. Hirano *et al*. (2000) reported that captan inhibits seedling growth by reducing both cell division and cell enlargement via directly inhibition of the production and activation of hormones in the seeds. Kabar and Baltepe (1990) supposed that cytokinin, auxin and kinetin levels could be a limitation factor under captan stress conditions. Therefore, their study explained that an exogenous application of chitosan like-phytohormone effects resulted in improved seed germination and growth of rice seedlings.

The present study found that CL and E+CL coated seeds were characterized by high GI, SGR, high shoot and root length. There is the possibility that cytokinin is the primary

factor in the initiation of radical growth. Alternatively, it may play an important role in nutrient mobilization, particularly during the germination process and seedling establishment (Scott, 1984). Staehelin *et al.* (1992) found that in soybean seeds, treated with chitosan for 24 hours, the nutritional reserve in the seed was rapidly metabolized and cytokinin was activated. As a result, the germination percentage was increased, seedling performances and resistance to chemical stress condition was better. Moreover, both GA3 and kinetin partially alleviated the effect of chemical stress conditions on seed germination and elongation of both root and coleoptile (Wanichpongpan, 2001). Kim *et al*. (2000) suggested that chitosan acted as GA3 and kinetin effect, which counteracted the fungicide stress condition by enhancing seed germination and early vegetative growth that led to better seedling growth. This was supported by other studies (Khan and Ungar, 1997b; Khan and Rizvi, 1994).

The result of our study suggested that stressful conditions like low or high temperatures, high moisture and certain fungicides affect seedling establishment. Therefore, biological coating substances could be used to ensure that the seed does not exposed to stress conditions. Moreover, in agreement with other studies (Hirano *et al.,* 2000; US patent, 6524998; US Patent 6927192), the current study found that the chitosan polymers enhanced seed germination, vigor, seedling performances and also plant regulators (act like a plant growth promoter) and produced the less phytotoxic effect to seeds than CA.

CA significantly increased the conductivity of rice seed. Most of the applied fungicides increased conductivity of seeds, which led of membrane degradation (Sung and Chiu, 1995). The present study also shows that in all seed vigor tests, CA declined seedling performances and led to the reduction of seed qualities and seedling vigor. Conversely, the applications of CL and E+CL maintain high seed qualities and seedling vigor. Many studies explained that the loss of seed viability was caused by damages of macromolecules within the embryogenic tissue, which was resulted from fungicide treatments (Villiers, 1972). However, such damages could be more after chemical than after biological seed treatment. Captan affects the repairing of the macromolecules damages in the seedlings during storage (Berjak and Villiers, 1972). In the current study an indication of the extended damages in some important seed components was given by the increase of the mean germination time during storage periods. Dalianis (1980) reported that the CA treatments reduced the number of germinating seed and inhibited seedlings growth during

storage. In contrast, the biological seed treatment could produce normal seedlings with longer shoots and roots and prolonged seed longevity than CA (Ellis and Roberts, 1980).

In CO, fungal infection is one most important factor that induces seed deterioration during storage. The previous study (Chapter 3) showed that E+CL acts as antifungal agent that can protect the seeds from fungal infection during storage. This finding was in agreement with Maffii *et al*. (1998) and Khin *et al*. (2006). Chitosan polymer has a very effective combination of attractive characteristics, i.e., stimulates plant growth, provides plant protection, and can be mixed well with fungicide agents. Therefore, chitosan could be a very useful biocontrol polymer with a good perspective for plant production systems (Struszczyk and Pospieszny, 1997; Bautista-Banos *et al*., 2003).

However, at high concentrations, essential oils might produce phytotoxicity, which affect the seed viability. Tworkoski (2002) found that essential oils from red thyme, summer savory, cinnamon, and clove were the most phytotoxic and caused electrolyte leakage resulting in cell death of rice seed when applied at high concentrations (5 to 10% v/v). Thymol, eugenol, and citronellal significantly inhibited corn seed germination and development if applying at the concentration higher than 4 %v/v (Waliwitiya *et al*., 2005). Others studies on rice (Paranagama *et al*., 2003), tomato, radish, lettuce, and wheat (Mazzafera, 2003) reported that eugenol affected the seed germination when treated at concentrations higher than 4 %v/v.

4.6 Conclusion

In order to ensure a successful rice production, a uniform and vigorous seedling is a key element. Without a good stand, the effectiveness of other agronomic inputs is drastically reduced, and usually such inputs can never compensate the negative impact of a poor stand. The current study has proved that seeds coated with biological substances showed less phytotoxic effects which indicated a good seed quality as high germination, vigor and seedling establishment. On the other hand, germination and vigor declined with increasing age of seeds. However, this study focuses only on seedling properties. It is necessary for future works to study the effects of both chemical and biological coating substances on chemical and biochemical seed properties. The results could be useful for better understanding of seed deterioration mechanisms that are affected by various coating substances.

4.7 References

Agrawal, A.A. (2002). Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. Ecology. 83, 3408-3415.

Anonymus. (2000). Natural Chemical Product Research and Technology Institute in Agriculture. Pli Bai Newsletter, Nov. vol. 10. Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

Anonymus. (2004) Available at http://home.tonline.de/home/hydrobio.hw/berms.html. Visited at 01.11.2004.

Basra, S.M.A., Farooq, M., and Tabassum, R. (2005). Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (*Oryza sativa* L.). Seed Science and Technology. 33, 623-628.

Bautista-Banos, S., Hernandez-Lopez, M., Bosquez-Molina, E., Wilson, C.L. (2003). Effects of chitosan and plant extracts on growth of Colletotrichum gloeosporioides, anthracnose levels and quality of papaya fruit. Crop Protect. 22, 1087–1092.

Berjak, P., and Villiers, T.A. (1972). Ageing in plant embryos. V. Lysis of the cytoplasm in non-viable embryos. New phytologist.71, 1075-1079.

Bewley, D.J., Black, M. (1994). Seeds: Physiology of Development and Germination. New York: Plenum Press.

Bozcuk, S. (1981). Effect of chitosan like kinetin effect on germination of tomato, barley and cotton seeds. Ann Bot, 48, 81-84.

Bradford, J.K. (1995). Water relations in seed germination. In: Kigel J., Galili G., eds. Seed Development and Germination. New York: Marcel Dekker, Inc., 351-396.

Bugbee, B., Spanarkel, B., Johnson, S., Monje, O., and Koerner, G. (1994). Crop Growth Enhancement and Toxicity in Wheat and Rice. Advances in Space Research 14, 257-267.

Chandrkrachang, S. (2002). The applications of chitin and chitosan in agriculture in Thailand. in: K. Suchiva, S. Chandrkrachang, P. Methacanon, M.G. Peter (Eds.). Advances in Chitin Science. 5, 458–462.

Dalianis, C.D. (1980). Effect of temperature and seed size on speed of germination, seedling elongation and emergence of berseem and Persian clovers (*Trifolium alexandrinum* and *T. resupinatum*). Seed Science and Technology. 8, 323-331.

Ellis, R.H., and Robert, E.H. (1980). Improved equations for the prediction of seed longevity. Annals of Botany. 45, 13-30.

Ester, A., De Putter, H., and Van Bilsen, J.G.P.M. (2003). Film coating the seed of cabbage (*Brassica oleracea* L. convar. Capitata L.) and Cauliflower (*Brassica oleracea* L. var. Botryti L.) with imidacloprid and spinosad to control insect pests. Crop Production. 22, 761-768.

Farooq, M., Basra, S.M.A., Kareem, H.A., and Afzal, I. (2004). Optimization of seed hardening techniques for rice seed invigoration. Emirates J Agric Sci. 16, 48-57.

Farooq, M., and Basra, S.M.A. (2006). Priming of field-sown rice seedb enhance germination,

seedling establishment, allometry and yield. Plant Growth Regul. 49, 285-294.

Hirano, S., Hayashi, M., and Okuno, S. (2000). Soybean seeds surface-coated with depolymerised chitins: chitinase activity as a predictive index for the harvest of beans in field culture. J. Sci. Food Agric. 81, 205-209.

Hutton, M.J., Van Staden, J. and Davey, J.E. (1982). Cytokinins in germinating seeds of *Phaseolus vulgaris* L. I. Changes in endogenous levels within the cotyledons. Ann. Bot. 49, 685-691.

International Seed Testing Association (ISTA). (2006). International Rules for seed testing. Seed Science and Technology, 21 (Supplement).

Kabar, K., Baltepe, S. (1990). Effect of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on the germination of Barley and Lettuce seeds. Phyton. 30, 65-74.

Khairullin, R.M., Akhmetova, I.E., and Yusupova, Z.R. (2002). Inhibition of IAA-induced growth of wheat coleoptile fragments by chitin-chitosan oligomers. Biology Bulletin. 29(2), 135-138.

Khan, I., Khan, M.A. and Khizar, T. (1976). Plant growth regulators from species differing in salt resistance as affected by soil salinity. Plant Soil. 45, 267-271.

Khan, M.A., Rizvi, Y (1994). Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. stocksii. Can. J. Bot. 72, 475-479.

Khan, M.A., Ungar, I.A. (1997b). Alleviation of seed dormancy in the desert for *Zygophyllum simplex* L. from Pakistan. Ann. Bot. 80, 395-400.

Khin, L.N., Nitar N., Chandrkrachang, S., and Willem, F.S. (2006). Chitosan as a growth stimulator in orchid tissue culture. Plant Science. 170, 1185–1190.

Kim, Y.S., Lee, J.H., Yoon, G.M., Cho, H.S., Park, S.W., Suh, M.C., Choi, D., Ha, H.J., Liu, J.R., and Pai, H.S. (2000). CHRK1, a Chitinase Related Receptor-Like Kinase in Tobacco. Plant Physiology. 123, 905–915.

Kurosaki, F., Tashiro, N., and Nishi, A. (1987). Chitinase induction in carrot cell culture treated with various chitosan components. Biochem Int. 20, 99-106.

Lin, J.M., and Sung, J.M. (2001). Pre-sowing treatments for improving emergence of bitter gourd seedlings under optimal and sub-optimal termperatures. Seed Science and Technology, 29, 39-50.

Maffii, D., Bassi, M., Brambillai, A., and Conti, C.G. (1998). Possible role of chitosan in the interaction between barley and *Erysiphe graminis* after tetraconazole treatment. Mycol. Res. 102 (5), 599-606.

Mazzafera, P. (2003). effect of the alcoholic extract of eugenol on seed germination. Revista Brasil. Bot., 26 (2), 231-238.

Mungkornasawakul, P., Pyne, S.G., Jatisatienr, A., Supyen D., Lie W., Ung, A.T., Skelton, B.W. and White, A.H. (2003). Stemocurtisine, the First Pyridol [1, 2-a] azaepine Stemona Alkaloid. J. Nat. Prod. 66, 980-982.

Neergaard, P. (1997). Seed Pathology, Vols. I, II. John Wiley&Sons, NY.

Paranagama, P., Abeysekera, T., Nugaliyadde, L., and Abeywickrama, K. (2003). Effect of the essential oils of *Cymbopogon citratus, C. nardus* and *Cinnamomum zeylanicum* on pest incidence and grain quality of rough rice (paddy) stored in an enclosed seed box. Food, Agriculture & Environment. 1(2), 134-136.

Paulsen, M.R., Nave, W.R., and Gray, L.E. (1981). Soybean quality as affected by impact damage. Transactions of the ASAE. 24(6), 1589. 1577-1582.

Petrukhina, M.T., Maksimov, V.I., Bordak, M.M., and Luntsevich, V.G. (1994). Chitodextrin as a promising regulator of plant growth. Ecologically Safe and Pesticide-Free Technologies of Plant Production. Materials of National Conference. 299-231.

Scott, T.K. (1984). Hormonal regulation of development. II the functions of hormones from the level of the cell to whole plant. In Encyclopedia of Plant Physiology. New Series. Berlin: Springer-Verlag, 180-185.

Silva, F.M.A., Melo, P.C., and Oak, R.L. (1996). Effect of insecticide in the germination and vigor of maize seeds (*Zea mays* L.) at two times of storage. In National Congress of Maize and Sorgo. 21. Summaries. Native of London: IAPAR. 276.

Simon, E.W. (1984). Early events in germination. In: Murray, D.R. Ed. Seed Physiology. Australia: Academic Press.

Sinkkonen A. 2004. A model describing chemical interference caused by decomposing residues at different densities of growing plants. Plant and Soil. October (29), 315-322.

Staehelin, C., Mueller, J., Mellor, R.B., Wiemken, A., and Boller, T. (1992). Chitinase and peroxidase in effective and ineffective soybean nodules. Plant 187, 295-300.

Struszczyk, H., and Pospieszny, H. (1997). New applications of chitosan and its derivatives in plant protection. in: M.F.A. Goosen (Ed.), Applications of Chitin and Chitosan, Technomic Publishing Co., Inc., USA. 171–184.

Sung, J.M., and Chiu, C.C. (1995). Lipid peroxidation and peroxide-scavenging enzymes of naturally aged soybean seed. Plant Science. 11, 45-52.

Tworkoski, T. (2002). Herbicide effects of essential oils. Weed Science. 50 (4), 425–431.

US Patent 4812159. Plant growth regulators derived from chitin**.** Available at http://www.freepatentsonline.com/4812159.html. Visited at 27/04/07.

US Patent 6524998. Biological compositions and methods for enhancing plant growth and health and producing disease-suppressive plants. Available at http://www.patentstorm.us/patents/6524998-claims.html. Visited at 27/04/07.

US Patent 6927192. Process to improve the quality of grains and seeds. Available at. http://www.patentstorm.us/patents/6927192-description.html. Visited at 27/04/07.

Villiers, T.A. (1972). Ageing and longevity of seeds in field conditions. In *Seed Ecology*, (ed. W. Heydecker), 265-286. Butterworths, London.

Von Pine, E.V.R., Silveira, J.F., Vieira, M.G.G.C. (1995). Influence of the size and the treatment of maize seeds in the preservation of the quality during the storage and posterior behavior in the field. Cultivate and Practical science. 19(1), 30-36.

Waliwitiya, R., Isman, M.B., Vernon, R.S., and Riseman, A. (2005). Insecticidal activity of selected monoterpenoids and rosemary oil to *Agriotes obscurus* (Coleoptera: Elateridae). Journal of Economic Entomology. 98(5), 1560–1565.

Wanichpongpan, P., Suriyachan, K., and Chandrkrachang, S. (2001). Effects of chitosan on the growth of Gerbera flower plant (Gerbera jamesonii).bin: T. Uragami, K. Kurita, T. Fukamizo (Eds.). Chitin and Chitosan in Life Science. 198–201.

Yildirim, E., And Hoy, C.W. (2003): Cyromazine Seed Treatments to Control Onion Maggot, *Delia antiqua*, on Green Onions J. Econ. Entomol. 96, 1494-1499.

5. The effects of seed coating substances on chemical and biochemical properties of rice seeds

5.1 Abstract

Fungicides may cause phytotoxic induced seeds deterioration. Thereafter, during storage a number of biochemical and physiological changes occur. The natural ageing process, which is caused by deterioration moves consequently toward seed death. Loss of viability is associated with disturbances of the cell membranes, nutrients losses and losses of enzyme activities. The aim of this study was to investigate the effects of CA, CL and E+CL seed coating substances on chemical and biochemical properties of rice seeds, which have been studied during storage. The results indicated that CA significantly affected the rice seed viability and the associated deterioration. After 12 months storage, total protein content decreased accompanied by declined of lipid content, increased FFA and activated LOX. In the case of biological fungicide coated seeds, the antioxidative scavenging enzymes as APX and SOD and a high antioxidant activity protected them. Moreover, the sugar content was positive correlated with seed germination and vigor. The biological treated seeds were found to maintain high seed quality throughout the storage period, because they could maintain high sugar contents inside the seeds. In contrast, under fungicide stress (CA), those compounds were lost that directly affected seed vigor during storage. This results support the idea that sucrose may serve as the principal agent of stress tolerance in the seeds, whereby the larger oligosaccharides serving to keep the sucrose from crystallizing.

5.2 Introduction

Rice seeds are attacked by many pathogenic fungi, which is a major cause leading to seed deterioration and degradation of rice grain qualities (Hewett, 1987). Treating seeds with fungicide to protect the seed from those organisms can improve stand quality, increase yields, and increase return on investment (Kaiser, 1987). Although, fungal pathogen normally lives in closed association with the seed, so it is difficult to find chemicals that kill the fungus without harming the seeds. The toxicity of fungicide was ascribed to produce phytotoxic compounds that induced seed deterioration (Milka *et al*., 1976). Rudrapal and Basu (1981) reported that chlorine, bromine, and iodine in fungicide compounds induced physiological deterioration of seeds. In seeds treated with the

fungicide chlorine, a loss of cell membrane function was found which might be the main reason of high respiration and subsequently, deterioration of the seeds (Tappel, 1973).

Storage life of seeds depends on varieties, conditioning, treatment, and storage conditions, and most losses resulting from infestation by microorganisms (FAO, 1997). The metabolic changes occurring in the early stages of germination are the result of the activity of various enzymes, which are either present in the dry seed or very rapidly become active as the seed imbibes water. Generally, during germination, the increase of enzyme activities, which break down starch, protein, lipids, and other storage materials, is essential for the germination process (Simon, 1984). Metabolic processes that occur upon imbibition are complex. They include three processes. First, the reserve compounds are broken down and then they are transported from one part to the other parts of the seed. Finally, new compounds are synthesized during growth. The large amounts of soluble sugars present in germinating seeds are apparently the result of the breakdown of reserve carbohydrates, such as starch and oligosaccharides (Salisbury and Ross, 1985). The ratio between the various sugars and oligosaccharides changes because of the activity of the enzymes discussed above (Perez *et al*., 1975). Most of seed proteins are metabolically inactive and serve merely as food reserves used by the growing embryo during germination. Protein synthesis begins in the various embryonic organs immediately with the beginning of their growth. Thus, the dry seed is a well functional unit, which can carry out a large number of biochemical reactions if placed into aquatic medium (Mayer, 1977).

Biochemical and physiological changes during storage are commonly termed ‘aging’. The natural aging process caused by deterioration, lead inevitably toward death. Deterioration cannot be reversed or eliminated, once it has occurred. A smaller but quite significant proportion of the total loss results from respiration and gradual deterioration of viability, nutritive quality, and the germination. The aged seed with loss of viability is associated not only with disturbances of the cell membranes, but also with biochemical nutrients losses. Changes in their compositions and losses of enzymes activities is a cause of rice seed deterioration (Pomeranz and Meloan, 1987). Thus, the aim of this study was to analyze the effects of seed coating substances on rice seed deterioration via biochemical changes during storage.

5.3 Materials and methods

5.3.1 Plant material and experimental design

Dry graded rice seeds (*Oryza sativa* L. cv. KDML 105) from one seed lot were supplied by Bureau of Seed Multiplication of Thailand. The seeds were submitted to three seed treatment variants and different storage durations. Chemical and biochemical properties of the seeds were determined during 12 months stored. The split-plot design with four replications was applied. The main plot was seed treatments; captan (CA), only chitosan lignosulphonate polymer (CL) eugenol incorporated into chitosan lignosulphonate polymer (E+CL) and control (CO). The sub-plot was the storage duration (12 months). The seeds of each treatment were randomly stored in plastic bag sealed in an incubation chamber, (KPB6395FL, Termaks, S/N 2-858 Germany), which controlled temperature at 30±2°C, and relative humidity at 40±5 %. Seeds were immediately examined, then, every month for 12 months.

5.3.2 Determination of the chemical composition

All experiments were done using dehulled grains with 12 ± 0.5 % MC. Freeze-dried rice seeds were ground (UDY, Cyclone Sample Mill No.2).

5.3.2.1 Determination of total protein content

The protein content was analyzed according to Kjeldahl method (AOAC, 2000). First, 4 ml of sulfuric acid and the catalyst (K_2SO_4: $CuSO_4.5H_2O$: Se; 10:1:0.1) was added to 0.25 g of ground rice seed. The sample was digested at 370°C. After complete the digestion, the solution was distilled using Kjeldahl Protein Analyzer, into a receiving flask containing boric acid indicator. The distilled sample was titrated by 0.1 N sulphuric acid. The protein content of the sample was obtained from the multiplying the nitrogen determined by 6.25.

$$\text{Total nitrogen content} = \frac{(\text{ml HCl} - \text{ml blank}) \times \text{molarity} \times 14.007 \times 100}{(\text{mg of sample})}$$

Total protein content (g $100g^{-1}$DM) = Total nitrogen content × 6.25

5.3.2.2 Determination of total lipid and free fatty acid (FFA) content

According to Lam and Proctor (2000), the gravimetric method was used to determine the total lipid content. Lipid content was extracted by vortexing 10 g of rice sample with 4 ml of isopropanol for 5 min. Then, added 5 ml of isopropanol into the sample and vortexed for

5 min. After that the extract was centrifuged at 2500 rpm for 10 min. The weight of extracted lipids was determined after evaporating the solvent on an electric hot plate at 40°C.

According to Walde and Nastruzzi (1991), FFA of the sample was determined by preparing an assay solution, which contained 0.375 ml of solution A (0.1 M tris/HCL; pH 9.0), 0.125 mlof solution B (2 mM phenol red in 0.1 M tris/HCl; pH 9.0), and 0.5 ml of solution C (50 mM Bis (2-ethylhexyl) sodium sulfosuccinate in isooctane). Then, 30 µL of isopropanol extract was mixed with 1 ml of assay solution in a 1-cm wide cuvette and was shaked for one minute before measuring absorbance at 560 nm. FFA of each extract was obtained from a calibration curve. The calibration curve was prepared by dissolving oleic acid in isopropanol to produce oleic acid solution of 0.001 to 0.02 %(w/w).

5.3.2.3 Determination of total ash content

Total ash content was measured by the standard method of AOAC (2000). Five grams of ground rice seed were placed in a tared silica dish. The sample was transferred to a muffle furnace and ash at a temperature of 550±10°C for 12 hrs. After the sample was heated at 550 ± 10°C for 30 min, it was cooled down in a desiccator. The sample was weighed. This heating, cooling down and weighing until the difference between two successive weights is less than 1 mg. The lowest weight was recorded and the ash content was calculated by the following equation:

$$\text{Total ash (\% on dry weight)} = \frac{(W2 - W) \times 100 \times 100}{(W1 - W) \times (100 - M)}$$

W1 = Weight in grams of Silica dish + Sample, W2 = Weight in grams of Silica dish + ash, W = Weight in grams of empty Silica dish, M = Moisture percentage of the sample

5.3.2.4 Determination of carbohydrate content

The carbohydrate content of the samples was calculated by residual weight after subtracting all amounts of fat, protein, water, and ash found in the nutrient chemical analysis with the following formula (Merrill and Watt, 1973):

Total carbohydrate = 100 - (protein + fat + water + ash) in 100 g of food

5.3.2.5 Determination of sugar content

Sugar content was determined by iodine titration of excess copper of Luff-Schoorl method (Alexander *et al*., 1985). Sample solution preparation: 5 g of ground sample was accurately weighed to nearest 1 mg and transferred to a 250 ml- volumetric flask, which contained 200 ml distilled water. Five milliliters of Carrez solution I, (21.795 g of zinc acetate dehydrate ($Zn(CH_3COO)_2.2H_2O$) and 3 ml of glacial acetic acid were dissolved and made up to 100 ml with distilled water) and 5 ml of Carrez solution II, (10.76 g of potassium hexacyanoferrate II trihydrate ($K_4[Fe(CN)_6].3H_2O$) in water and make up to 100 ml with water), were mixed after each addition and made up to 250 ml with water and mix well.

Luff-Schoorl reagent preparation: citric acid solution (50 g of citric acid dihydrate ($C_6H_8O_7.2H_2O$) in 50 ml of water) was added to sodium carbonate solution (143.78 g of anhydrous sodium carbonate in 300 ml of warm water and allow to cool) in a one-liter volumetric flask with gentle swirling. Then, copper II sulphate pentahydrate solution (25 g of $CuSO_4.5H_2O$ in 100 ml of water) was added and made up to 1000 ml with water. The solution was allowed to stand overnight and then filtered.

Twenty-five milliliters of Luff-Schoorl reagent and 25 ml of sample solution were transferred into 250 conical flasks and extracted at 80°C in ultrasonic bath (Bandelin, Germany) for 10 min. After that, the sample was immediately cooled for 5 min. Then, 10 ml of 30 %(w/v) potassium iodide solution were added, and immediately followed by 25 ml of 3 M sulphuric acid adding. Titration was done with 0.71 M sodium thiosulphate solution until the solution was almost colorless, then added a few milliliters of starch indicator (5 g of soluble starch; Sigma, St. Louis, MO, USA.) slurred in 30 ml of water, boil for 3 min, allowed the mixture to cool and added water to make the 1 L solution). The titration was continued until the blue color disappears.

Finally, sugar content was calculated from the standard table by using the weight of glucose or the weight of invert sugar in percentage corresponding to the difference between the two-titration readings, expressed in milliliter of 0.71 M sodium thiosulphate. Express the results in terms of invert sugar or D-glucose as percentage of the dry matter.

5.3.2.6 Determination of total antioxidant activity

Total antioxidant activity was determined by the diphenylpicrylhydrazyl (DPPH) radical scavenging effect according to Kim *et al*. (2002). To obtain the concentration of crude methanolic extract, one gram of ground rice seed sample was dissolved in 50 ml methanol (Merk, Germany) and the ultrasonic solvent extraction was carried out in an ultrasonic bath (Bandelin, Germany) for one hour. The sample solution was then filtered. The filtrate was evaporated with rotary evaporator (Buchi, Switzerland) at 40°C until dried. Crude extract was then weighed and calculated back to obtain the concentration in mg (crude) per ml (methanol).

To determine the antioxidant activity, three milliliters of methanol solution of each treatment at various concentrations (1.5-45 mg ml^{-1}) were added to 1 ml of $1x10^{-4}$ M DPPH (Fluka, Germany) in methanol. The reaction mixture was shaken vigorously. After leaving the mixture at the room temperature for 30 min, the optical density was measured at 520 nm using a UV-VIS spectrophotometer (SPECCORD 40). The radical scavenging activity of each sample was expressed by the ratio of lowering of the absorption of DPPH (%) and the absorption (ASB) (100%) of DPPH solution in the absence of test sample (control). The mean value was obtained from four replication experiments. The percent inhibition was calculated by the following equation:

$$\text{Inhibition percentage} = \frac{1 - \text{mean of sample ASB}}{\text{mean of control ASB}} \times 100$$

The antioxidant activity of each sample was expressed in terms of fifty percent of effective concentration (IC_{50}) value, which required inhibition of DPPH radical formation by 50%. The IC_{50} value was calculated by plotting the inhibition percentage of each tested concentrations in the logarithmic graph. A logarithmic equation was used to calculate IC_{50} value.

5.3.2.7 Determination of ascorbate peroxidase antivity (APX)

Enzyme extraction: 5.0 g of rice powder was weighed into a centrifuge tube. Five milliliters of extraction buffer (0.1 M phosphate buffer, pH 6.0) were added and mix well on shaker for 15 min. The mixture was then centrifuged at 12 000 rpm at 4°C for 30 min.

Supernatants were used for enzyme assays. All steps of extraction procedure were carried out at 1-4°C.

Soluble protein content of the extracts was determined by the method of Bradford (1976), which used a Bio-Rad protein assay kit with bovine serum albumin (Sigma, St. Louis, MO, USA.) as the calibration standard (150-900 μg ml^{-1}) at 595 nm. Fifty micro- litters of extracted solution added to 2.5 ml Biorad before it was vortexed for 5 min. Then the mixture was shaken for one minute before measuring the absorbance at 595 nm.

APX was determined according to Nakano and Asada (1981), with some modifications. The reaction mixture (2.0 ml) contained 0.05 M phosphate buffer (pH 7), 0.1 mM EDTA, 5 mM ascorbate, and 800 μL of crude enzyme extract. The reaction was initiated by adding 200 μL H_2O_2 (2 mM). The decreasing of absorbance from the oxidation of ascorbate at 290 nm was recorded using a spectrophotometer (Hewlett Packard 8453, Germany) at 1,200 s after the adding H_2O_2. The activity of APX was expressed as μmole min^{-1} 100 mg^{-1} protein.

5.3.2.8 Determination of superoxide dismutase activity (SOD)

The method of Oberley and Spitz (1985), with some modifications was used to determine the activity of SOD. One milliliter of reaction mixture for the determination of SOD activity contained 800 μL of reaction mixture (0.1 mM Xanthine, 0.056 mM NBT, 1.0 mM DETAPAC, and 1 U CAT in 0.05 M phosphate buffer (pH 7.8)), 100 μL of enzyme extracted, and 100 μL of 0.1 U/ml xanthine oxidase. SOD was evaluated by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction was initiated by illuminating the reaction mixtures at 26-28°C for 30 min and absorbance was read at 590 nm. One unit of SOD was defined as the enzyme activity that inhibited photoreaction of NBT to blue formation by 50%. SOD activity of the extracts was expressed as Δ activity mg^{-1}protein.

5.3.2.9 Determination of lipoxygenase activity (LOX)

Enzyme extraction: 3.0 g of rice powder from the seeds subjected to various duration of ageing were homogenized with 5 ml of extraction buffer (0.05 M sodium borate buffer, pH 9.0) and shaken for 30 s. The mixture was centrifuge at 12 000 rpm at 26-28°C for 5 min. For LOX assay, 5 ml of 96 %(v/v) Ethanol were added and incubated at 26-28°C for 15 s,

then filtered. Afterwards, 3 ml of 70 %(v/v) Ethanol were added to 50 µL of extract solution and absorbance was read at 234 nm using 70 %(v/v) Ethanol for blank. LOX activity of the extracts was expressed as Δ activity mg^{-1} protein (Meshehdani, 1990).

5.3.3 Calculation and statistical analysis

The data are presented as mean ± standard deviation. The analysis of variance was performed for data analysis and differentiated with LSD test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

5.4 Results

The ANOVA results indicated that seed treatment and storage time influenced the total protein content in the seeds but interaction between them had no significant effect (Table 5.1). The loss of total protein content in CA was significantly different when compared with CO, CL and E+ CL treatments (Table 5.2). Storage decreased significantly the total protein content in all treatments (Appendix 4, Table 5.6).

The storage time significantly influenced the total lipid content (Table 5.6) but seed treatment variants and the interaction between them were not significant different (Table 5.1, Table 5.2).

The seed treatment variants and storage duration had a significant effect on FFA in the seeds (Table 5.1, Table 5.6). CA treatment significantly increased FFA in the seeds when compared with CL and E+CL (Table 5.3). Appendix 6 indicated that during storage the FFA in the seeds increased significantly in all seed treatments.

Total carbohydrate content was significantly influenced by the seed treatment variants and storage duration (Table 5.1, Table 5.6). At the beginning of storage (0 month), CA treatment showed higher total carbohydrate content than CO, CL and E+CL treatments (Table 5.2) did. Appendix 7 indicated that during storage the carbohydrate content in the seeds decreased significantly in all seed treatments.

The ANOVA results indicated that seed treatment variants, storage duration and the interaction between them influenced glucose, sucrose and lactose content in the seeds. However, the seed treatment variants showed no effect on the seed maltose content, but the storage duration was the main impact factors of maltose content (Table 5.1, Table 5.6). As

shown in Table 5.4, at the beginning of storage (0 month), glucose, sucrose and lactose content significantly decreased in CA treatment. When compare with the CL and E+CL, it provided significantly different results. After 12-months storage, the content of glucose, sucrose, and lactose in CA treated seeds tended to be lower than in CO, CL and E+CL treated seeds. The effect of seed treatment variants and storage duration on sugar content in the seeds is shown in Appendices 8-11.

Seed treatment variants, storage duration as well as the interaction between them had significant effects on LOX, APX and SOD (Table 5.1). The seed treatment variants influenced APX and SOD. Especially in CA treatment, the lowest activity of both enzymes was measured. CL and E+CL treatments had no significant effect on both enzyme activities when compared with CO (Table 5.5). APX and SOD were significantly affected by the storage duration (Table 5.7). Appendices 12 and 13 indicated that during storage the activity of both enzymes decreased significantly in all treatments. LOX was significantly higher in the CA treated seeds when compared with CO, CL and E+CL treatments (Table 5.5). Moreover, LOX was significantly affected by the storage duration. The LOX activity significantly decreased when the storage duration was increased (Table 5.7). Appendix 14 indicated that the storage duration had a significant effect on LOX in all seed treatment variants.

The seed treatment variants, storage duration and their interaction had a significant effect on the antioxidant activity (Table 5.1.). Total antioxidant activity showed significant differences among the treatment variants. CA treated seeds showed significantly decrease of total antioxidant activity while the CL and E+CL treated seeds showed no significant difference when compared with CO (Table 5.3). The total antioxidant activity was also found to be significantly affected by the storage duration (Table 5.7). Appendix 15 indicated that the storage decreased significantly the total antioxidant activity in all seed treatments.

Table 5.1: Analysis of variance (ANOVA) for seed treatments and storage time on protein, lipid, free fatty acid (FFA), carbohydrate (Carb), glucose (Glu), sucrose (Suc), lactose (Lac), maltose (Mal), lipoxygenase (LOX), superoxide dismutase (SOD), ascorbate peroxidase (APX), and total antioxidant activity (Antiox)

Source of variation	DF	Statistic significant (P)											
		Protein	Lipid	FFA	Carb	Glu	Suc	Lac	Mal	LOX	SOD	APX	Antiox
Treatment (T)	3	*	NS	*	*	*	*	*	NS	*	*	*	*
Storage time (S)	12	*	*	*	*	*	*	*	*	*	*	*	*
T x S	36	NS	NS	NS	NS	*	*	*	NS	*	*	*	*

*: significant different at the 0.05 level of probability, NS: not significant different

Table 5.2: The effect of seed treatment variants on total protein, carbohydrate, and lipid content changes during storage for 12 months

Treatment	Mean ± SD*					
	Total protein (g $100g^{-1}$DM)		Carbohydrate (g $100g^{-1}$DM)		Lipid (g $100g^{-1}$DM)	
	0 month	12 months	0 month	12 months	0 month	12 months
CO	7.84±0.18a	6.88±0.04a	89.10±0.22ab	90.22±0.39a	2.65±0.09a	2.15±0.02a
CA	7.60±0.08b	6.39±0.51b	89.25±0.47a	90.30±0.22a	2.72±0.49a	2.21±0.15a
CL	7.87±0.05a	6.95±0.05a	88.75±0.16b	90.23±0.21a	3.00±0.19a	2.31±0.04a
E+CL	7.96±0.19a	6.88±0.02a	88.76±0.29b	90.27±0.21a	2.92±0.17a	2.22±0.26a

*: The different letters indicate the statistically significant difference by LSD at 5% level.

Table 5.3: The effect of seed treatment variants on free fatty acid and antioxidant activity changes during storage for 12 months

Treatment	Mean ± SD*			
	Free fatty acid (g $100g^{-1}$DM)		Antioxidant activity (IC_{50})**	
	0 month	12 months	0 month	12 months
CO	0.0362±1.15E-04ab	0.0381±4.16E-04ab	0.1177±8.89E-03ab	0.9662±0.03a
CA	0.0370±5.77E-05a	0.0384±4.00E-04a	0.1260±2.50E-03a	0.8934±0.07ab
CL	0.0355±1.21E-03bc	0.0369±1.03E-03b	0.1145±4.80E-03b	0.8093±0.06b
E+CL	0.0349±4.16E-04c	0.0372±3.46E-04b	0.1174±2.51E-03ab	0.8256±0.07b

* The different letters indicate the statistically significant difference by LSD at 5% level. **IC_{50} was expressed in terms of the fifty percent of effective concentration (IC_{50}) value, which required inhibiting DPPH radical formation by 50%. This IC_{50} parameter has the drawback value with the total antioxidant activity.

Table 5.4: The effect of seed treatment variants on glucose, sucrose, lactose, and maltose content changes during storage for 12 months

Treatment	Mean ± SD*							
	Glucose (g 100g^{-1}DM)		Sucrose (g 100g^{-1}DM)		Lactose (g 100g^{-1}DM)		Maltose (g 100g^{-1}DM)	
	0 month	12 months	0 month	12 months	0 month	12 months	0 month	12 months
CO	6.42±0.29ab	4.12±0.72a	3.21±0.14ab	2.05±0.36a	9.51±0.40b	6.23±1.09a	10.16±0.44a	6.58±1.13a
CA	6.06±0.82b	2.97±5.77E-0.3b	3.03±0.44b	1.83±0.17a	9.89±0.40b	4.50±0.02b	10.97±1.03a	5.13±0.60a
CL	9.10±2.77ab	3.54±0.19ab	4.55±1.39ab	1.76±0.10a	11.82±1.76ab	5.34±0.29ab	13.85±4.41a	5.67±0.32a
E+CL	9.44±1.86b	4.11±0.80a	4.72±0.93a	2.05±0.40a	13.59±1.59a	6.20±1.20a	14.52±3.04a	6.59±1.26a

*: The different letters indicate the statistically significant difference by LSD at 5% level.

Table 5.5: The effect of seed treatment variants on ascorbate peroxidase (APX), superoxide dismutase (SOD), and lipoxygenase (LOX) enzymes activity changes during storage for 12 months

Treatment	Mean ± SD					
	APX (µmol min^{-1} 100 mg-1 protein)		SOD (∆activity mg^{-1}protein)		LOX (∆activity mg^{-1}protein)	
	0 month	12 months	0 month	12 months	0 month	12 months
CO	0.88±0.10a	0.05±0.02ab	3.52±0.14a	1.86±0.28a	0.0226±1.23E-03b	0.0059±8.02E-04ab
CA	0.82±0.06b	0.03±0.01b	3.47±0.13b	1.33±0.28b	0.0358±3.21E-03a	0.0072±1.79E-03a
CL	0.89±0.07a	0.07±0.02a	3.60±0.11a	2.07±0.02a	0.0204±1.41E-03b	0.0038±1.27E-03b
E+CL	0.88±0.04a	0.07±3.53E-03a	3.60±0.08a	2.09±0.04a	0.0195±2.12E-03b	0.0042±1.40E-03b

* The different letters indicate the statistically significant difference by LSD at 5% level.

Table 5.6: The effect of storage duration on total protein, lipid, free fatty acid (FFA), carbohydrate, glucose, sucrose, lactose and maltose changes*

Storage duration (month)	Mean ± SD**							
	Protein (g $100g^{-1}$DM)	Lipid (g $100g^{-1}$DM)	FFA (g $100g^{-1}$DM)	Carbohydrate (g $100g^{-1}$DM)	Glucose (g $100g^{-1}$DM)	Sucrose (g $100g^{-1}$DM)	Lactose (g $100g^{-1}$DM)	Maltose (g $100g^{-1}$DM)
0	8.38±0.67a	2.82±0.29a	0.0359±9.90E-4c	88.97±0.36d	7.76±2.17a	3.88±1.09a	11.20±1.99a	12.37±3.03a
4	7.91±0.41b	2.68±0.21a	0.0363±7.63E-4bc	89.27±0.25c	5.86±0.27b	2.93±0.14b	8.75±0.39b	9.33±0.43b
8	7.65±0.39c	2.38±0.15b	0.0368±7.94E-4b	89.65±0.25b	5.20±0.57b	2.60±0.28b	8.51±1.03b	8.15±1.76b
12	6.77±0.32d	2.22±0.14c	0.0377±8.18E-4a	90.25±0.24a	3.68±0.68c	1.92±0.28c	5.57±1.02c	5.99±1.01c

* The data compared from each treatments and mean separation in a column by LSD at 5% level.
** The different letters indicate the statistically significant difference by LSD at 5% level.

Table 5.7: The effect of storage duration on ascorbate peroxidase (APX), superoxide dismutase (SOD), lipoxygenase (LOX) activity and total antioxidant activity changes*

Storage duration (month)	Mean ± SD**			
	APX (µmole min^{-1} 100 mg^{-1} protein)	SOD (Δactivity mg^{-1}protein)	LOX (Δactivity mg^{-1}protein)	Antioxidant activity (IC_{50})
0	0.8721±0.07a	3.55±0.12a	0.0246±7.10E-3a	0.1189±0.07a
4	0.6374±0.03b	3.02±0.11b	0.0173±2.76E-3b	0.1852±001b
8	0.4394±0.04c	2.33±0.07c	0.0117±1.92E-3c	0.3099±0.02c
12	0.0565±0.02d	1.84±0.36d	0.0053±1.83E-3d	0.8736±0.08d

* The data compared from each treatments and mean separation in a column by LSD at 5% level.
** The different letters indicate the statistically significant difference by LSD at 5% level.

Table 5.8: Pearson correlation coefficients of seed coating substances on chemical and biochemical properties of rice seeds**

	MC	Inf	Pro	Lipid	FFA	Carb	Glu	Suc	Lac	Mal	LOX	SOD	APX	Antiox	Germ	SL	RL	SDw	GI	SGR	Brix	Cond
Aw	0.90*	0.56	-0.67*	-0.74*	0.65*	0.81*	-0.82*	-0.81*	-0.81*	-0.75*	-0.73*	-0.88*	-0.91*	0.88*	-0.86*	-0.93*	-0.93*	-0.59*	-0.81*	-0.77*	-0.90*	0.82*
MC		0.53	-0.68*	-0.73*	0.67*	0.86*	-0.77*	-0.73*	-0.78*	-0.71*	-0.72*	-0.82*	-0.92*	0.95*	-0.93*	-0.90*	-0.94*	-0.51*	-0.78*	-0.78*	-0.94*	0.81*
Inf			-0.48	-0.59	0.29	-0.49	-0.62	-0.63	-0.54	-0.52	0.75	-0.62	-0.68	0.61	-0.54	-0.60	-0.58	-0.45	-0.55	-0.53	-0.58	0.55
Pro				0.71*	-0.76*	-0.64*	0.63*	0.57*	0.60*	0.55*	0.35*	0.71*	0.67*	-0.63*	0.72*	0.69*	0.73*	0.68*	0.84*	0.74*	0.74*	-0.56*
Lipid					-0.61*	-0.80*	0.68*	0.67*	0.62*	0.62*	-0.62*	0.83*	0.80*	-0.73*	0.73*	0.79*	0.80*	0.56*	0.81*	0.75*	0.79*	-0.80*
FFA						0.65*	-0.52*	-0.49*	-0.48*	-0.43*	0.25*	-0.63*	-0.61*	0.58*	-0.66*	-0.66*	-0.70*	-0.54*	-0.77*	-0.75*	-0.72*	0.55*
Carb							-0.69*	-0.67*	-0.67*	-0.63*	-0.65*	-0.77*	-0.86*	0.85*	-0.83*	-0.84*	-0.86*	-0.44*	-0.75*	-0.80*	-0.87*	0.82*
Glu								0.97*	0.90*	0.91*	0.72*	0.85*	0.83*	-0.75*	0.73*	0.82*	0.79*	0.61*	0.72*	0.64*	0.75*	-0.75*
Suc									0.85*	0.88*	0.73*	0.81*	0.81*	-0.72*	0.69*	0.79*	0.79*	0.60*	0.70*	0.65*	0.72*	-0.76*
Lac										0.92*	0.66*	0.79*	0.80*	-0.75*	0.74*	0.80*	0.74*	0.57*	0.66*	0.57*	0.73*	-0.67*
Mal											0.68*	0.77*	0.74*	-0.67*	0.65*	0.74*	0.72*	0.59*	0.64*	0.51*	0.65*	-0.68*
LOX												0.73*	0.82*	-0.77*	0.68*	0.76*	0.76*	0.45*	0.60*	0.52*	0.72*	-0.77*
SOD													0.89*	-0.80*	0.80*	0.90*	0.90*	0.66*	0.84*	0.76*	0.86*	-0.86*
APX														-0.94*	0.91*	0.96*	0.92*	0.56*	0.83*	0.82*	0.94*	-0.88*
Antiox															-0.94*	-0.89*	-0.93*	-0.48*	-0.75*	-0.76*	-0.93*	0.78*
Germ																0.88*	0.94*	0.52*	0.79*	0.78*	0.93*	-0.76*
SL																	0.95*	0.62*	0.86*	0.82*	0.91*	-0.86*
RL																		0.72*	0.85*	0.83*	0.95*	-0.85*
SDw																			0.72*	0.54*	0.60*	-0.52*
GI																				0.83*	0.85*	-0.78*
SGR																					0.84*	-0.75*
Brix																						-0.84*

*: Significant level at α = 0.05; NS = not significant different at α = 0.05 level. **: Data are involved in all treatments

Aw: Water activity, MC: Seed moisture content, Inf: seed infection, Pro: Protein content, Lipid: Lipid content, FFA: Total free fatty acid content, Carb: Carbohydrate content, Glu: Glucrose, Suc: Sucrose, Lac: Lactose, Mal: Maltose, LOX: Lipoxygenase activity, SOD: Superoxide dismutase activity, APX: Ascorbate peroxidase activity, Germ: Seed germination, SL: Shoot length, RL: Root length, SDw: Seedling dry weight, GI: Germination index, SGR: Seedling growth rate, Brix: Field emergence, Cond: Conductivity

5.5 Discussion

In rice seeds, chemical and biochemical properties are commonly used criteria for measuring the seed deterioration. Most of the usually applied seed treatment substances directly affected the mobilization of food reserves in the seeds, which is essential for the growth of embryogenic axis during early seed germination. The present results showed that storage time, CA treatment, and the interaction of seed treatment and storage time affected the biochemical degradation in rice seeds significantly higher than the biological seed treatment. In congruence with Kozlowski (1986), seeds injury was reported after the use of captan. Marc and Bugbee (1996) described that the metabolism of captan is activated as aqueous slurry; it released tetrahydrophthalimide, tetrahydrophthalic acid and three molecules of chloride (Cl^-). The penetration of these compounds into the cell can cause phytotoxic effects (Yupsanis *et al*., 1994).

In our experiments, CA treated seeds had higher amount of FFA and a higher LOX activity than biological coated seeds and seeds without treatment (CO) (Table 5.5). The experiments suggested that under the stress conditions and during storage, lipid in rice seeds are broken down by LOX into FFA. According to Narvaey-Vasquez *et al*. (1999), the deterioration of rice seeds is generally accompanied with lipid peroxidation. An interesting result of the present study was that the CA treatment induced lipid peroxidation, which was seen in an inverse relationship between lipid and FFA content, lipid and LOX and a positive correlation between LOX and FFA as well (Table 5.8). This relationship was also found by Rosahl (1996) who reported that CA induced LOX activity. The degradation of lipids in senescing membranes and the release of FFA initiate oxidative deterioration by providing substrate for LOX (Wang *et al*., 1990). This mechanism releasing reactive oxygen species (ROS) from membrane phospholipids in aleurone layer of rice seeds in response to chemical stress conditions could induced cell senescence (McCord, 2000). The ROS have the potential to damage cell membranes and is likely to be a primary cause of deterioration of seeds (Sung and Chiu, 1995). Moreover, several comprehensive reviews have identified ROS mediated lipid peroxidation, enzyme inactivation, protein degradation, disruption of cellular membranes and damage to genetic (nucleic acids) integrity as major cause of seed deterioration (Smith and Berjak, 1995; Mcdonald, 1999). As shown in Table 5.8, the results showed a positive correlation between cell membranes damage (conductivity results) and lipid peroxidation.

In consequence, the deterioration of cellular membranes affected the seeds viability in the CA treatment. Progress in membrane deterioration can lead to increase in membrane leakage during seed imbibition (Chapter 4). These findings are in agreement with Ryu and Wang (1996) who reported that CA induced the cell membrane senescence, which led to an increase of the susceptibility of membrane lipids against enzymatic degradation.

The CA treatment decreased also the total protein content in the seeds. However, the biological coated seed enhanced the relative synthesis of proteins of the seeds (Table 5.2). Apparently, CA stress altered the turnover of proteins. According to Slavin (2003), ROS can activate proteins by reacting with specific amino acids (amine group), which resulted in DNA degradation and impaired transcription which causes incomplete protein synthesis. Smith (1981) reported that CA had a multi-site activity, which inhibited the tubulin formation, affecting DNA and mRNA synthesis and metabolism (effects on cell division) as well as site I and II of electron transport pathways. Therefore, the degradation of protein content was assumed to be associated with the change of nitrogenous fractions and a decrease in "true protein" nitrogen (Boora and Kapoor, 1985). Dell'Aquila and Spada (1992) reported that CA inhibited amino acids synthesis, especially glutamine. Moreover, it acted like a glutamine synthetase inhibitor, which directly affected the total protein content. Moreover, hydrolytic enzymes were inhibited by CA, which might influence the protein turnover in germinating seeds. Xu *et al*. (1997) also reported that CA inhibited such enzymes that decreased the protein content during rice seed storage. Abernathy *et al*., (1989) found that CA affected mRNAs stability and activity, which led to an inhibition of the protein synthesis (Munnik *et al*., 1995). The present results reported that CA stress inhibited protein synthesis. However, it does not identify specific mechanisms. Therefore, future studies about the effect of CA stress on protein degradation could be interesting.

The experiment showed that the seed treatment had an inverse relationship to biochemical degradation and physical seed properties (Table 5.8). According to Touati (1990), ROS inhibit not only the protein synthesis but they have also an impact on other major classes of macromolecules in deteriorated seed. The ROS are highly cytotoxic and can actively react with lipids, proteins, and nucleic acids, which can lead to seed injury or seed death (Rathinavel and Dharmalingam, 2001).

This study demonstrated a clear relationship between the detoxifying enzymes (APX and SOD), total antioxidant activity, the lipid peroxidation, and the seed deterioration depending on seed treatments (Table 5.8). The oxidative scavenging enzymes (APX and SOD) as well as total antioxidant capacity were in biological coated seeds more active than in the CA treatment and CO (Table 5.3 and 5.5). The experiments suggested that those are important factors for scavenging ROS, which induced the seeds tolerance to oxidative stress conditions. These findings are similar to that of Esfandiari *et al*., (2007). Additionally, the loss of seed viability was associated with the decrease of SOD, APX, and antioxidant activity in the seeds as reported by Zhao *et al*. (2006). Asada (2000) found that higher activities of SOD and APX decreased the level of ROS in the cells and increased the stability of cell membrane, as well as activate the Calvin cycle that could maintain the seed viability as well.

During the early period of seedling growth, the main source of energy is derived from the sugars. This sugar is released after enzymatic hydrolysis starch that is stored in the endosperm of the rice seeds (Dhingra, 1984). According to the experiment results explained in Chapter 4, CA treated with slurry dust application increased MC and Aw content of the seeds. The water was consumed and induced the starch hydrolysis and the sugars were converted to CO_2 and water via the respiration. These reactions led to the decrease of the sugar content in the seeds (Table 5.4). The sugar content was also positive correlated with seed germination and vigor (Table 5.8). The biological treated seeds had a higher seed quality throughout the storage period, because they could maintain high sugar contents inside the seeds (Table 5.4). According to Dhingra, (1984), glucose was derived from the endosperm in the amylolytic breakdown process. Following that, it was mobilized to the scutellum, where the sucrose synthesis occurred. After that, the glucose was transported to the embryonic axis for further seedling development.

In the control variant, the seeds had a decreased germination percentage after eight months of storage (Chapter 4). The decrease of seed germination might be a consequence of the damage of embryos and meristematic cells by fungi. Moreover, fungi also influenced the depletion of food reserves in the endosperm. The results showed that seed infection had an inverse relationship with biochemical degradation and seed properties (Table 5.8). According to Chandrashekar and Sandhyarani, (1996), fungi utilized sucrose as a nutrient or carbon source, which caused a decrease in seed vigor by inhibiting the

nutrient availability in the seed. It appears that a decrease in viability and germination of the seed because of fungi development led to the inhibition of amino acid and protein synthesis (Senaratna *et al*., 1988) and the decrease in sugar and starch contents (Kriedemann and Beevers, 1967).

The present results suggested that Maillard reaction might be a mechanism that declined seed viability. This reaction occurred after an initial simple non-enzymatic attack on amino groups of protein and nucleic acid/protein complexes by reducing sugars (Bradbeer, 1988). The AGE products (Advanced Glycosylation End-products) from Maillard reactions occur in both aged seed and deteriorated seed (Sun and Leopold, 1995). In addition, lipid peroxidation and sugar hydrolysis were coupled to the Maillard reactions during seed storage (Wettlaufer and Leopold, 1991).

The experimental data demonstrated the inverse relationship between MC, Aw and the biochemical degradation, which affected seed deterioration (Table 5.8). The experiment suggested, even at low moisture contents, rice seeds contained a considerable amount of reducing sugars that were able to initiate the Maillard reaction, seed aging during storage could well be due to the slow Maillard reactions. As seed moisture content increased, enzymatic reactions are expected to play a role in seed aging, thus lipid peroxidation and sugar hydrolysis are similarly increased (Priestley, 1986). As the seed moisture content increased further, lipid peroxidation was reduced and sugar hydrolysis increased rapidly. These data indicated that the products of lipid peroxidation are a main driving force of deterioration reactions, whereas reducing sugars might become a major driving force at high moisture content (Murthy and Sun, 2000). Finally, these complex mechanisms affected directly seed viability and storability (Chen and Chen, 2003).

5.6 Conclusions

The results clearly explained that seeds coated with CL and E+CL decreased to a lesser extend rice seed quality compared to CA treatment or no seed treatment (CO). The storage time was probably the main factor that affected biochemical degradation. However, the results indicated that CA treatment affected rice seed viability significantly, which was associated with biochemical deterioration. After 12 months storage, total protein content decreased and was accompanied by declined of lipid content, which was activated by LOX and produced more FFA . These mechanisms showed that an increasing

of lipid peroxidation led to the rapid loss of seed viability. In the case of biological fungicide coating substances, the lipid peroxidation was alleviated by the APX and SOD enzymes and showed a high antioxidant activity. These mechanisms induced the seed tolerance to oxidative stress conditions. Moreover, the sugar content was positive correlated with seed germination and vigor. Preliminary evidence under fungicide stress showed that sucrose and larger oligosaccharides were lost but in the biological coated seeds their compounds were consistently presented and therefore, this treatment maintained high seed quality throughout the storage.

5.7 References

Abernathy, R.H., Thiel, D.S., Petersen, N.S., Helm, K. (1989). Thermoresistance is developmentally dependent in germinating wheat seeds. Plant Physiol. 89, 569-576.

Alexander, R.R., Griffiths, J.M., and Willkinson, M.L. (1985). Basic biochemical methods, John Wiley&Sons, New York.

AOAC 17th edn. (2000). Official Method 920.91 Rice–Determination of total ash content.

AOAC 17th edn. (2000). Official Method 960.52 Rice–Determination of total nitrogen content.

Asada, K. (2000). The water-water cycle as alternative photon and electron sinks. Phill Trans R. Soc. Lond. B. 355, 1419-1431.

Boora, P., and Kapoor, A. C. (1985). Influence of storage on the protein quality of pearl millet flour. J. Sci. Food. Agric. 36, 59-62.

Bradbeer, J.W. (1988). Seed dormancy and germination. Blackie and Son Ltd. Glasgow and London. 367.

Bradford, M.M. (1976). Rapid and Sensitive Method for the Quantization of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical biochemistry. 72, 248-254.

Chandrashekar, K.R., and Sandhyarani, S. (1996). Salinity induced chemical changes in *Crotalaria striata* dc. plants. Indian Journal Plant Physiology. 1, 44-48.

Chen, T.F., and Chen, C.L. (2003). Analyzing the freshness of intact rice grain by color determination of peroxidase activity. J Sci Food Agric. 83, 1214-1218.

Dell'Aquila, A., Spada, P. (1992). Regulation of protein synthesis in germinating wheat embryos under polyethylene glycol and salt stress. Seed Sci. Res., 2, 75-80.

Dhingra, H.R. (1984). Physiological and biochemical studies on the effect of salt stress on maize (*Zea mays* L.) pollen. Ph.D. Thesis: HAU, Hisar.

Downton, W.J.S. (1977). Photosynthesis in salt stressed grapevines. Australian Journal of Plant Physiology. 4, 183-92.

Esfandiari, E.O., Shakiba, M.R., Mahboob, S.A., Alyari, H., and Toorchi, M. (2007). Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seed. Journal of Food, Agriculture & Environment. 5(1), 149-153.

Food and Agriculture Organization of the United Nations (FAO). (1997). Estimated Post-Harvest Losses of Rice in Southeast Asia. Available online at: http://www.fao.org/News/FACTFILE/FF9712- E.HTM (viewed July 16, 1997).

Hewett, P.D. (1987). Pathogen viability on seed in deep freeze storage. Seed Science and Technology. 15, 73-77.

Kaiser, W.J. (1987). Testing and production of healthy plant germplasm. Tech. Bull. 2 Dan. Gov. Inst. Seed Pathol. Dev. Countries, Copenhagen, Denmark. 30.

Kim, J.K., Jung, H.N., Sun, G.L., Jae, S.C., Hong, S.S., Hae, Y.C., Yeong, O.S., and Won, C.C. (2002). The First Total Synthesis of 2, 3, 6-Tribromo-4, 5-dihydroxybenzyl Methyl Ether (TDB) and Its Antioxidant Activity. Bull. Korean Chem. Soc. 23(5), 66-662.

Kriedemann, P., and Beevers, H. (1967). Sugar uptake and translocation in the rice seedling. I. Charateristics of transfer in intact and excised seedlings. Plant Physiol. 42, 161-173.

Kozlowski, T.T. (1986). Effects on seedling development of direct contact of Pinus resinosa seeds or young seedlings with Captan. Forest Pathology, 16(2), 87–90.

Lam, H.S., and Proctor, A. (2000). Rapid methods for milled rice total lipid and free fatty acid determination. AAES Research Series. 291-295.

Lee, A.T., and Cerami, A. (1989). Non-enzymatic glycosylation of DNA by reducing sugars. In: Baynes JW, Monnier VM, Eds. The Amadori and Maillard reaction in ageing, diabetes and nutrition. New York: Alan R Liss, Inc, 291-299.

Marc, W. van I., and Bugbee, B. (1996). Phytotoxic effects of fungicides on bedding plants. J. Amer. Soc. Hort. Sci. 121(6), 1095-1102.

Mayer, A.H. (1977). Metabolic control of germination. In Khan, A.A. (Ed): The Physiology and Biochemistry of Seed Dormancy and Germination, North Holland.

McCord, J.M. (2000). The evolution of free radicals and oxidative stress. The American Journal of Medicine. 108, 652-658.

McDonald, M.B. (1999). Seed deterioration: physiology, repair and assessment. Seed Sci. and Technol. 27, 177-237.

Merrill, A.L., and Watt, B.K. (1973). Energy value of foods: basis and derivation. Agriculture Handbook No. 74. Washington, DC, ARS United States Department of Agriculture.

Meshehdani, T., Pokorný, J., Davídek, J., and Pánek J. (2006). The lipoxygenase activity of rapeseed. Food. 34(8**)**, 727 – 734**.**

Milka B., Marijana, P., and Kljalic R. (1976). Effect of the herbicide 2, 4-DB and fungicide captan on reaction of mitochondria and chloroplasts. Archives of Environmental Contamination and Toxicology. 4, 166-174.

Munnik, T., Arisz, S.A., de Vrije, T., Musgrave, A**.** (1995). G-protein activation stimulates phospholipase D signaling in plants. Plant Cell. 7, 2197-2210

Murthy, U.M.N., Sun, W.Q. (2000). Protein modification by the Amadori and Amadori and Maillard reaction during seed storage: roles of sugar hydrolysis and lipid peroxidation. Journal of Experimental Botany. 51, 1221-1228.

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant & Cell Physiol. 22(5), 867-880.

Narvaey-Vasquez, J., Florin-Christensen, J., and Ryan, C.A. (1999). Positional specificity of a phospholipase an activity induced by wounding systemic and oligosaccharide elicitors in tomato leaves. Plant cell. 11, 2249-2260.

Oberley, L.W., and Spitz, D.R. (1985). Assay of superoxide dismutase using nitroblue tetrazolium. Greenwald, R.A. (Ed.). Handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL. 217-221.

Perez, L.M., Perdon, A.A., Resursection, A.P., Villareal, R.M., and Juliano, B.O. (1975).Enzymes of carbohydrate metabolism in the developing rice grain. Plant Physiol., Lancaster 56, 579-83.

Pomeranz, Y., and Meloan, C.E. (1987). Food analysis: Theory and Practice, 2nd ed. Van Nostrand Reinhold Co., New York.

Priestley, D.A. (1986). Seed aging: Implications for seed storage and persistence in soil, pp. 1–304. Comstock Publ. Assoc., Ithaca and London.

Rathinavel, K., and Dharmalingam, C. (2001). Efficiency of seed treatment on storability of cottonseeds and seed vigor. Journal of Tropical Agriculture. 39, 128-133.

Rosahl, S. (1996). Lipoxygenase in plants-their in development and stress response. ZS Naturforsch Sect CJ Biosci 51C, 123-138.

Rudrapal, A.B., and Basu, R. N. (1981). Use of chlorine and bromine in controlling mustard seed deterioration. *Seed* Res. 9, 188-191.

Ryu, S. B., Wang, X**.** (1995). Expression of phospholipase D during castor bean leaf senescence. Plant Physiol 108: 713-719

Ryu, S. B., Wang, X. **(**1996). Activation of phospholipase D and the possible mechanism of activation in wound-induced lipid hydrolysis in castor bean leaves. Biochim Biophys Acta, 1303.

Salisbury, F.B., and Ross, C.W. (1985). Plant Physiology. Wadsworth Publishing Company, Inc. Belmont, California.

Senaratna, T., Gusse, J.F., and McKersie, B.D. (1988). Ageing induced changes in cellular membranes of imbibed soybean seed axes. Physiol. Plant. 73, 85-91.

Simon, E.W. (1984). Early events in germination. In: Murray, D.R. Ed. Seed Physiology. Australia: Academic Press. Sen Gupta, A., Webb, R. P., Holaday, A. S., and Allen, R. D. (1993). Over expression of superoxide dismutase protects plants from oxidative stress. Plant Physiology. 103, 1067-1073.

Slavin, J. (2003). Why whole grains are protective: Biological mechanisms. Proceeding of the Nutrition Society. 62, 129-134.

Smith, M.T. (1981). Changes during imbibition of irradiated dry seed of lettuce. Proceeding of the Electron Microscopy Society of Southern Africa. 11, 167-168.

Smith, M.T., and Berjak, P. (1995). Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation-sensitive seeds. In:Kigel, J.; G. Galili (eds.) Seed development and germination. New York, USA: Marcel Dekker.

Sun, W.Q., Leopold, A.C. (1995). The Maillard reaction and oxidative stress during ageing of soybean seeds. Physiologia Plantarum. 94, 94-105.

Sung, J.M., and Chiu, C.C. (1995). Lipid peroxidation and peroxide-scavenging enzymes of naturally aged soybean seed. Plant Science. 11, 45-52.

Taniguchi, N., Kinoshita, N., Arai, Iizuka, S., Usui, M., and Naito, T. (1989). Inactivation of erythrocyte Cu-Zn-superoxide dismutase through non-enzymatic glycosylation. In: Baynes JW, Monnier VM, Eds, the Maillard reaction in ageing, diabetes and nutrition. New York: Alan R Liss, Inc, 277-290.

Tappel, A.L. (1973). Lipid peroxidation damage to cell components. Federation Proc. 32, 1870-1874.

Touati, D., Farr, S.B., (1990). Elevated mutagenesis in bacterial mutants lacking superoxide dismutase. Methods Enzymol. 186, 646-650.

Walde. P., and Nastruzzi, C. (1991). Application of a new, simple and economic colorimetric method for the determination of non-esterified fatty acids in vegetable oils. Food Chemistry. 39, 249-256.

Wang, J., Fujimoto, K., Miyazawa, T., Endo, Y., and Kitamura, K. (1990). Sensitivity of lipoxygenase-lacking soybean seeds to accelerated ageing and their chemiluminescence levels. Phytochemistry. 29, 3739- 3742.

Wettlaufer, S.H., Leopold, A.C. (1991). Relevance of Amadori and Maillard products to seed deterioration. Plant Physiology. 97, 165-169.

Xu, L., Zheng, S., Zheng, L., and Wang, X. (1997). Promoter Analysis and Expression of a Phospholipase D Gene from Castor Bean. Plant Physiol. 11(5), 387-395.

Yupsanis, T., Moustakas, M., Eleftherrious, P., and Damalanidou, K. (1994). Protein phosporylation-dephosphorylation in alfalfa seeds germinating under salt stress. J Plant Physiol. 143, 234-240.

Zhao, F., Guo, S., Zhang, H., and Zhao, Y. (2006). Expression of yeast SOD2 in transgenic rice results in increased salt tolerance. Plant Science. 170, 216-224.

6. The effects of seed coating substances on plant growth, grain yield, and fungicide residues in direct-seed rice production

6.1 Abstract

Seed and soil-borne infections are major courses reducing seedling population in the direct seed rice production system. Poor seedling establishment is a deterrent in adopting direct seed rice production. Seed coating, an application to improve crop stand, could be an attractive approach. The aim of this experiment was to investigate the effectiveness of seed coating strategies on agronomic characters, yield, nutrient uptake and fungicide residue in direct-sown rice. Seeds were treated with various substances i.e. captan (conventional treatment) (CA), only chitosan-lignosulphonate polymer (CL) and eugenol incorporated into chitosan-lignosulphonate polymer (E+CL). Untreated seeds were used as control (CO). The results of growing in seasons 2005 and 2006 indicated that CL and E+CL coating polymer improved seed germination, and seedling establishment under field conditions. Moreover, this seed coating substances improved plant growth, kernel yield, and plant nutrients uptake efficiency. CA treatment led to residues of organochloride fungicides in soil and rice grains. The results indicate that the application of biological substances for seed coating as CL or E+CL polymer may be a promising strategy to improve the direct-seed rice production system.

6.2 Introduction

Rice occupies a conspicuous position in the agro-based economy of Thailand. It is grown on an area of 3.4 million ha with the average yield of 4.70 t ha^{-1} and accounts for 6.6% value added in the agriculture system and 1.6% in GDP in the country. (Office of Agricultural Economics, 2006). Besides meeting the dietary requirements of the people, it has emerged as a major export product contributing about 13% to the total foreign exchange earnings of the country (Anonymous, 2004).

Transplanting is a principle method of rice cultivation in the world, which nursery seedlings are raised and then transplanted into flooded fields. However, in Thailand, the farmers have been used germinated seeds for rice nursery sowing, which resulted in poor, delayed germination and seriously infection by several fungi. This resulted in lower tillering capacity and thus reducing the final yield. It is the limiting factors in the traditional rice production system (Reddy, 2004).

Seed coating technique are used to protect the seeds from fungi infection. Moreover, this application may also improve the seedling performances, i.e. the seedling emergence time, synchronized emergence, improve emergence rate, and better seedling stand production in many field crops (Khan 1992; Jett *et al*., 1996) like wheat (Chowdhary and Baset, 1994), maize (Dell Aquilla and Tritto, 1990) and rice (Basra *et al*., 2005).

The technique of film coating involves the application of thin layer of a polymer binder often in combination with other components onto the seed coat. These other components may include color (effective pigment) and/or a variety of active additives, i.e. pesticides, fertilizers or plant regulators. The objectives of film coating seeds include the safe, accurate and dust free incorporation of seed treatment chemicals, the addition of nutrients or plant strengtheners for the improvement of the seeds ability (Du and Toung, 2002). In the present study, chitosan lignosulphonate polymer or eugenol incorporated into chitosan lignosulphonate polymer were found to be the most effective seed coating materials for rice seed borne fungi control. Furthermore, these applications led to vigorous seedlings, decreased germination time, and they improved the uniformity of seedling stand population under field conditions (see Chapter 4 and 5).

The presented research was aimed to study the appropriate technique for improving direct-seed rice cultivation, to evaluate the effect of seed coating substances on plant productivity, agronomical traits, nutrient uptake, plant yield and fungicide residues in soil and harvested grain.

6.3 Materials and methods

Dry graded rice seeds (Oryza sativa L. cv. KDML 105) from one seed lot were supplied from Bureau of Seed Multiplication of Thailand. The initial seed moisture content and germination percentage were 10.65% and 96.00%, respectively. The field experiments were conducted at the Agronomy Research Station, Faculty of Agriculture, Department of Agronomy, Chiang Mai University, Chiang Mai, Thailand during August - December in 2005 and 2006. The randomized complete block design (RCBD) with four replications was applied. The treatments were: control (untreated seeds) (CO), captan (CA), only chitosan lignosulphonate polymer (CL) and eugenol incorporated into chitosan lignosulphonate polymer (E+CL).

6.3.1 Crop husbandry

The soil properties and mineral contents before planting in both seasons 2005 and 2006 are presented in the Appendix 18. For soil preparation, the wetland preparation method was used before sowing. The field was flooded with water and puddle with the help of a tractor drawn cage-wheel puddler in standing water. The puddle field was left for 5 days for the soil particles to settle before sowing. The experiment plot sizes were 6 x 4 m^2 (Figure 6.1). As fertilizers were 150 kg N as urea (46% N), 150 kg P_2O_5 as single super phosphate (18% P_2O_5) and 100 kg K_2O potassium sulphate (50% K_2O) per hectare applied. The whole quantity of P, K, and a half of the N were incorporated into the soil as basal dose. The remaining N was brought out in two equal splits each at tillering and panicle initiation.

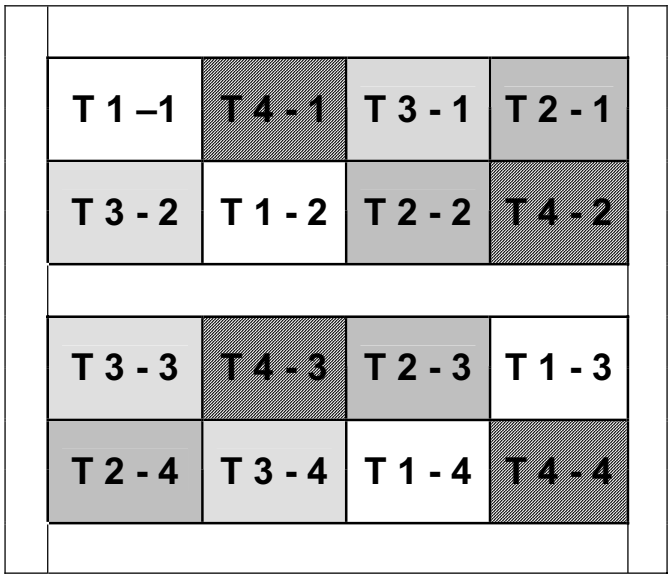


Figure 6.1: Field experimental design with randomized complete block design (RCBD) and four replications
= Control (CO), = captan seed treatment (CA), = Only chitosan-lignosulphonate polymer treatment (CL) and = Eugenol incorporated into chitosan-lignosulphonate polymer treatment (E+CL)

For irrigation, the field was flooded in the first week after sowing until water depth of about 0.5 cm. During growing, water was flooded continuously every week to maintain a constant water depth of 5-6 cm in the field. In total, 16 irrigations were applied during the crop growth period. The irrigation was stopped about one week before harvesting when the physiological maturity appeared (110 days after sowing). Pest control was done manually without any chemical pesticides. Harvesting was done manually at harvest maturity stage, the approximate grain moisture content was about 22%, and threshing was done separately for each plot.

6.3.2 Seedling properties

At 14 days after sowing, the field emergence was evaluated as follows: from 10 points of 1x1 m^2 areas the numbers of germinated seeds were determined. At the 30^{th} day after sowing, the seedlings were tested for vigor and the seedling dry weights were taken after drying at 70°C for 48 hrs (Basra *et al*., 2006).

6.3.3 Agronomic traits, yield components and growth analysis

During planting, the observations regarding agronomic traits, i.e. recording of plant high followed the standard procedures (Farooq, 2007). Chlorophyll content was determined by chlorophyll meter (MINOLTA, SPAD 502). At harvesting time, yield components as panicle m^{-2}, kernels $panicle^{-1}$, 1000 seeds weight (g) and yield (ton ha^{-1}) were determined according Farooq (2007). At 30 days after sowing, the plant infection was recorded at 10 points of 1x1 m^2 area in each plot. At 100 days after sowing, rice grain infection was recorded.

6.3.4 Plant and soil analysis

At 60 days after sowing (before flowering), 25 whole plants were randomly sampled in each replication plot. Plants were cleaned by distilled water for four times and were dried at 60°C for 72 hrs. Dried samples were ground and analyzed for macro- and microelements content. The rice grain samples were also randomly sampled from 10 points per plot. Then, they were mixed together and separated into four sub-samples, approximately one kilogram of each sub-sample. These samples were dried at 45°C for adjust the grain moisture content to about 12±5 %, milled and sieved through a U.S. Standard # 16 mesh.

The mineral content analysis was performed as follow: 0.4 g sample of known moisture content was accuracy weighed in the reaction vessel, 4 ml 65% nitric acid was added. Samples were digested in the oven which was allowed to stand at 175°C for 12 hrs in order to remove the organic matter in the sample. The cleared solution was collected by raising the digestion vessel, and made up to 10 ml in volumetric flask. The samples were kept under cool conditions before using for measurement of the mineral elements. The mineral elements, i.e. phosphorus (P), potassium (K), sulphur (S), iron (Fe), sodium (Na), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), copper (Cu), aluminum (Al) and boron (B) were determined by using atomic absorption spectrophotometer (AAS, Analytik Jena model ZEEnit 700PC), flame emission spectrometer (FES) and spectrophotometer.

Before planting, 10 points of randomly 20 cm depth soil samples were collected in each replicate. Soil samples were air dried for 96 hrs, then grounded and analyzed for the physical properties, i.e. soil pH, soil organic matter, cation exchange capacity (CEC) and soil texture. Mineral contents were analyzed by the following order: total nitrogen (N, Kjeldahl method), exchangeable P, (Bray and Kurtz P-41 method), exchangeable K, Na, Ca and Mg (NH_4OAC pH7/FES method), exchangeable S, ($BaCI_2$ method), exchangeable Al, Cl, S, Zn, Mn, Fe and Cu (DTPA/AAS method) and exchangeable B (hot water extraction method). The results are shown in the Appendix 18.

6.3.5 Organochloride fungicide residue analysis

6.3.5.1 Sample collection

Soil samples

Representative soil samples were collected from 10 points per plot. After systematic random sampling, the soil was digged by V-shape to 15 cm depth and in a depth of 5 cm soil was collected as a composite sample. Samples were mixed, and separated into four sub-samples for fungicide residue analysis.

Rice grain samples

Rice grain samples were randomly collected from 10 points per plot. Then, samples were mixed and separated into four sub-samples which was approximately one kilogram of each sub-sample. The sub-samples were immediately taken to the laboratory for fungicide residue analysis.

6.3.5.2 Sample preparation

Soil samples

At first, stones and other foreign materials were removed from the soil samples. Soil samples were air dried for 96 hrs, then grounded and sieved through a U.S. Standard #16 mesh. Finally, ground samples were stored in sealed glass and kept frozen at -20°C in a refrigerator before analyses.

Rice grain samples

At first, rice grain samples were dried for adjusting the moisture content to 12±0.5 %. Then, the samples were ground and sieved through a U.S. Standard # 16 mesh. Ground samples were kept in sealed glasses and stored in a refrigerator at -20°C before extraction.

6.3.5.3 Samples extraction and analysis

Soil sample

First, a soil sample of 50 g was accuracy weighed into the extraction thimble. Then, sample was extracted by adding of 200 ml 1:1 (v/v) hexane/acetone solvent and shaken for eight hours at 40-50^{o}C. After cooling down, the sample was filtrated and transferred to 500 ml separator funnel. The sample was partitioned by adding 100 ml 2% sodium sulfate, and then vigorously shook for 5 minutes. The sample was allowed to stay until the hexane and water phases separated and drew off aqueous layer (bottom), this step was repeated twice. After partitioning, sample was evaporated in rotary evaporator until approximately 5 ml left. Then, sample was washed with 200 ml of 6% diethyl ether in petroleum ether and 200 ml of 15% diethyl ether in petroleum ether solution, respectively. The clean-up column was packed with glass wool, 40 g florisil and 20 g anhydrous sodium sulfate. Florisil must be heated in oven at 130°C ± 1°C for at least 16 hours before using. Finally, after clean-up operation, the sample was evaporated to dryness by using the rotary evaporator and rinsed with n-hexane about 5 times and adjusted to exactly 5 ml for GLC injection.

Rice grain sample

First, a 100 g ground sample was homogenized in blender cup. Then, added 200 ml acetonitrile and sample was vortexed for 10 min. The extract was centrifuged at 2500 rpm for 10 min and removed the supernatants which was repeated this step for three times. The supernatants were transferred to 2 L separator funnel and adding 100 ml petroleum ether, 500 ml distilled water and 10 ml saturated NaCl solution. Then, the extract was vigorously shaken for 5 minutes and let the layers separated and discarded the aqueous (bottom) layer. The samples were filtrated by passing through 15 g anhydrous Na_2SO_4 in funnel with glass wool. The filtrated solution was evaporated in a rotary evaporator until 5 ml retained, sample was washed with 200 ml of 6% diethyl ether in petroleum ether and 200 ml of 15% diethyl ether in petroleum ether solution, respectively. The clean-up column was packed with glass wool, 40 g florisil and 20 g anhydrous sodium sulfate. Finally, after clean-up operation, the sample was evaporated to dryness by using the rotary evaporator and rinsed with n-hexane about five times and adjusted to exactly 5 ml for GLC injection.

The TRACOR MT-220 gas chromatograph was used for organochloride fungicide residue determination. An electron capture detector was used as detector at 275°C. Type of column used as 1.5% SP-2250 / 1.95% SP-2401 on 100/120 mesh Supelcon AW DMCS at 200°C. Carrier gas was nitrogen at 3600 ml s^{-1} flow rate and 0.508 lb m^{-2} pressure.

The concentration of organochloride fungicide residue was calculated by the following equations (Duggen, 1969):

$$= \frac{(\text{mg of Std.}) \times (\text{Sample area})}{\text{Std. area}} \times \frac{\text{Sample adjusted (ml)}}{\text{Sample injected (}\mu\text{L)}} \times \text{Sample size factor}$$

Sample size factors: Soil sample = 1/5, Rice grain sample = 1/100

6.3.6 Calculation and statistical analysis

The data are presented as mean ± standard deviation. The analysis of variance was performed for data analysis and differentiated with LSD test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

6.4 Results

Comparison of the effects of seed treatment variants on rice production revealed that CA treatment significant reduced the seedling emergence and establishment under field conditions (Figure 6.2, Table 6.1). This result was significant different from CO, CL and E+CL treatments. CL and E+CL led to higher seedling emergence under field conditions. However, these results were non-significant different from the control plot.

Table 6.1: The effect of seed coating substances on field emergence in season 2005 and 2006*

Treatment	Field emergence (%) (Mean ± SD)	
	2005	2006
CO	**80±4.16a**	**81±7.72a**
CA	**57±19.17b**	**64±4.79b**
CL	**84±7.95a**	**82±8.88a**
E+CL	**76±5.00a**	**80±7.80a**

* The different letters indicate the statistically significant difference by LSD at 5% level.

Figure 6.3 demonstrated that CA treatment significantly affected seedling establishment under field conditions. As shown in Table 6.2, at 2 weeks after sowing, the CA treatment significantly decreased seedling dry weight, shoot and root dry weight when compared with the biological seed coating substances. CL and E+CL improved the seedling establishment. Figures 6.4 and 6.5 demonstrated the different effects of the studied seed treatment variants on shoot length and root system of rice plants.

Table 6.2: The effect of seed coating substances on seedling performances after germination (2 weeks after sowing) in season 2005 and 2006*

Treatment	(Mean ± SD)					
	Seedling dry weight (mg)		Shoot dry weight (mg)		Root dry weight (mg)	
	2005	2006	2005	2006	2005	2006
CO	46.11±1.63b	35.05±1.24b	25.78±2.03ab	19.60±1.55ab	20.33±0.58bc	15.45±0.43b
CA	44.24±3.94b	34.51±3.07b	24.05±3.73b	18.76±2.91b	20.18±0.29c	15.75±0.23b
CL	52.79±3.53a	36.95±2.48ab	28.74±0.71a	20.12±0.50ab	24.04±3.10a	16.83±2.17ab
E+CL	50.64±1.23a	39.50±0.96a	27.67±0.54a	21.60±0.42a	22.97±0.86ab	17.92±0.67a

* The different letters indicate the statistically significant difference by LSD at 5% level.

The chlorophyll accumulation in the rice plant during growing is presented in Table 6.3. The results indicated that in leafs from CA treatment the chlorophyll content was markedly lower compared to the other treatments. On the other hand, the biological seed coating substances did not affect the chlorophyll accumulation during plant growth.

As shown in Table 6.4, the CA treatment retarded the growth of etiolate rice plants over the period of growth. Additionally, it caused not only growth retardation, but led also to leaf dehydration, causing shrinkage and a decrease in leaf length and width. CL and E+CL did not affect the plant growth. Biological coating substances exhibited plant growth rate similar to the control seeds. Furthermore, Figure 6.3 showed clear differences of plant growth characteristics when compared between seed treatment variants (4 weeks after sowing).

At four weeks after sowing, the plants of the control plot were impaired as a consequence of the highest percentage of seedlings infected (Table 6.6., Figure 6.6). The plants from E+CL coated seeds were less infected than those from the CO. CA treatment completely inhibited seedlings infection.

At twelfth weeks after sowing, pre-harvesting grain infection was significantly higher in the CO plot than in the plots with the other treatments. The results demonstrated that the percentage of infected grains was significantly different between grains from CA treatment plot and E+CL polymer plot.

Table 6.3: The influence of seed coating substances on the leaf chlorophyll accumulation in rice plants during season 2005 and 2006*

Treatment	Chlorophyll content (SPAD value) (Mean ± SD)					Mean
	Time (weeks)					
	2	4	8	12	16	
Season 2005						
CO	**18.53±1.00a**	**34.20±0.55ab**	**31.32±0.99a**	**31.42±0.43a**	**28.45±0.78a**	**28.76ab**
CA	**16.75±1.27b**	**31.87±0.76c**	**28.07±1.41b**	**27.12±0.91b**	**25.05±2.50b**	**25.76c**
CL	**18.50±0.78a**	**34.62±1.00a**	**32.40±1.11a**	**32.10±0.75a**	**29.20±0.64a**	**29.36a**
E+CL	**18.20±0.50a**	**33.45±0.54b**	**30.82±0.55a**	**31.77±0.71a**	**28.52±0.79a**	**28.5b**
Season 2006						
CO	**18.30±0.38b**	**34.77±0.68b**	**31.85±1.58b**	**31.43±1.54b**	**29.22±0.97b**	**28.76ab**
CA	**18.71±1.61b**	**33.93±1.23b**	**29.40±0.84c**	**27.77±1.51c**	**26.11±2.71c**	**25.76c**
CL	**20.15±1.50ab**	**36.77±0.25a**	**34.47±2.23a**	**34.14±0.81a**	**31.11±0.68a**	**29.36a**
E+CL	**21.12±0.89a**	**36.62±0.29a**	**33.72±1.40ab**	**34.77±0.83a**	**31.13±0.96a**	**28.5b**

* The different letters indicate the statistically significant difference by LSD at 5% level.

Table 6.4: The influence of seed coating substances on rice plant growth in season 2005 and 2006*

Treatment	Plant hight (cm) (Mean ± SD)					Mean
	Time (weeks)					
	2	4	8	12	16	
Season 2005:						
CO	**23.7±1.92a**	**59.9±2.01bc**	**104.9±5.25b**	**105.3±3.64bc**	**116.9±2.76b**	**82.2b**
CA	**20.4±0.95b**	**56.9±4.17c**	**95.0±2.26c**	**103.7±2.76c**	**110.8±1.53c**	**77.4c**
CL	**23.3±1.03a**	**62.8±1.85ab**	**117.2±7.13a**	**118.4±3.09a**	**122.6±2.20a**	**87.65a**
E+CL	**23.8±2.10a**	**64.0±1.64a**	**106.8±5.21b**	**106.8±3.20b**	**118.2±1.27b**	**83.9b**
Season 2006:						
CO	**43.7±1.99bc**	**78.7±1.76a**	**98.8±12.92a**	**91.7±4.18a**	**93.6±2.23b**	**82.2b**
CA	**45.9±4.10b**	**73.2±3.72b**	**80.8±2.12b**	**87.8±2.57a**	**88.6±1.20c**	**77.4c**
CL	**42.3±0.88c**	**81.6±3.25a**	**78.1±3.12b**	**94.7±1.19a**	**98.1 ±1.76a**	**87.65a**
E+CL	**50.1±1.83a**	**81.8±1.23a**	**82.9±2.06b**	**114.6±41.52a**	**94.6±0.98b**	**83.9b**

* The different letters indicate the statistically significant difference by LSD at 5% level.

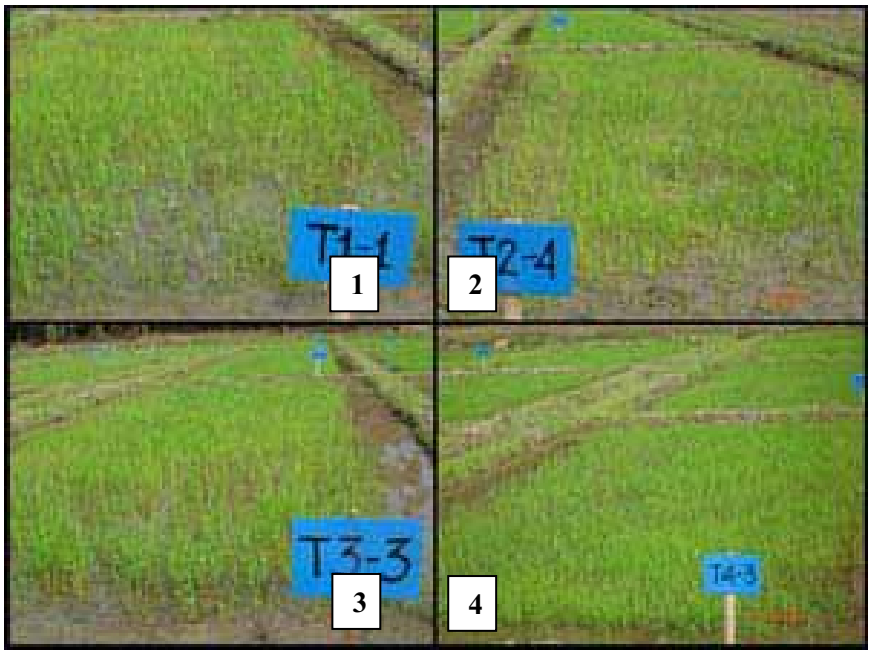


Figure 6.2: The effect of seed coating substances on seedling emergence at two weeks after sowing **1:** CO, **2:** CA, **3:** CL, and **4:** E+CL

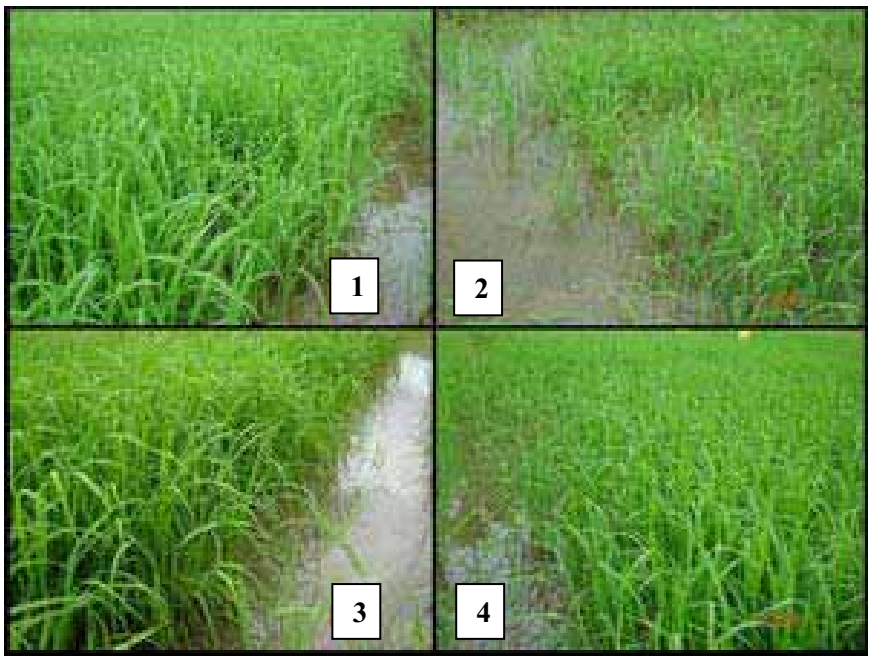


Figure 6.3: The effect of seed coating substances on seedling establishment at four weeks after sowing **1:** CO, **2:** CA, **3:** CL, and **4:** E+CL

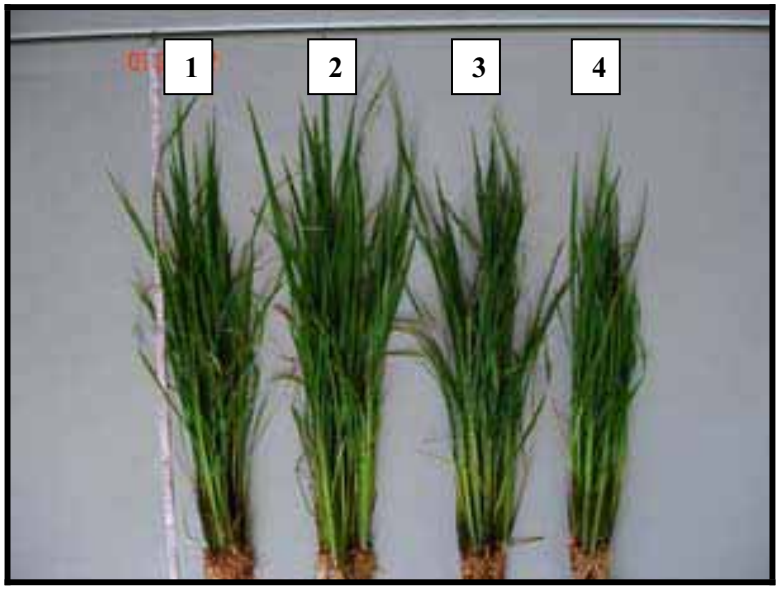


Figure 6.4: The effect of seed coating substances on shoot length at four weeks after sowing **1:** CO, **2:** CA, **3:** CL, and **4:** E+CL

Figure 6.5: The effect of seed coating substances on root system at four weeks after sowing **1:** CO, **2:** CA, **3:** CL, and **4:** E+CL

Figure 6.6: The grain infection in control check plot at early harvesting stage (110 days after planting)

Table 6.5: The effect of seed coating substances on seedlings and rice grain infection in season 2005 and 2006*

Treatment	Seedlings infection[1/] (%) (Mean ± SD)		Grain infection[2/] (%) (Mean ± SD)	
	2005	2006	2005	2006
CO	16.91±0.01a	16.18±0.02a	44.44±0.57a	50.00±2.59a
CA	0d	0d	0d	0d
CL	11.09±0.03b	10.15±0.04b	16.67±0.06b	27.78±0.36b
E+CL	5.06±0.04c	8.04±0.07c	6.95±0.10c	16.67±0.11c

* The different letters indicate the statistically significant difference by LSD at 5% level.

1/ : Seedling infection data were recorded at 14 days after sowing based on averaged 100 seedlings of 10 points 1 m^2 sampling area/plot for each treatments.

2/ : Rice grain infection data were recorded at 110 days after sowing (10 days before harvesting) based on averaged 25 panicles in 10 points of 1 m^2 sampling area/plot for each treatments.

The influence of seed coating substances on yield and yield components of rice grain are presented in Table 6.6. The results demonstrated that in both seasons 2005 and 2006 the influence of seed coating substances did not affect the number of panicles per area. These results were none significantly different. The seed coating substances influenced the number of bearing kernels per panicle. Especially, CO and CA treatment showed the lowest number of bearing kernels per panicle. However, higher numbers of panicle were recorded from plants raised from CL and E+CL. The seed coating substances affected also the 1,000 seeds weight. Table 6.6 indicated that the variant with E+ CL had the highest 1,000 seeds weight and the seeds obtained from the control plot had the lowest 1,000 seed weight. The studied seed treatment variants had no influence on the grain yield. However, compared to CA treatment, the use of biological seed coating substance treatments showed a tendency in both seasons to increase the yield.

The results demonstrated a positive correlation between field emergence and agronomic yield (Figure 6.7). The reduction of grain yield was the result of nitrogen deficiency. Positive correlations were noted between total nitrogen accumulation and grain yields (Figure 6.8).

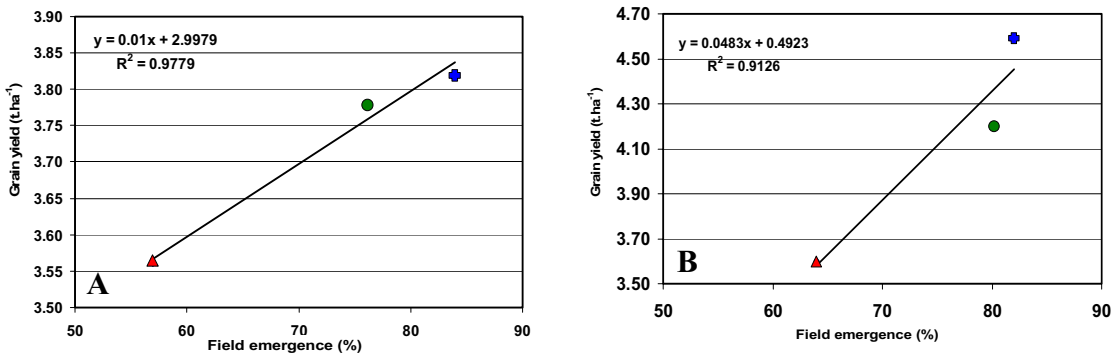


Figure 6.7: Correlation between mean of field emergence and yield in direct-seed rice production as affected by seed coating substance treatments. A: Season 2005, B: Season 2006 ▲ : CA, ✚ : CL, ● : E+CL

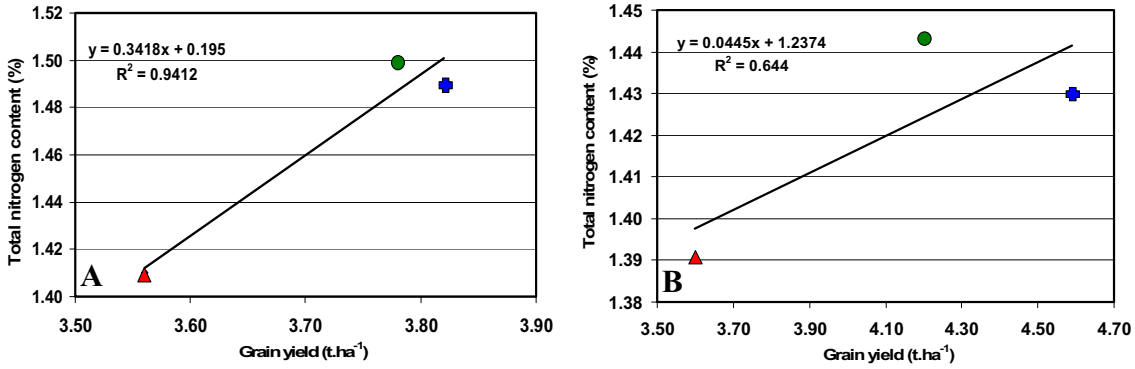


Figure 6.8: Correlation between mean total nitrogen content and grain yield in direct-seed rice production as affected by seed coating substance treatments. A: Season 2005, B: Season 2006 ▲ : CA, ✚ : CL, ● : E+CL

The contents of various minerals were determined in the whole plant tissue (60 days after sowing) and rice grains as presented in Tables 6.7, 6.8 and 6.9. The mineral contents in plants and grains from biological seed coating treatments were higher than from CA treatment. However, no significant differences to the grains from the control plot were found.

The results of the fungicide residue contamination in the soil and rice grain in both seasons 2005 and 2006 are presented in Table 6.10. As expected, the amounts of organochloride fungicide residue after sowing of CA treated seeds were the highest both in soil and grain whereas the other treatments led to significantly lower values without any significant differences between them.

Table 6.6: The efficiency of seed coating substances on yield components and agronomic yield of direct-seeded rice production

Treatment	Yield component evaluation (Mean ± SD)							
	Panicle m^{-2}		kernels/Panicle		1000 kernels weight (g)		Yield (ton ha^{-1})	
	2005	2006	2005	2006	2005	2006	2005	2006
CO	204±37.78a	208±38.71a	128±9.43b	131±9.43b	26.75±0.30b	25.41±0.27b	3.49±0.70a	3.65±0.71b
CA	189±24.01a	193±24.43a	137±7.34ab	140±7.35b	27.03±0.53ab	25.63±0.53ab	3.56±0.43a	3.60±0.39b
CL	198±22.28a	218±24.70a	142±6.08a	156±6.65a	27.17±0.22ab	25.81±0.21ab	3.82±0.45a	4.59±0.54a
E+CL	189±25.31a	199±26.70a	146±5.25a	154±5.25a	27.50±0.36a	26.09±0.35a	3.78±0.61a	4.20±0.67ab

* The different letters indicate the statistically significant difference by LSD at 5% level.

Table 6.7: The efficiency of seed coating substances on N, P, K and S uptake of the rice plants and grains

Treatment	N (g $100g^{-1}$ DM)		P (g $100g^{-1}$ DM)		K (g $100g^{-1}$ DM)		S (g $100g^{-1}$ DM)	
	2005	2006	2005	2006	2005	2006	2005	2006
Whole plant								
CO	0.70	0.91	0.22	0.19	0.86	1.51	0.096	0.101
CA	0.66	0.89	0.21	0.17	0.85	1.49	0.117	0.116
CL	0.70	0.94	0.22	0.20	0.93	1.54	0.142	0.132
E+CL	0.68	0.96	0.23	0.21	1.25	1.56	0.191	0.130
Harvested grain					(Mean ± SD)			
CO	1.47±0.05a	1.41±0.03a	0.37±0.01ab	0.37±5.77x10^{-3}a	0.29 ±0.02ab	0.31±0.03a	0.124±0.01a	0.138±5.57x10^{-3}b
CA	1.41±0.01b	1.39±0.01a	0.36±5.77x10^{-3}b	0.35±5.77x10^{-3}b	0.28±5.77x10^{-3}b	0.28±5.77x10^{-3}a	0.124±9.54x10^{-3}a	0.123±0.05b
CL	1.49±0.01a	1.43±0.06a	0.36±0.01b	0.37±0.01a	0.31±0.03ab	0.31±0.01a	0.134±0.01a	0.236±2.88x10^{-3}a
E+CL	1.50±0.01a	1.46±0.05a	0.38±5.77x10^{-3}a	0.37±0.01a	0.32±5.77x10^{-3}a	0.30±0.01a	0.129±1.52x10^{-3}a	0.230±5.51x10^{-3}a

* The different letters indicate the statistically significant difference by LSD at 5% level.

Table 6.8: The efficiency of seed coating substances on Fe, Na, Ca, and Mg uptake of direct-seeded coarse rice

Treatment	Fe (mg kg^{-1} DM)		Na (g 100g^{-1} DM)		Ca (g 100g^{-1} DM)		Mg(g 100g^{-1} DM)	
	2005	2006	2005	2006	2005	2006	2005	2006
Whole plant								
CO	73.99	55.96	1.92	3.25	0.584	1.523	0.104	0.192
CA	87.59	45.87	1.97	3.14	1.168	0.767	0.097	0.174
CL	81.42	57.06	2.26	3.21	1.311	1.883	0.108	0.189
E+CL	96.89	61.29	2.93	3.35	1.563	1.648	0.119	0.201
Harvested grain					(Mean ± SD)			
CO	1.95±0.09a	1.46±0.29b	0.62±0.05bc	0.62±0.02a	1.083±0.09a	0.963±0.02b	0.132 ±1.52x10^{-3}b	0.132±1.00x10^{-3}a
CA	1.79±0.62a	1.34±0.10b	0.60±0.01c	0.60±0.04a	1.164±0.06a	0.910±1.52x10^{-3}b	0.130±8.00x10^{-3}b	0.130±2.00x10^{-3}a
CL	1.88±0.20a	2.35±0.28a	0.74±0.02a	0.64±0.04a	1.322±0.13a	1.174±0.02a	0.141±4.16x10^{-3}a	0.135±9.45x10^{-3}a
E+CL	2.04±0.87a	2.43±0.37a	0.73±0.13ab	0.65±0.11a	1.233±0.14a	1.182±0.05a	0.135±2.08x10^{-3}ab	0.131±1.15x10^{-3}a

* The different letters indicate the statistically significant difference by LSD at 5% level.

Table 6.9: The efficiency of seed coating substances on Mn, Zn, Cu, Al and B uptake of direct-seeded coarse rice

Treatment	Mn (mg kg^{-1} DM)		Zn (mg kg^{-1} DM)		Cu (mg kg^{-1} DM)		Al (mg kg^{-1} DM)		B (mg kg^{-1} DM)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Whole plant										
CO	161.1	320.2	35.0	37.9	1.89	4.03	79.2	98.29	2.74	3.18
CA	160.5	214.1	30.0	33.7	1.51	3.21	58.55	46.83	2.82	2.57
CL	178.9	291.2	32.4	34.9	1.93	4.16	135.45	68.68	2.73	2.83
E+CL	167.2	252.3	33.0	34.2	1.90	4.15	99.25	78.97	3.57	2.92
Harvested grain						(Mean ± SD)				
CO	8.89±0.86a	8.37±0.05a	22.067±0.70a	22.500±0.76a	2.376±0.14ab	2.243±0.14a	28.250±0.42ab	17.650±1.22ab	0.65±0.01b	0.72±5.77x10^{-3}b
CA	8.47±0.17a	8.71±0.28a	21.867±0.50a	21.867±0.50a	2.226±0.08b	1.760±0.07b	25.877±3.21b	12.337±0.36b	0.62±0.01b	0.73±0.06b
CL	8.65±0.22a	8.92±0.21a	22.433±1.15a	22.967±0.72a	2.573±0.27a	2.107±0.03a	29.240±3.76ab	22.273±6.71a	0.66±5.77x10^{-3}b	0.77±0.08ab
E+CL	8.98±0.08a	8.72±0.70a	22.067±0.35a	22.333±0.20a	2.376±0.05ab	2.280±0.11a	31.290±2.87a	17.553±3.12ab	0.80±0.12a	0.81±0.01a

* The different letters indicate the statistically significant difference by LSD at 5% level.

Table 6.10: The effect of seed coating substances on total organochlorine fungicides residue contamination in soil and harvested rice grain

Treatment	Organochlorine residue in soil sample (mg L^{-1})		Organochlorine residue in harvested grain (mg L^{-1})	
	(Mean ± SD)			
	2005	2006	2005	2006
CO	**0.2222±0.06b**	**0.2970±0.03b**	**0.0216±6.27x10^{-3}b**	**0.0167±3.05 x10^{-3}b**
CA	**0.4403±0.07a**	**0.6470±0.10a**	**0.0540±0.01a**	**0.0413±0.01a**
CL	**0.1900±0.02b**	**0.2590±5.00 x10^{-3}b**	**0.0217±2.51E-3b**	**0.0143±4.93 x10^{-3}b**
E+CL	**0.2117±0.03b**	**0.2880±0.08b**	**0.0203±1.53E-3b**	**0.0163±1.52 x10^{-3}b**

* The different letters indicate the statistically significant difference by LSD at 5% level.

6.5 Discussion

The used biological seed coating treatments improved first of all the seedling vigor. Farooq *et al*. (2004) and Farooq *et al*. (2007) also reported that biological seed coating application increased crop stand via improving the seedling emergence and seedling vigor under field conditions. High seed vigor is positive correlated with field performance of rice. Hampton and Tekrony (1995) and Zheng *et al*. (2002) also obtained vigorous and uniform seedlings after using biological seed coating technique. In the present study, the biological seed coating treatments significantly promoted plant growth, improved the chlorophyll accumulation and other agronomic characters throughout the ontogeny of rice. The improvement of seedling establishment in coated seeds could be related to a rapid and regulated production of emergence metabolites (Basra *et al*., 2005). Lee and Kim (2000) found that the biological seed coating application enhanced the activity of α-amylase or other essential enzymes and increased the levels of soluble sugars in rice seed. Thus, germination metabolites could be provided in required amounts for improving the germination process. However, in the present study, the CA seed treatment caused poor performance of seedling including delaying and uncertainly emerging of the seedling. Farooq (2006) reported that the poor seedling performances are results of the impaired ability of the seeds in utilizing the germination metabolites. These findings are also in agreement with Ruan *et al*. (2002).

Field assessment of seed coating strategies was made in term of plant growth, and the grain yield. The improvement of plant growth in this experiment resulted from the early, uniform and vigorous seedling. Wang *et al.,* (1999) found that seed coating application improved the growth and quality of seedlings. Harris *et al*. (2002) and Farooq *et al*. (2006) emphasized that the improvement of harvest index, and kernel and straw yield

after biological seed coating application are possibly related to the increasing of dry matter partitioning and grain development. As a result, crop growth rate (CGR), net assimilation rate (NAR) and leaf area index (LAI) were increased. Moreover, the inverse relationship of mean emergence time with kernel yield and harvest index suggested that earlier establishment of seedling had persistent effect on subsequent plant growth. Yamauchi and Winn (1996)'s finding supported that the biological seed coating application (based on chitosan coating polymer) really improved vigorous seedling, plant height and reduced the period of emergence to heading and period of heading to maturity.

The current experiments found that the biological seed coating treatment enhanced the seedling vigor, growth, and chlorophyll accumulation. Furthermore, the improvement of yield can be correlated to the number of panicle bearing and leaf area duration, which is confirmed by the positive correlation between numbers of panicle bearing and kernel yield per area. These results are in agreement with Reddy (2004) who reported that healthy seedlings resulted in a higher number and uniform of stand per area and number of fertile kernels per area. Literatures supported that the increase of chlorophyll accumulation, LAI, LAD, CGR, and NAR by the use of biological seed coating substances improved the efficiency of the plant in the production and the partitioning of photoassimilates to the developing of reproductive parts (sink) (Niklas, 1994; Ashraf and Foolad, 2005; Farooq *et al*., 2006d). A high yield requires a proper balance between the size of the photosynthetic apparatus and the duration of its activity. The performance of the photosynthetic apparatus include the rate of net-photosynthesis, the rate of transportation and distribution of photoassimilates into individual organs (sink), the number and size of caryopses and their accumulation capacity of the photosynthesis products. This result was supported by other findings, e.g. the improvement of nutrient uptake and photoassimilates was increased after biological seed coating treatment (Basra *et al*., 2005, 2006; Yamauchi and Winn, 1996; Thakuria and Choudhary, 1995). Zheng *et al.*, (2002), Paul and Choudhary (1991)'s results suggested that the high minerals uptake, assimilation, partitioning and uniform distribution of photoassimilates due to biological seeds coating substances treatments results in a higher number of maturity kernels with higher crude protein content and reduced amylose content and lower number of opaque, abortive and chalky kernels.

The seed treatments affected also the 1,000 kernels weight. The CA treatment had a negative effect on the ripening ability and percentage of filled grains, whereas the biological seed coating treatments had a positive impact on 1,000 grains weight and ripening ability. Moreau (1987) and Foulkes *et al.* (1998) described five factors affecting grains filling: temperature, light, water and environmental stress, and availability of nutrients. Our results suggested that biological seed coating treatment might affected the percentage of filled grains through an increasing of nutrients uptake and better utilization of them through the rice plant resulting in increased canopy persistence. The dry matter distribution and the transport of photoassimilates toward to the panicles could be enhanced, which lead to higher final kernel yield.

Normally, the 1000-grains weight is usually more or less stable because the grain is rigidly controlled by the size of the hull, which is only subjected to slight modifications by solar radiation during the 2 weeks before anthesis (Yoshida, 1981). The filling of the hull depends on the concept: the more grains there are, the more difficult they are to fill (Moreau, 1987). The CA treatment affected the sink capacity, which decreased the 1000-grains weight and ripening ability. Such discrepancies might indicate that the rice plant lacked resources for grain filling (Moreau 1987), probably because the number of kernels was too high as compared to available resources. Moreover, fungicide injury by CA treatment also affected the LAI, LAD, CGR, NAR, chlorophyll accumulation and nutrient deficiency (Yang *et al.*, 2000). Following that, the photosynthesis rate decreased. If the photosynthesis is hindered, the grain filling will also be restricted (Borrell *el al*., 1989).

The diseases transmitted by seeds, such as root rots or leaf blight, can reduce the number of plants population throughout the vegetative period. Evidences supported that early diseases can affect sink capacity, for example, by reducing the number of fertile florets or affecting the chlorophyll content (Thomson and Gaunt, 1986). The present results confirm that grain infection and associated premature senescence of the flag leaf can be connected with reduced grain filling, final grain dry matter, and grain nutrient content (Schnyder, 1993). Fungal infection can damage photoassimilates and nutrients accumulation and partly they are important for accumulation and partitioning of dry matter associated with extended grain filling (Kraus, 1994; Ruske *et al*., 2003).

Pathogenic fungi always depend on the host tissue for their nourishment. Their aggressiveness in the host tissue varies depending upon the ecological conditions and other competing factors like the protecting compounds. In the present study, the plants growth from the control plots showed aggressive plant infection accompanied by reduced seedling establishment, plant growth rate, chlorophyll accumulation, minerals uptake efficiency, grain assimilation and yield. Fungal infection affected to damage the leaf canopy, plant stem, and grain. The effect of plant infection on reduced grain yield was confirmed by the data, which described a negative correlation between seedling infection, grain infection and grain yield. This effect on the crop growth and yield reduction was supported by other authors. Hwang *et al*., (1991) found that the percentage of emerged seedling from the seed lot with a high rate of infection was lower than the healthy seed. They also described reduced yield and product qualities. Moreover, Sürek and Beşer (1997) reported that plant infection reduced the grain yield up to 35%. Our results are also supported by other authors (Bandong *et al*., 1979; Groth *et al*., 1990; Prabhu and Filippi, 1993; Filippi and Prabhu, 1997).

The use of seed treatments can protect plant from diseases infection. Eugenol is a phenolic compound produced from plants, which are known to inhibit pathogenic fungi. On the other hand, eugenol acts like a fungicide when fungi come in contact with the host plant tissue. Host cells might produce more defense enzymes which includes peroxidase, chitinase-1,3-gluconases involved in the synthesis of phytoalexins (Van Peer *et al*., 1991; M'pigo *et al*., 1997). Hence, the effectiveness of eugenol fungicides may induce the development of resistance of host plants against plant infection.

The reduction of grain yield is correlated with minerals utilization. Our results demonstrated that CA seed treatment inhibited minerals uptake and accumulation. It inhibited plant nutrients assimilation system that have a directly impact on the reduction of minerals uptake efficiency and decrease of the capacity of minerals accumulated in the rice grain. The grain yield reduction correlated with the nitrogen deficiency results mainly from a distinctly decreased number of panicles per area, and affected to grain filling which result in term of lower 1,000-grains weight. Bould *et al*. (1984) and Schrader (1984) reported about a high yield response to the accumulation of nitrogen when at least 70% of nitrogen contained in the leaves and the culm can be transported to the developing grains and improve the grain filling. Peter *et al*. (1988) reported that captan had

depressive effects on soil nitrification. It directly affects some soil organism variables, as ureolytic bacteria like *Proteus* sp., and *Micrococcus* sp. These soil organisms produced urease enzyme, which is an enzyme containing several –SH groups in its active site. Captan is known to exert its toxic effect through interaction with this group (Banerjee and Banerjee, 1987). Moreover, our results corroborate those of Chamber and Montes (1982) reported that captan reduced plant growth, seed yield, and was correlated with nitrogen uptake and accumulation. Thus, a number of studies have found that captan seed treatment had a direct affect on nitrogen assimilation and accumulation, which affected grain filling (Dillwith and Lewis, 1982; Tam and Trevors, 1981).

In the present study, CA treatment inhibited not only nitrogen uptake but also the uptake of other minerals. The other minerals also play an important role in the overall cell metabolism. They are incorporated into the structure of some components (N, S, P, and Mg), participate in energy-metabolism reactions (P), act as electron donors in redox reactions (Fe, Cu, Mn, Co) and perform the function of cofactors in many enzyme systems (K, Na, Mg, Ca) (De Datta, 1981). Finally, being unevenly distributed on either side of a cell membrane (K, Na), it gives rise to electric potential difference (Wells *et al*., 1988). The evidences shown that, in spit of the inhibition of assimilate efflux from leaves, occurring already at an early stage of nitrogen deficiency, and then reduced the other minerals uptake, the loading of photosynthesis products into phloem endings and their propagation through leaf veins to accumulate into the sink continue unabated during the grain-filling period (Tanaka and Yoshita, 1970).

P is involved in a broad range of metabolic reactions. In the case of P deficiency, the cytoplasm of photosynthesis cells produces little transportable sucrose because triose phosphates are retained in chloroplasts and converted to starch (Shu *et al*., 2004). More generally, the role of P boils down to its involvement in energy transport in the nucleoside-phosphate systems as well as in activation of sugars and intermediate metabolites, which is to some or other degree essential for all transport stages. Growth, or the activity of sink tissue meristems, is highly sensitive to P deficiency (Sung, 1990). This is why the decreasing of the plant productivity associated with the increasing of P deficiency occurs already before inhibition of export of triose phosphates from chloroplast, chiefly as result of lack of demand on the part of sink tissues (Kursanov, 1984). While, K is also essential for the action potentials (excitation waves) spreading in

the phloem. K deficiency resulted in a marked decrease in the cross-section area of conducting bundles and shortening of sieve tubes. The effect of K on phloem transport has long been speculated that the K promotes the translocation of sugars from the leaves to storing into the sink, which directly affected grain filling (Zhou *et al*., 2007). Moreover, Na deficiency leads to the lack of phytohormones in the plant tissues, their addition (GA_3 and cytokinin), when phytohormones play an essential role in regulating plant growth and development. Phytohormones have been implicated in many developmental processes and environmental responses of plants, including leaf senescence, apical dominance, chloroplast development, and the regulation of cell division and sink/source relationships (Hutchison and Kieber, 2002). Phytohormones are regarded as the most important senescence-retarding plant hormone (Fenn and Gobran, 1999) and their deficiency resulted in the degradation of chlorophyll and photosynthetic proteins (Wingler *et al.*, 1998). It has been demonstrated that the role of Ca deficiency indirectly caused changes of phloem transport, linked to the Ca-enhanced activities of glutamine synthetase (GS), glutamate synthase (GOGAT), ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and correlated to N utilization, which directly declined grain filling (Fenn and Gobran, 1999).

It is assumed that B caused heavy callose deposition on transverse and longitudinal sieve tube walls, which may seem to be a variance on phoem transport. It influences transpiration through the control of sugar and starch formation. It also influences on cell development and elongation. In annual crops, the symptoms of B deficiency vary from one species to another. B affects carbohydrate metabolism and play a role in amino acid formation and the synthesis of protein (Gupta, 1979). B deficiency resulted on the retarded new growth and development. In black gram, the B deficiency is not visible in the seed, but the yield may fall by as much as 50%. In peanut or soybean, B deficiency often results in an empty space within the seed, known as "hollow heart" (Tisdale *et al*., 1985). A common result of boron deficiency in all crops is an interruption of flowering and fruiting. Yields are poor, and the fruit or grain is deformed or discolored (Kingsolver *et al*., 1984).

Cu deficiency affects the formation of grains, seeds and fruit (Penney *et al*., 1988). The main reason for the poor development of seeds and fruits because Cu-deficiency affects the viability of pollen (Marschner, 1986). The main symptom of Fe deficiency is chlorosis

or yellowing between the veins of new leaves (Cheng and Rerkasem, 1993). Fe is essential for the synthesis of chlorophyll. It is involved in N fixation, photosynthesis, and electron transport. Fe is required in protein synthesis and several key enzymes in plants (Tisdale, 1985). Fe deficiency severely depresses nodule mass, hemoglobin content and crop yield, which affected the distribution of the photosynthates assimilating toward to grain (Tang *et al.* 1992). Similar effects are observed from Mn deficiency. Mg declined assimilates for grain filling and showed a correlation with the shoot dry weight and nitrogen content (Ratanarat *et al.*, 1990). Our results showed that the biological treatment led to improved nutrients uptake, e.g. P, K, S, Fe, Na, Ca, Mg, Cu, Al and B. On the other hand, the uptake of some nutrients was not affected, e.g. N, S, Mn and Zn. CA treatment led to the lowest minerals uptake and accumulation. In consequence, decreased final grain yields were obtained.

In the present experiment, captan fungicide residue was found in both soil and rice grain. Although, the amounts of captan residue found in the soil and grain sample were not high, the concentration of these persistend fungicide will cause serious problems in long term scales. Nevertheless, the concentrations found in both season surveys were different. It was relevant to the amount of precipitation when season 2005 had higher precipitation than season 2006 (Appendix 17). Edwards (1970) demonstrated that when the water flooding led to anaerobic conditions, it resulted in rapid degradation of fungicide residues. Furthermore, previous study have reported that the soil properties i.e. soil pH, organic content, minerals content and soil texture influenced the quantity of fungicide residues in the soil (Soil Survey Division, 2000). The amount of captan residues found in rice grain was not over the maximum residue limit (MRL) of the FAO (1.0 mg kg^{-1} DM) (FAO, 2004). The investigations confirmed that most of rice grain sample collected from control and biological seed coating substance treatments contained less amounts of organochloride fungicide residues than the tolerance limit of FAO. However, captan residue was found at high levels, especially, on the plot that used captan seed treatment application. These grain samples were not considered as safe for human or animal consumption because of the chronic toxicity and multiple accumulations in trophic level. Captan residue can be accumulated in kidney and blood and it is mutagenic and clastogenic (Tinston, 1995).

The results are significantly different between growing seasons that caused by the environmental factors i.e. light intensity, precipitation, day length, temperature, and relative humidity (Appendix 17). Drought stress is the most important factors reducing the grain yield (Woodruff, 1983). Late seedling diminished the growth, leaf viability, and grain yield, because the delay in development exposed plants to the increasingly adverse temperatures during maturation (Paulsen, 1994). The optimum sowing date of August is obviously a compromise among conservation of moisture, day length, date of maturation, and weather conditions. Moreover, in this study, the two-year experiments had been conducted in the same field, and it led to a prediction that climatic factors might influence the yield and subsequently the grain qualities.

6.6 Conclusion

This experiment found that the CA seed treatment application completely controlled fungi infection in seedling and grain. However, the chemical compounds, which were used here, influenced seed germination, seedling establishment, plant growth rate and other mechanisms related to the plant productivity. Therefore, many academic researches are explored and examined the new seed treatment application that has fewer side effects on plant production system. Seed coating with biological substances is a possibility, which provides a satisfied accomplishment in plant production system. This technique can protect plants from various kinds of pest, improve stand quality and uniformity, increase yields, and increase return on investment.

These findings strongly suggest that seed coating with E+CL, which was studied here, is an interesting strategy in direct-seed rice production system. Physiological changes occurring in kernels led to the increase of yield. E+CL polymer improved seedlings vigor, caused better plant growth, improved chlorophyll accumulation, yield attributes, and minerals uptake efficiency. Moreover, it did not produce any undesirable chemical residue contaminating soil and grain.

6.7 References

Anonymous. (2004). http://home.t-online.de/home/hydrobio.hw/bterms.htm, visited at 01.11.2004

Ashraf, M., and Foolad, M.R. (2005). Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv Agron. 88, 223-271.

Bandong, J.M., Nuque, F.L., Torres, C. and Crill, J.P. (1979). Leaf blast control by seed treatment with systemic fungicide. Int. Rice Res. Newsl. 4, 16-17.

Banerjee, A., and Banerjee, A.K. (1987). Influence of Captan on some organisms and microbial process related to the nitrogen cycle. Plant and Soil. 102, 239-245.

Basra, S.M.A., Farooq, M., Hafeez, K., Ahmad, N. (2004). Osmohardening: a new technique for rice seed invigoration. Inter Rice Res Notes. 29, 80-81.

Basra, S.M.A., Farooq, M., and Tabassum, R. (2005). Physiological and biochemical aspects of seed vigor enhancement treatments in fine rice (*Oryza sativa* L.). Seed Science and Technology. 33, 623-628.

Basra, S.M.A., Farooq, M., Afzal, I., and Hussian, M. (2006). Influence of osmopriming on the germination and early seedling growth of coarse and fine rice. International Journal of Agriculture & Biology. 1, 19-22.

Borrell, A.K., Incoll, L.D., Simpson, R.J., and Dalling, M.J. (1989). Partitioning of dry matter and the deposition and use of stem reserves in a semi-dwarf wheat crop. Ann. Bot. 63, 527-539.

Bould, C., Hewitt, E. J., and Needham, P. (1984). Diagnosis of mineral disorders in plants. Vol. 1, Principles. Chemical Publishing, New York.

Chamber, M.A., and Montes, F.J. (1982) Effects of some seeds disinfectants and methods of rhizobial inoculation on soybeans (Glycine max (L.) Merrill). Plant and Soil 66, 350-360

Cheng, C., and Rerkasem, B. (1993). Effects of boron on pollen viability in wheat. Plant and Soil 156, 313-315.

Chowdhary, A.Q., and Baset, Q.A. (1994). Effect of soaking period and aerobic condition on germination of wheat seeds. J. Chittagang Univ., 18, 83-87.

De Datta, S.K. (1981). Principles and practices of rice production. John Wiley&Sons, New York.

Dell Aquilla, A., and Tritto, V. (1990). Ageing and osmotic priming in wheat seeds: effects upon certain components of seed quality. Ann Bot., 65, 21-26.

Dillwith, J.W., and Lewis R.A. (1982). Inhibition of DNA polymerase by Captan. Biochem Biophys. Acta. 696, 245-252.

Duggen, R.E. (1969). Pesticide analytical manual volume I: Method, which detect multiple pesticides. Us. Department of Health, Education and Welfare, Food and Drug Administration.

Du, L.V., Tuong, T.P. (2002). Enhancing the performance of dry-seeded rice: effects of seed priming, seedling rate, and time of seedling. In: Pandey S., Mortimer M., Wade L., Tuong T.P., Lopes, K., Hardy B. (eds) Direct seeding: research strategies and opportunities. International Rice Research Institute, Manila, Philippines, 241-256.

Edwards, C.A. (1970). Persistent pesticides in environment. Critical review in environmental control. 1(1), 7-67.

FAO. (2004). FAO committee on pesticides in agriculture. Rome PL/2004. 9.

Farooq, M., Basra, S.M.A., Kareem, H.A., and Afzal, I. (2004). Optimization of seed hardening techniques for rice seed invigoration. Emirates J Agric Sci. 16, 48-57.

Farooq, M., Basra, S.M.A., Haffez, K. (2005). Thermal hardening: a new seed vigor enhancement tool in rice. J Integr Plant Biol. 47, 187-193.

Farooq, M., and Basra, S.M.A. (2006). Priming of field-sown rice seed enhance germination, seedling establishment, allometry and yield. Plant Growth Regul. 49, 285-294.

Farooq, M., Basra, S.M.A., Khalid, M., Tabassum, R., and Mehmood, T. (2006d). Nutrient homeostasis, reserve metabolism and seedling vigor as affected by seed priming in coarse rice. Can J Bot. 84, 1196-1202.

Farooq, M., Basra, S.M.A., Ahmed, N. (2007). Improving the performance of transplanted rice by seed priming. Plant Growth Regul. 51, 129-137.

Fenn, L.B., and Gobran G.R. (1999). Willow tree productivity on fertilizer solutions containing various Ca/Al ratios. Nutrient Cycling in Agro ecosystems. 53(2), 121-132.

Filippi, M.C., and Prabhu, A.S. (1997). Integrated Effect of Host Plant Resistance and Fungicidal Seed Treatment on Rice Blast Control. The American Phytopathological. 351-355.

Foulkes, M.J., Sylvester-Bradley, R., and Scott, R.K. (1998). Evidence for differences between wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. Journal of Agricultural Science. Cambridge 130, 29–44.

Groth, D.E., Rush, M.C., and Lindberg, C.D. (1990). Foliar fungicides for control of rice diseases in the United States. in: Pest Management in Rice. B. T. Grayson, M. B. Green, and L. G. Copping eds. Elsevier Applied Science, London. 31-52.

Gupta, U. C. (1979). Boron nutrition of crops. Adv. Agron. 31, 273-303.

Hampton, J.G., and Tekrony, D.M. (1995). Handbook of ISTA vigor test methods, 3rd ed. ISTA, Zurich. 10.

Harris, D., Tripathi, R.S., and Joshi, A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. In:Pandey S, Mortimer M, Wade L, Tuong TP, Lopes K, Hardy B (eds) Direct seeding : research strategies and opportunities. International Research Institute, Manila, Philippines, 231-240.

Hwang, S.F., Lopetinsky, K., and Evans, I.R. (1991). Effects of seed infection by *Ascochyta* spp. Fungicidal seed treatment and cultivar on yield parameters of rice under field conditions. Canadian Plant Disease Survey. 71(2), 169-172.

Hutchison, C.E., and Kieber, J.J. (2002). Cytokinin signaling in Arabidopsis. Plant Cell 14, 47-59.

Jett, L.W., Welbaum, G.E., and Morse, R.D. (1996). Effects of matric and osmotic priming treatments on broccoli seed germination. J Am Soc Hort Sci. 12, 423-429.

Khan, A.A. (1992). Pre-plant physiological seed conditioning. In: Janick J. Hort (ed) Reviews, vol. 14. Willey, NY, 131-181.

Kingsolver, C.H., Barksdale, T.H., and Marchetti, M.A. (1984). Rice blast epidemiology. Bull. Agric. Exp. Stn., The Pennsylvania State University, University Park. 853.

Kraus, T.E. (1994). Paclobutrazol protects wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? Plant and cell physiology. 35, 45-52.

Kursanov, A.L. (1984). Assimilate transport in plants. K.A. Timiryazev Institute of Plant Physiology, USSR Academy of Sciences. Elsevier. 410-418.

Lee, S.S., and Kim, J.H. (2000). Total sugars, α-amylase activity, and emergence after priming of normal and aged rice seeds. Kor. J Crop Sci. 45, 108-111.

Marschner, H. (1986). Mineral nutrition of higher plants. Academic Press, Orlando, FL.

M'pigo, P., Belaner, R.R., Paulitz, T.C., and Benhamou, N. (1997). Increased resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* in tomato plants treated with endophytic bacterium *Pseudomonas fluorescens* strain 63-628. Physiological and Molecular Plant Pathology. 50, 301-320.

Moreau, D. (1987). *L'analyse de L'e_laboration du Rendement comme Outil de Diagnostic*. Paris: Groupe de Recherché et d'e_ changes technologiques.

Niklas, K.J. (1994). Plant allometry. The University of Chicago Press, Chicago, Illinois.

Office of Agricultural Economics. (2006). Area, production, yield, farm price and farm value, 1996-2005. Available at http://www.oae.go.th/ in 4-05-07.

Paul, S.R., and Choudhary, A.K. (1991). Effect of seed priming with salts on growth and yield of wheat under rain fed conditions. Ann Agric Res. 12, 415-418.

Paulsen, G.M. (1994). High temperature responses of crop plants. *In* K.J. Boote et al. (ed.) Physiology and determination of crop yield. ASA, CSSA, and SSSA, Madison, WI. 365–389.

Reddy, S. (2004). Agronomy field crops. Kalyani Publishers, New Delhi, India.

Penney, D.C., Solberg, E.D., Evans, I.R., and Piening, L.J. (1988). The copper fertility of Alberta soils. Great Plains Soil Fertility Workshop Proceedings, Vol.2 Kansas State University. Manhattan, KS 66506.

Peter, J., Ĉernŷ, V., and Hruška, L. (1988). Yield formation in the main field corp. Development in crop science (13). University of Agriculture, Prague-Suchdol, Czechoslovakia. Elsevier. 72-151.

Prabhu, A.S., and Filippi, M.C. (1993). Seed treatment with pyroquilon for the control of leaf blast in Brazilian upland rice. Int. J. Pest Manage. 39, 347-353

Ratanarat, S., Masangsan, W., Vadeesirisak, P., Ditsantia C., and Phanuvas, V. (1990). Effects of ion foliar spray and specific Rhizobium strains on yield components of three peanut cultivars. In: Proceedings, 8th National Groundnut Research Meeting. 3-5 May 1989, Roi-Et, Thailand. (In Thai).

Ruan, S., Xue, Q., Tylkowska, K. (2002). Effects of seed priming on emergence and health of rice (*Oryza sativa* L.) seeds. Seed Science and Technology 30, 451-458.

Ruske, R.E., Gooding, M.J., and Jones, S.A. (2003). The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning, remobilization and grain N accumulation in winter wheat cultivars. Journal of Agricultural Science. 140, 395–407.

Schrader, L. E. (1984). Functions and transformations of nitrogen in higher plants. Pages 55-66 in: Nitrogen in Crop Production. R. D. Hauck, ed. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Schnyder, H. (1993). The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling. New Phytol. 123, 233-246.

Shu Q.C., Rong X.Z., Wei, L., Zhi R.D., and Qi M.Z. (2004). The Involvement of cytokinin and abscisic acid levels in roots in the regulation of photosynthesis function in flag leaves during grain filling in super high-yielding rice (Oryza sativa). J. Agronomy and Crop Science. 190, 73-80.

Soil Survey Division. (2000). The detailed soil survey of Nong Wai Pioneer Agriculture Project. Bangkok: Department of Land Development.

Sung, F.J.M., and Lo, W.S. (1990). Growth responses of rice in ammonium-based nutrient solution with variable calcium supply. Plant and Soil. 125(2), 239-244.

Sürek, H., Beşer, N. (1997). Effect of blast disease infection on rice and milling yield. In the international symposium on rice quality Nottingham, U.K. November 24-27, 1997. Chair optiuns Med 24(3) and paper no: 35

Tam, T.Y., and Trevors, J.T. (1981). Effect of pentachlorophenol on symbiotic nitrogen fixation in soil. Water Air Soil Pollut. 16, 409-414.

Tang, C., Robson, A.D., Dilworth M.J., and Kuo., J. (1992). Microscopic evidence on how iron deficiency limits nodule initiation in Lupinus angustifolius L. New Phytol. 121, 457-467

Tanaka, A., and Yoshita, S. (1970). Nutritional disorders of the rice plant in Asia. Int. Rice Res. Inst. Tech. Bull. 10.

Thakuria, R.K., and Choudhary, J.K. (1995). Effect of seed priming, potassium and anti-transparent on dry-seeded rain fed rice (*Oryza sativa* L.). Ind J Agron. 40, 412-414.

Thomson, W.J., and Gaunt, R.E. (1986). The effect of speckled leaf blotch on apical development and yield in winter wheat in New Zealand. Ann. Bot. 58, 39-48.

Tinston, D.J. (1995). Captan: Investigation of duodenal hyperplasia in mice. Unpublished report No. CTL/P/4532 from Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, United Kingdom. Submitted to WHO by ZENECA Agrochemicals, Fenhurst, Haslemere, Surrey, United Kingdom.

Tisdale, S. L., Nelson, W. L., and Beaton, J. D. (1985). Soil: Fertility and fertilizer. 4th ed. MacMillan, New York.

Van Peer, G.V.N., and Scheppers, B. (1991). Induced resistance and phytoalexin accumulation in biocontrol of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS 417 r. Phytopathology. 81, 728-734.

Wang, Y., Tasaka, K., Ogura, A., and Maruyama, S. (1999). Growth and physiological characteristics of rice seedlings raised with long mat by hydroponics-Comparison with young seedlings raised in soil. Plant Prod. Sci. 2(2), 115-120.

Wells, B.R., Norman, R.J., Lee, F.N., and Moldenhauer, K.A.K. (1988). Integrating management practices to maximize rice yields. J. Fert. Issures. 5(1), 14-18.

Wingler, A., Von Schaewen, A., Leegood, R.C., Lea, P.J., and Quick, W.P. (1998). Regulation of leaf senescence by cytokinin, sugars, and light. Plant Physiol. 116, 329-335.

Woodruff, D.R. (1983). The effect of a common date of either anthesis or planting on the rate of development and grain yield of wheat. Aust. J. Agric. Res. 34, 13-22.

Yamauchi, M., and Winn, T. (1996). Rice seed vigor and seedlings establishment in anaerobic soil. Crop Sci. 36. 680-686.

Yang, J.C., Peng, S.B., Visperas, R.M., Sanico, A.L., Zhu, Q.S., and Gu, S.L. (2000). Grain filling pattern and cytokinin content in the grains and roots of rice plants. Plant Growth Regul. 30, 261-270.

Yoshida, S. (1981). Fundamentals of Rice Crop Science. Los Banos: IRRI.

Zheng, H.C., Jin, H.U., Zhi, Z., Ruan, S.L., and Song, W.J. (2002). Effect of seed priming with mixed-salt solution on emergence and physiological characteristics of seedling in rice (*Oriza sativa* L.) under stress conditions. J. Zhejiang Uni. 28, 175-178.

Zhou, A., Lam, H.M., and Zhang, J. (2007). Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice**.** Journal of Experimental Botany. 58(5), 1207-1217.

7. Effects of seed coating substances on rice grain quality

7.1 Abstract

High water requirement and increasing labor costs are the major problems of the traditional rice production system. Direct seed rice production, growing rice without standing water, can be an attractive alternative. However, seedling attacking by fungi results in poor emergence and seedling establishment is the main hindrance in the adoption of this cultural practice. An attempt to improve the performance of direct seed rice, which influences on rice grain nutritional value and physicochemical properties by seed coating applications, was made in this study. Seeds were treated with various substances i.e. captan (CA), only chitosan-lignosulphonate polymer (CL) and eugenol incorporated into chitosan-lignosulphonate polymer (E+CL). Untreated seeds were used as control (CO). The results of growing in seasons 2005 and 2006 indicated that biological coating treatments, CL and E+CL improved harvested grain qualities. However, CA treatment impaired products qualities more than CO did. Furthermore, the pasting properties of harvested rice grains were not affected by seed treatment variants. On the other hand, the climatic environment was a major factor which influenced the product qualities. The results confirm that E+CL may be a potential seed coating substance which can improve direct seed rice production system.

7.2 Introduction

Food security in the world is challenged by increasing food demand and is also threatened by declining water availability. More than 75% of rice is produced on 79 million ha of irrigated land. Thus, the present and the future food security depend largely on the irrigated rice production systems (Office of Agricultural Economics, 2006). However, the water-use efficiency of rice is low whereas growing rice requires large amount of water. In Asia, irrigated agriculture accounts for 90% of total diverted freshwater and more than 50% of this is required to irrigate rice field (Huang *et al*., 1998). Until recently, this amount of water has been granted, but now “the global water crisis” is threatening the sustainability of irrigated rice production. The researchers are looking for possibilities to decrease water use in rice production, and to increase the efficiency of water use at the same time (Huaqi *et al*., 2002). Alternative methods of establishing crops, especially rice, that require less labor and water without sacrificing productivity are needed (IRRI, 2007). A fundamental approach to reduce water inputs in rice is to grow it in irrigated upland like wheat and

maize. Balasubramanian and Hill (2002) hypothesized that the direct seeding is an appropriate alternative to the traditional transplanting method. Poor germination, uneven crop stand and high seedling damage due to diseases infection are the main constraints to its adoption (Du and Tuong, 2002).

Seed coating technology is a seed invigoration techniques being used to reduce the germination time, get synchronized germination, improve germination rate, protect seedling infection and provide better seedling stand in many field crops like wheat and maize (Basra *et al*., 2002) and rice (Basra *et al*., 2003; Farooq *et al*., 2004a). Furthermore, the invigoration persists under high and low temperature (Lee *et al.*, 1998; Ruan *et al*., 2002) and both at high and low soil moisture content (Du and Tuong, 2002). These invigoration techniques can be applied by coating the seed with various substances, which provided the vigorous and clean seedling, or coating with growth promoters or pesticides which protect seedlings from disease or insect attack (Loehken, 1990). The implications of seed coating application, which might have an impact on the nutrition value and processing properties of harvested grain are lacking or not reported along with morphological characters (Du and Tuong, 2002). Thus, the present study aimed to investigate the effects of appropriate seed coating substances on the quality of harvested grain.

7.3 Materials and methods

Dry graded rice seeds (*Oryza sativa* L. cv. KDML 105) from one seed lot were supplied from Bureau of Seed Multiplication of Thailand. The initial seed moisture content and germination percentage were 10.65% and 96.00%, respectively. The field experiments were conducted at the Agronomy Research Station, Faculty of Agriculture, Department of Agronomy, Chiang Mai University, Chiang Mai, Thailand during August - December in 2005 and 2006. The randomized complete block design (RCBD) with four replications was applied. The treatments were: control (CO), captan (CA), only chitosan lignosulphonate polymer (CL) and eugenol incorporated into chitosan lignosulphonate polymer (E+CL). The field management as soil preparation, fertilizer and irrigation application, and harvesting process is described in chapter 6.

The experiments were done by using dehulled grains with 12±0.5 % MC. Then, freeze-dried materials of rice grains were grounded (UDY, Cyclone Sample Mill No.2). Nutrition values were analyzed by using different standard procedures: the contents of total protein (AOAC, 2000), lipids (Lam and Proctor, 2000), free fatty acid (FFA; Lam and Proctor,

2000), carbohydrates (calculation according to Merrill and Watt, 1973), amylose content (Perez and Juliano, 1979), sugar content (Luff-Schoorl method, 1929), total antioxidant activity (Kim *et al*., 2002), lipoxygenase activity (LOX; Altenbrunn, 1995), superoxide dismutase activity (SOD; Oberley and Spitz 1985) and ascorbate peroxidase activity (APX; Nakano and Asada, 1981). All samples were tested in four replications. The detail of each analyze is presented in chapter 5.

Viscosity and pasting properties of rice flour were analyzed using a Rapid Visco Analyser (RVA) Series 4 instruments (Newport Scientific, Sydney-Australia). For viscosity and pasting analysis using a standard method (Newport Scientific Method 10, Version 4, December 1997), 25 ± 0.1 ml of distilled water was added to 3.00±0.01 g of ground rice (12 % MC) in the canister. The samples were heated under standard stirring conditions first held at 50°C for 1 min, and then heated to 95°C with 11.84 °C/min. The samples were then held at 95°C for 7 minutes followed by cooling to 50 °C for 5 min and holding for 1.4 min at a rotation speed of 160 rpm. Viscosity was measured in Rapid Viscosity Units (RVU). All samples were tested in four replications.

From the obtained RVA curves the following specific pasting properties were read: pasting temperature (PT): temperature indicating an initial increase in viscosity, (°C), peak viscosity (PV): maximum viscosity during the heating cycle, (RVA units), trough: hot viscosity (HV) at the beginning of temperature decline, breakdown viscosity (BD): peak viscosity minus hot viscosity (HV) at 95 °C (PV-HV), setback (SB): final viscosity (FV) minus peak viscosity (FV-PV). A typical pasting curve is presented in Figure 7.1.

The data are presented as mean ± standard deviation. The analysis of variance was performed for data analysis and differentiated with LSD test at $p<0.05$ using the software SX release 8.0 (Analytical software, Tallahassee, USA).

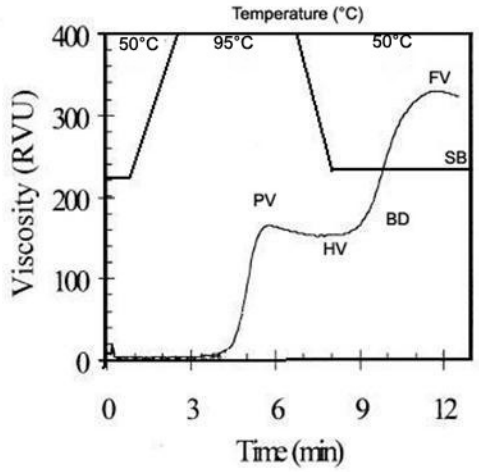


Figure 7.1: Typical pasting profiles of rice flour (cv. KDML105) obtained from RVA showing peak viscosity (PV), the hot viscosities at 95°C (HV), breakdown viscosity (BD), final viscosity (FV) and setback (SB)

7.4 Results

The ANOVA results indicated that the applied seed treatment variants significantly affected FFA, LOX, SOD and total antioxidant activity in the rice grain, whereas the growing season significantly affected FFA, SOD, HV, BD and FV (Table 7.3). However, the interaction between seed treatment variants and growing season did not affected rice grain qualities (Table 7.3). The data of total protein content of rice grain in season 2005 and 2006 are presented in Figure 7.2A. The results indicated no significant difference between seed treatment variants. Nevertheless, CL and E+CL treatments provided a higher tendency of protein content than the CA treatment did. As shown in Table 7.1, the growing season did also not affect the total protein content significantly. However, the total protein content in the rice grain was positively correlated with N, P, K, Mg but negatively correlated with total carbohydrate content, total antioxidant activity and LOX (Table 7.5 and 7.6).

The total lipid contents of rice grain in season 2005 and 2006 are presented in Figure 7.2B. No significant differences were found among seed treatment variants. Nevertheless, the CL and E+CL treatments provided a higher tendency of total lipid content than the CA treatment did. The data in Table 7.1 shows that no significant differences were found between growing seasons. Table 7.5 and 7.6 showed positive correlations among total lipid content, N, S and Mg but negative correlations with FFA, total antioxidant activity and LOX.

Figure 7.2C shows the amount of FFA content which were harvested from the different of seed treatment variants. These variants had no effects on the FFA content. Seeds from CO and CA treatments showed a tendency to higher amounts of FFA than the seeds from the biological treatments. Table 7.1 indicated that the growing season significantly affected the FFA content. It had a negative correlation with amylose and sugars content and SOD (Table 7.5 and 7.6).

Both the seed treatment variants and the growing season did not affect the amylose content (Figure 7.2D, Table 7.1) which was positive correlated with the S content (Table 7.5 and 7.6).

The data of carbohydrate content are presented in Figure 7.2E. No significant differences were found between seed treatment variants and the growing season did not affect the total carbohydrate content (Table 7.1). Correlation coefficient analysis shows that carbohydrate content had a high positive correlation with K content. However, carbohydrate content was negative correlated with protein and sugar content (Table 7.5 and 7.6).

As shown in Figure 7.3, sugar content was not affected by seed treatment variants and growing seasons (Table 7.1). It had high positive correlations with K, Mg and Mn content and negative correlations with carbohydrate content (Table 7.5 and 7.6).

The effect of seed treatment variants on the activity of the two free radical scavenging enzymes ascorbate peroxidase (APX) and superoxide dismutase (SOD) is presented in Figure 7.4. As shown in Figure 7.4A, seed treatment variants did not affect the activity of APX. The CL and E+CL treatments provided a trend of higher APX than CA seed treatment did. The result suggested that the growing season did not affect the activity of APX (Table 7.1). The correlation coefficient analysis shows that the activity of APX had a positive correlation with N content and a negative correlation with LOX (Table 7.5 and 7.6).

Figure 7.4B summarized the activity of SOD. In season 2005, there was a significant difference between seed treatment variants. The CA treatment caused the lowest SOD activity. In 2006, no significant differences were found between seed treatment variants. However, the results revealed that the CL and E+CL treatments provided a higher SOD activity than CA treatment did. The activity of SOD was significant different between the

growing seasons (Table 7.1). The correlation coefficient analysis (Table 7.5 and 7.6) showed that the activity of SOD had a high positive correlation with sugars content and a negative correlation with FFA content.

In season 2005, the seeds of the CA treatment showed the significantly highest LOX activity compared to the seeds of the other three treatments (Figure 7.4C). However, in season 2006, none of these differences was statistically significant. The growing season did not have a significant effect on the activity of LOX. The correlation coefficient analysis showed that the activity of LOX was positive correlated with FFA content and negative correlated with sugars content and the activity of APX and SOD (Table 7.5 and 7.6).

The seed treatment variants significantly affected the total antioxidant activity (Table 7.2). The CA treatment provided the lowest total antioxidant activity. There was a significant difference when compared CO, CL and E+CL treatments. The growing season did not have a significant effect on the total antioxidant activity in the rice grain (Table 7.1). The correlation coefficient analysis that showed the total antioxidant activity had a high positive correlation with total carbohydrate content and negative correlation with protein, lipid and sugar content (Table 7.5 and 7.6).

Viscosity profiles of rice grains were analyzed to study the impact of seed treatment variants on seed flour pasting characteristics. Table 7.4 demonstrated that no significant differences were found among seed treatment variants on the pasting profile (time to gelatinization, time to peak viscosity and PV) and viscosity attributes (HV, BD, FV and SB). Table 7.1 provided the effects of growing seasons on the pasting characteristics of harvested rice grains. Significant differences were found among peak time, BD, HV and FV, but not among PT, PV and SB.

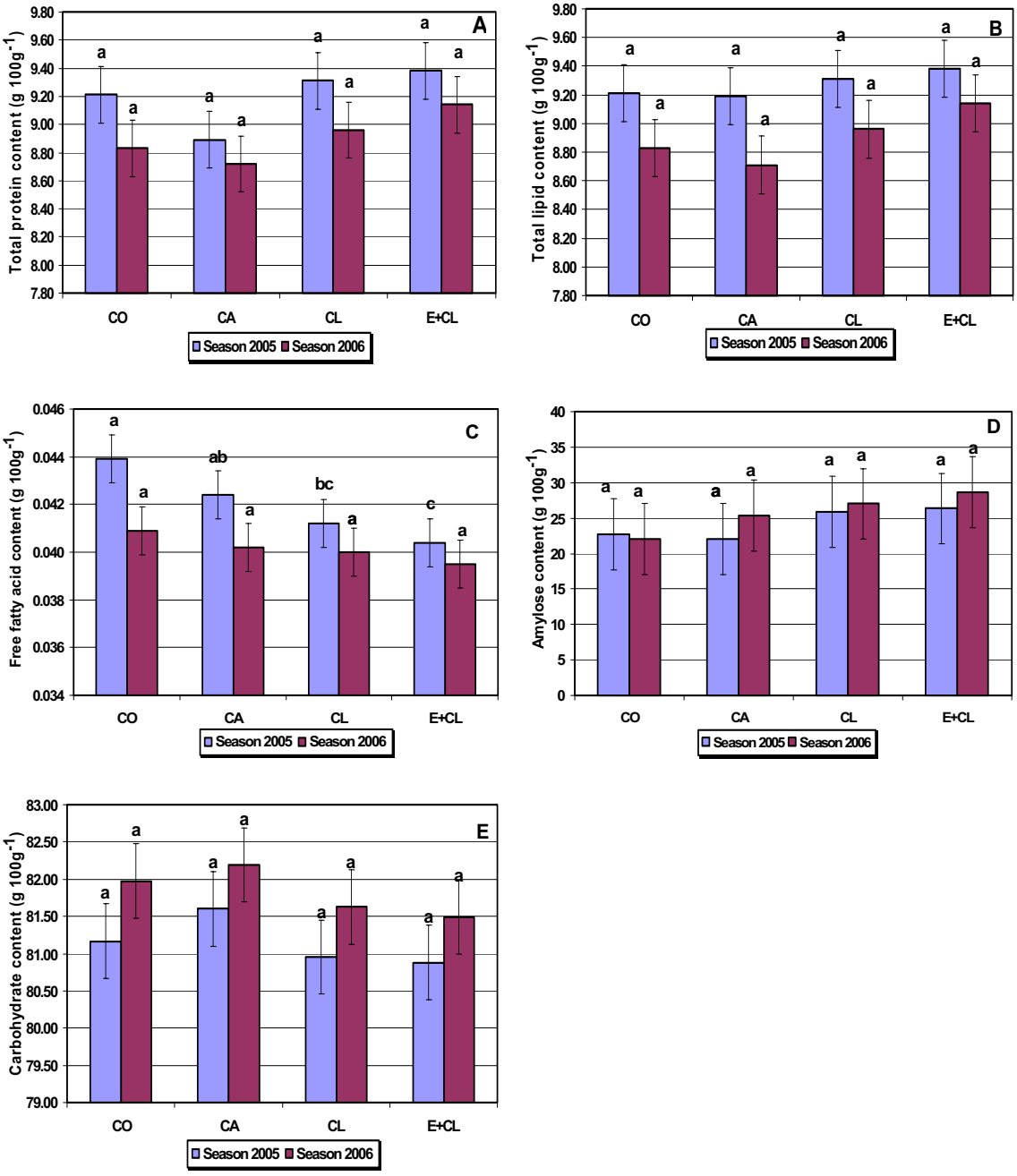


Figure 7.2: Effects of seed treatment variants on the protein content (A), total lipid content (B), free fatty acid content (C), amylose content (D), and carbohydrate content (E) of rice grains harvested on seasons 2005 and 2006. The data are presented as mean ± SD*
*: The different letters indicate the statistically significant difference by LSD at 5% level

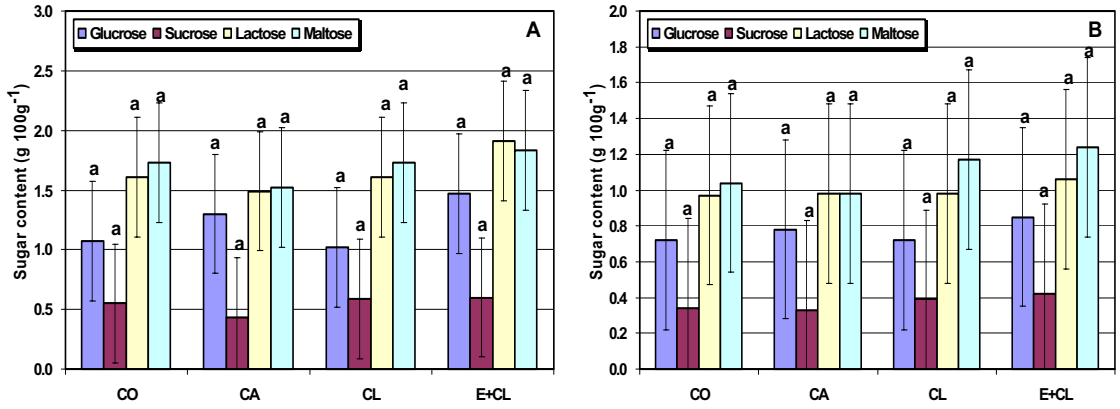


Figure 7.3: Effects of seed treatment variants on sugar content of rice grains harvested on seasons 2005 (A) and 2006 (B).
The data are presented as mean ± SD*
*: The different letters indicate the statistically significant difference by LSD at 5% level

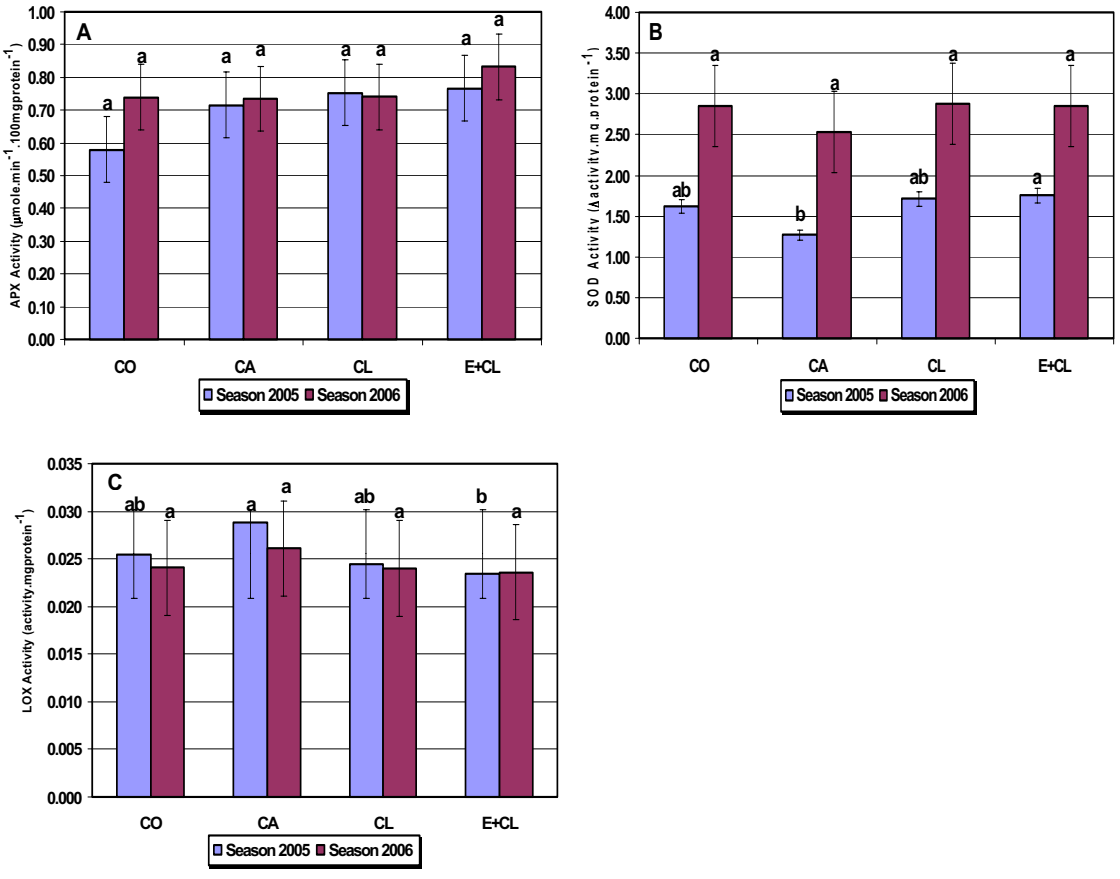


Figure 7.4: Effects of seed treatment variants on enzyme activities in rice grains harvested in seasons 2005 and 2006: APX (A). SOD (B), and LOX (C).
The data are presented as mean ± SD*
*: The different letters indicate the statistically significant difference by LSD at 5% level

Table 7.1: Differences between growing seasons in average yield, quality parameters and pasting properties in rice grains

Parameter**	Planting season (Mean ± SD)		Statistic significance
	2005	2006	
Yield (t.ha^{-1})	**3.862 ± 0.381**	**3.923 ± 0.635**	**NS**
Rice grain quality:			
Total protein content (g 100g^{-1}DM)	**9.18 ± 0.287**	**8.92 ± 0.297**	**NS**
Total lipid content (g 100g^{-1}DM)	**9.27 ± 0.232**	**8.92 ± 0.395**	**NS**
Free fatty acid content (g 100g^{-1}DM)	**0.0420±1.529E-03**	**0.0402±8.806E-04**	*****
Carbohydrate content (g 100g^{-1}DM)	**81.15 ± 0.338**	**81.82 ± 0.385**	**NS**
Amylose content (g 100g^{-1}DM)	**25.75 ± 3.247**	**24.23 ± 3.010**	**NS**
Total antioxidant activity (IC_{50})	**0.1317 ± 0.022**	**0.1046 ± 0.014**	**NS**
APX activity (µmole min^{-1} 100 mg^{-1}protein)	**0.6684 ± 0.125**	**0.7390 ± 0.123**	**NS**
SOD activity (Δactivity mg^{-1}protein)	**1.5389 ± 0.278**	**2.7825 ± 0.556**	*****
LOX activity (Δactivity mg^{-1}protein)	**0.0261±2.837E-03**	**0.0247±1.892E-03**	**NS**
Sugar content:			
Glucose (g 100g^{-1}DM)	**1.00 ± 0.348**	**0.80 ± 0.297**	**NS**
Sucrose (g 100g^{-1}DM)	**0.54 ± 0.186**	**0.47 ± 0.272**	**NS**
Lactose (g 100g^{-1}DM)	**1.66 ± 0.523**	**1.00 ± 0.596**	**NS**
Maltose (g 100g^{-1}DM)	**1.70 ± 0.553**	**1.12 ± 0.443**	**NS**
Pasting properties:			
Peak time (min)	**5.72±0.02**	**5.79±0.05**	*****
Pasting temperature (PT; °C)	**90**	**90**	**NS**
Peak viscosity (PV; RVU)	**198.83±5.76**	**192.44±11.38**	**NS**
Breakdown (BD; RVU)	**47.02±3.72**	**37.74±7.81**	*****
Hot viscosity (HV; RVU)	**150.15±3.62**	**155.49±6.95**	*****
Setback (SB; RVU)	**123.22±9.42**	**122.93±8.54**	**NS**
Final viscosity (FV; RVU)	**320.87±2.90**	**314.90±7.56**	*****

*: Significantly differently at P≤0.05, NS: Not significantly different

**: The data are expressed in the mean of all treatments

Table 7.2: Effects of seed treatment variants on the total antioxidant activity in rice grains harvested in seasons 2005 and 2006

Treatment	Antioxidant activity (IC_{50}**) (Mean ± SD*)	
	Season	
	2005	2006
CO	**0.1231±0.018b**	**0.1008±0.008b**
CA	**0.1582±0.009a**	**0.1240±0.003a**
CL	**0.1232±0.020b**	**0.0978±0.009b**
E+CL	**0.1225±0.019b**	**0.0956±0.008b**

* The different letters indicate the statistically significant difference by LSD at 5% level

** IC_{50} was expressed in terms of the fifty percent of effective concentration (IC_{50}) value, which required inhibiting DPPH radical formation by 50%. This IC_{50} parameter has the drawback value with the total antioxidant activity.

Table 7.3: Analysis of variance (ANOVA) for seed treatment variants and growing season on rice grain qualities

		Statistic significant (P)																		
Source of variation	**DF**	**Protein**	**Lipid**	**FFA**	**Carb**	**Amy**	**Glu**	**Suc**	**Lac**	**Mal**	**LOX**	**SOD**	**APX**	**Antiox**	**PV**	**HV**	**BD**	**FV**	**SB**	**PT**
Treatment (T)	**3**	**NS**	**NS**	*	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	*	*	**NS**	*	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Season (S)	**2**	**NS**	**NS**	*	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	*	**NS**	**NS**	**NS**	*	*	*	**NS**	**NS**
T x S	**6**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

*: significant different at the 0.05 level of probability, NS: not significant different

Table 7.4: Effects of seed treatment variants on pasting properties of rice flour (seasons 2005 and 2006)

Treatments	**(Mean ± SD)***						
	PV (RVU)	**Peak Time (min)**	**PT (°C)**	**HV (RVU)**	**BD (RVU)**	**FV (RVU)**	**SB (RVU)**
Season 2005							
CO	**194.00±4.18a**	**5.75±0.01a**	**90**	**149.99±1.93a**	**44.01±3.07a**	**320.81±1.77a**	**130.16±6.77a**
CA	**202.32±7.08a**	**5.71±0.03a**	**90**	**153.26±4.16a**	**45.72±3.73a**	**323.53±4.17a**	**115.21±12.13a**
CL	**199.14±5.87a**	**5.72±0.02a**	**90**	**147.32±2.22a**	**48.49±1.01a**	**319.46±1.37a**	**122.99±7.14a**
E+CL	**199.87±5.03a**	**5.71±0.01a**	**90**	**150.00±4.40a**	**49.87±4.53a**	**319.69±2.86a**	**124.50±8.52a**
Mean	**199.83**	**5.72**	**90**	**150.14**	**47.02**	**320.87**	**123.21**

Treatments	**(Mean ± SD)***						
	PV (RVU)	**Peak Time (min)**	**PT (°C)**	**HV (RVU)**	**BD (RVU)**	**FV (RVU)**	**SB (RVU)**
Season 2006							
CO	**192.20±18.26a**	**5.79±0.10a**	**90**	**159.71±11.94a**	**32.49±6.32a**	**317.85±9.04a**	**126.66±5.51a**
CA	**192.74±13.29a**	**5.80±0.04a**	**90**	**158.42±3.76a**	**34.32±10.00a**	**320.85±6.10a**	**128.11±7.17a**
CL	**187.53±8.52a**	**5.77±0.03a**	**90**	**150.71±4.91a**	**39.32±2.32a**	**309.63±0.47a**	**122.93±5.09a**
E+CL	**197.28±7.33a**	**5.82±0.01a**	**90**	**153.13±1.60a**	**44.84±7.20a**	**311.29±8.27a**	**114.02±10.64a**
Mean	**192.44**	**5.80**	**90**	**155.49**	**37.34**	**314.91**	**122.93**

* The different letters indicate the statistically significant difference by LSD at 5% level

Table 7.5: Pearson correlation coefficients between quality parameters of rice grains harvested in season 2005*

	N	P	K	S	Mg	Mn	Pro	Lip	FFA	Car	Amy	Glu	Suc	Lac	Mal	Anti	APX	LOX	SOD	PV	HV	BD	FV	SB	PT
Inf	-0.55	NS	NS	NS	NS	NS	-0.28	-0.32	0.47	-0.22	NS	-0.81	NS	NS	NS	-0.35	-0.15	0.11	NS	NS	-0.26	-0.23	NS	NS	NS
N		NS	NS	NS	NS	NS	0.68	0.62	NS	NS	NS	NS	NS	NS	NS	NS	0.60	-0.65	NS	NS	NS	NS	NS	NS	NS
P			NS	NS	NS	NS	0.43	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
K				NS	NS	0.66	0.77	NS	NS	0.75	NS	0.76	0.76	0.78	0.79	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S					NS	NS	NS	0.67	NS	NS	0.61	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mg						NS	NS	0.63	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mn							0.72	NS	NS	0.65	NS	0.64	NS	0.63	0.63	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pro								NS	NS	-0.99	NS	0.72	0.71	0.73	0.74	-0.82	NS	-0.60	NS	NS	NS	NS	NS	NS	NS
Lip									-0.74	NS	NS	NS	NS	NS	NS	-0.77	NS	-0.72	NS	NS	NS	NS	NS	NS	NS
FFA										NS	NS	NS	NS	NS	NS	NS	NS	0.75	-0.71	NS	NS	NS	NS	NS	NS
Car											NS	-0.72	-0.71	-0.74	-0.74	0.81	NS	NS	NS	NS	NS	NS	NS	NS	NS
Amy												-0.84	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Glu													0.86	0.98	0.98	-0.58	NS	-0.85	0.60	NS	NS	NS	NS	NS	NS
Suc														0.95	0.95	-0.79	NS	-0.64	0.58	NS	NS	NS	NS	NS	NS
Lac															0.99	-0.67	NS	-0.80	0.59	NS	NS	NS	NS	NS	NS
Mal																-0.67	NS	-0.80	0.60	NS	NS	NS	NS	NS	NS
Anti																	NS	NS	NS	NS	NS	NS	NS	NS	NS
APX																		-0.66	-0.42	NS	NS	NS	NS	NS	NS
LOX																			NS	NS	NS	NS	NS	NS	NS
SOD																				NS	NS	NS	NS	NS	NS
PV																					NS	NS	NS	-0.79	NS
HV																						NS	NS	NS	NS
BD																							NS	NS	NS
FV																								NS	NS
SB																									NS
PT																									

The numbers show a significant correlation at $\alpha = 0.05$ level. NS: Not significant at $\alpha = 0.05$.*: Data are involved in all treatments
Inf: Grain infection, Pro: Protein content, Lip: Lipid content, FFA: Free fatty acid content, Car: Carbohydrate content, Amy: Amylose content, Glu: Glucose, Suc: Sucrose, Lac: Lactose, Mal: Maltose, Anti: Antioxidant activity, APX: Ascorbate peroxidase activity, LOX: Lipoxygenase activity, SOD: Superoxide dismutase activity, PV: Peak viscosity, HV: Hot viscosity, BD: Breakdown, FV: Final viscosity, SB: Setback, PT: Pasting temperature

Table 7.6: Pearson correlation coefficients between quality parameters of rice grains harvested in season 2006*

	N	P	K	S	Mg	Mn	Pro	Lip	FFA	Car	Amy	Glu	Suc	Lac	Mal	Anti	APX	LOX	SOD	PV	HV	BD	FV	SB	PT
Inf	-0.83	-0.40	NS	NS	NS	NS	-0.49	-0.67	-0.55	-0.29	NS	-0.51	NS	NS	-0.42	-0.51	NS	0.36	-0.57	NS	NS	-0.66	NS	0.35	-0.25
N		NS	0.58	NS	NS	0.71	0.50	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.63	NS	NS	NS	NS	NS	NS	NS	NS
P			0.68	NS	NS	NS	NS	NS	-0.60	-0.62	NS	0.61	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
K				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S						NS	NS	NS	NS	NS	0.66	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mg						NS	NS	-0.62	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mn							NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pro								NS	-0.62	-0.96	NS	NS	NS	NS	0.68	-0.63	NS	-0.64	NS	NS	NS	NS	NS	NS	NS
Lip									-0.54	NS	NS	NS	NS	NS	NS	-0.72	NS	-0.62	NS	NS	NS	NS	NS	NS	NS
FFA										NS	-0.67	-0.64	NS	NS	-0.66	NS	NS	0.68	NS	NS	NS	NS	NS	NS	NS
Car											NS	-0.69	NS	NS	-0.76	0.75	NS	NS	NS	NS	NS	NS	NS	NS	NS
Amy												-0.72	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Glu													NS	NS	0.93	-0.76	NS	NS	NS	NS	NS	NS	0.58	NS	NS
Suc														0.87	NS	-0.83	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lac															0.61	NS	NS	NS	NS	NS	NS	0.62	NS	NS	NS
Mal																-0.78	NS	NS	NS	NS	NS	NS	0.66	NS	NS
Anti																	NS	NS	NS	NS	NS	NS	NS	NS	NS
APX																		-0.61	-0.54	NS	NS	NS	NS	NS	NS
LOX																			NS	NS	NS	NS	NS	NS	NS
SOD																				NS	NS	NS	NS	NS	NS
PV																					0.60	0.74	NS	NS	NS
HV																						NS	NS	NS	NS
BD																							NS	NS	NS
FV																								NS	NS
SB																									NS
PT																									

The numbers show a significant correlation at α = 0.05 level. NS: Not significant at α = 0.05.*: Data are involved in all treatments

Inf: Grain infection, Pro: Protein content, Lip: Lipid content, FFA: Free fatty acid content, Car: Carbohydrate content, Amy: Amylose content, Glu: Glucose, Suc: Sucrose, Lac: Lactose, Mal: Maltose, Anti: Antioxidant activity, APX: Ascorbate peroxidase activity, LOX: Lipoxygenase activity, SOD: Superoxide dismutase activity, PV: Peak viscosity, HV: Hot viscosity, BD: Breakdown, FV: Final viscosity, SB: Setback, PT: Pasting temperature

7.5 Discussion

Based on this study, year-to-year variation is commonly found in the field experiments. The nutrition stability is provided by plant genetic backup. In the present study, the two-year experiments have been conducted in the same field, and it led to the suggestion that climatic factors might influence the yield and subsequently the grain qualities. In 2005, the weather was warmer (higher temperature) and more humid (precipitation and humidity) with a lower photoperiod than the growing season 2006 (Appendix 17). It could suggest that the variation of weather conditions between the years could be the source of difference in rice grain quality stronger than cultural practices do. Ritchie and NeSmith, (1991) also reported that at least three environmental factors influenced the yield and grain quality, i.e. temperature, radiation and precipitation.

One of environmental factor that might be responsible for the different results in this study was the temperature. The optimum temperature for rice growth is around 28°C (Ritchie and NeSmith, 1991); high temperature could impair the metabolic performance of rice crops and altered assimilate partitioning (Ishimaru, 2003). Increasing the temperature led to increase of respiration rate, decrease of starch synthesis, and increase of sucrose synthesis of rice grain (Weschke *et al*., 2000). Sugar and amylose content of rice grain was in 2005 higher than in 2006 because of relatively higher temperature during the growing season in 2005. Although the yield was high in 2005, high precipitation before and until harvesting date, from October to November 2005, may also play a role in this case. Precipitation and humidity are closely related to the availability and uptake of minerals by the root system (Liang, 2001). This can be seen in the results of 2005, where the minerals content were higher than in 2006 (Chapter 6, Table 6.6-6.9). Increase in rainfall can also influence the mineralization in the soil, subsequently affecting the growth of the vegetative parts and grain filling (Chandraratna, 1994). Another possible explanation on this discrepancy may be the difference between the photoperiods. As described by Yin and Martin (1998), the length of the photoperiod effect flowering, heading and grain filling stages of the tested variety (cv. KDML 105), therefore, the photoperiod affected also the final rice grain product. However, it is impossible to select one individually factor and interpret the final rice grain quality.

Among the three commonly applied fertilizer elements (N, P, and K), N is the most important element required for obtaining high yields. It is also known to have an effect on

the protein content of the rice grain (Patrick *et al*., 1986). The present results showed a positive correlation between the efficiency of N accumulation and the protein content (Table 7.5 and 7.6). Mae (1997) reported that the N requirement in rice plants during the vegetative stage is to promote the growth and tiller that may influence the quality of the kernels. Based on the studies reviewed, Mosse's (1990) finding supported the observation that grain protein concentration is directly related to the N concentration in the grain. Nishizawa *et al.* (1977) reported that the application of N at heading stage increases the percentage of N utilization from 0.84% to 1.50% (increase about 55.90%). Stanley *et al*. (2006) reported that protein content, especially glutelin (or oryzenin), was markedly increased by the application of N after heading stage.

The rice grain quality in the present study was correlated with other minerals: P, K, Mg, Ca and Mn contents. These results are confirmed by Singh *et al.* (2007) who reported that the use of different organic seed treatments could improve the nutrient uptake. Supradip *et al*. (2007) reported that Fe and Zn deficiencies are the most prevalent micronutrient deficiencies. In our experiment, the Fe and Zn accumulation was improved by using the biological seed treatments (Chapter 6). Krebs *et al.* (2002) also reported that in organic rice production the absorption, especially of Fe, Zn and Ca is enhanced compared to conventional production.

Brown rice contains about 75-85% starch. Starch properties are therefore an important factor determining the grain quality. Sano (1984) found that the biological seed treatment improved minerals uptake and supplied them to starch synthesis pathway. Sucrose is the basic substrate for starch synthesis in the grain filling stage. In our experiment, grains from the biological seed coating treatments contained high amounts of sucrose and minerals. A sufficient sugar and mineral accumulation does support the starch synthesis (Preiss and Levi, 1980). Together with P in inorganic phosphate (Pi) form, these compounds regulate the starch synthesis in the pathways. Weschke *et al*. (2000) have argued that minerals and sugar content should be essential in both starch mobilization and the accumulation in rice grain.

The sugar content can reach about 6%DM. The sugar content reported here is slightly different. The present results, both 2005 and 2006, showed inverse relationship between carbohydrate and sugar content, amylose and sugar content respectively (Table 7.5 and

7.6). It can be suggested that the starch was degraded to CO_2 and water due to the respiration. It is a generally evidence before and after grains harvest. Changes in the molecular weights of starch and its components (amylose and amylopectin) may occur. Preiss and Levi (1980) reported that phosphorylytic breakdown of starch would supply substrates for respiration and growth. These results are also supported by Shock *et al*. (1993), who found that the sugars content was decreased because of α-amylase activity especially during storage.

The results showed inverse relationship between lipid and FFA content and positive correlation between LOX and FFA as well (Table 7.5 and 7.6). It can be suggested that the lipids were degraded via lipid peroxidation that altered the lipid content of rice grain before and after harvesting. According to Aoki *et al*. (2003) lipases and LOX could hydrolyzed the kernel lipids. Lipolytic enzymes and lipids are located in the testa layer, the aleurone and germ, respectively. Therefore, these reactions occur principally in the outer layer of the rice kernel where lipids are concentrated. During dehulling of the rice grains, these outer layers disrupt and the hydrolysis of lipids by lipolytic enzymes into free fatty acids will start. Furthermore, the LOX is an important enzyme involved in the oxidation of lipid to fatty acid, especially in the aleurone layer and germ (Yamamoto *et al*., 1980a). Yamamoto *et al*. (1980b) reported that during storage, approximately 30% of the kernel oil content could be converted into fatty acids within a week. These processes lead to increasing of acidity, deterioration of taste, and the production of rancid odors. According to Sauer (1992), the deterioration of brown rice during storage is very fast because of the large amount of lipids and high lipolytic enzyme activity in the bran. The rice lipids also undergo non-enzymatic oxidation (Zhong *et al*., 2005). Exogenous metal ions, as well as light-energy and heat, catalyze non-enzymatic oxidation that similar to enzymatic oxidation, hydroperoxides will be formed during this reaction, subsequently producing the radical free oxygen species (ROS), undesirable odors, and flavors of rice grain (Reddy *et al*., 1992). Slavin *et al*. (1997) reported that ROS might not only affect plant products degradation, but also trigger the initiation phase of several diseases. Antioxidant compounds can scavenge ROS. Brown rice is rich of antioxidant compounds such as α-tocopherol, oryzanine and gamma oryzanol. Jones *et al*. (2002a) reported that enzymes like SOD and APX are also important for alleviating ROS. Our study shows that the use of biological seed treatments promotes SOD, APX (Figure 7.4) and the total

antioxidant activity (Table 7.3) better than the chemical seed treatment does. It can thus be suggested that biological products are valuable sources of antioxidant compounds.

The results of correlation calculation between quality components and pasting properties of rice grain showed that no single compound was correlated with pasting attributes of rice flour. The result suggested that for a complex compound like flour, all constituents should contribute to the pasting properties, with regard to pasting and viscosity properties. The variation in rice grain quality parameters between growing seasons are also exhibited by the difference in relationship of the grain components with the pasting properties for each respective year. This also confirms the former suggestion that the planting season does significantly influence the rice grain quality and subsequently the quality of flour obtained. It has been noted that viscosity properties of product are influenced by multiple factors that include amylose-amylopectin ratio, starch content and associated constituents such as P, Ca and Mg. Despite the already mentioned effect between the years, it is interesting to note that provided data of amylose content of season 2005 was higher than those of season 2006 which exhibited a higher PV, BV and FV, but a lower HV. This could be an indication that in the flour solubilized starch releases after granules disruption was stronger, followed by a higher number of re-associated granules upon cooling which led to a higher FV and SB. However, the seed treatment variants had no significant effect on viscosity properties because starch properties as size, number of starch granule and amylose-amylopectin ratio, were not greatly affected by the seed treatment variants. Zhong *et al* (2005) also reported that viscosity properties of rice grains are affected mainly by environmental factors, especially temperature during grain filling. Huang *et al.* (1998) reported that high temperature during grain filling decreased amylose content might in part explain the worsening of viscosity properties. Suzuki *et al.* (2004) reported that the viscosity properties of rice flour were affected by the temperature during grain development that associated with the proportion of amylose and amylopectin content. According to Tan and Corke (2002), pasting properties were dependent on not only amylose or amylopectin content, but also on starch chain-length distribution. They concluded that the seed treatment did not affect the pasting properties of rice flour.

Crop losses and grain quality degradation can occur due to the fungi infection. The control plots showed high level of diseases infection during vegetative growth and grain filling stage (Data showed in Chapter 6) which had inverse relationship with the studied

quality parameters of rice grains (Table 7.5 and 7.6). According to Abdullah and Othman (2000), the grains are blackened due to fungi infection, which causes the total loss of grain quality. Huaqi *et al*. (2002) reported that the fungal infection influences the chemical processes and increases the degradation of rice quality. Imura and Sinha (2006) described that fungi and other microorganisms cause a principal economic damage. A discolored grain is conspicuous in milled rice and reduces the quality; thereby reduce the value that imparts unpleasant odors and flavors of the cooked rice. Miller and Trenholm (1994) reported that in fungi infected rice grain some of the lipase and LOX present in the bran enters the endosperm and react with the oil in the rice grain. The lipolysis results in the formation of FFA followed by peroxidation and the production of ketoaldehydes and ROS. Several biochemical processes lead also to high levels of glucose, which are utilized by the developing organisms. Moreover, the use of infected rice might pose health hazards due to possible mycotoxin production (Evandro *et al*., 2005).

7.6 Conclusion

Apart from many other advantages, rice seed treatments did not have any side effect on product qualities, which were demonstrated by the stability of nutrients and pasting properties of rice grains. Anyhow, the use of biological seed coating treatments showed the tendency to improve the nutritional quality better than CA treatment did. From the field experiments, year-to-year variation is a common case. The variation could be related to various environmental factors, which can influence the performance of product quality stronger than cultural practices do.

In conclusion, the investigation of seed treatment variants confirm that E+CL is a promising seed coating substance for the future respecting due to its high potential on plant diseases control and improving crop growth and grain yield. In addition, this coating substance tends to improve grain quality and decrease grain infection. Thus, pre-sowing, seed coating with E+CL polymer could be a recommended seed invigoration application for direct seed rice production.

7.7 References

Abdullah and Othman. (2000). Fungal spoilage of starch-based foods in relation to its water activity. J Stored Prod. Res, 36(200), 47-54.

Aoki, N., Hirose, T., Scofield, G.N., Whitfeld, P.R. and Furbank, T. (2003) The sucrose transporter gene family in rice. Plant Cell Physiol. 44, 223-232.

Applications Manual for the Rapid™ Visco Analyser. (1998). RVA Rice method. Newport Scientific Pty. Ltd. Australia. 54-55.

Balasubramanian, V. and Hill, J.E. (2002). Direct seeding of rice in Asia: emerging issues and strategic research needs for 21st century. In Pandey, S., Mortimer, M., Wade, L., Tuong, T. P., Lopes, K., and Hardy, B. eds, Direct seedling: Research strategies and opportunities. International research Institute, Manila, Philippines. 15-39.

Basra, S.M.A., Zia, M.N., Mehmood, T., Afzal, I., and Khaliq, A. (2002). Comparision of different invigoration techniques in wheat seeds. J. of Arid Agric. 5, 11-16.

Basra, S.M.A., Farooq, M., and Khaliq, A. (2003). Comparative study of pro-sowing seed enhancement treatments in indica rice. Pak. J. of Life and Social Sci. 1, 5-9.

Chandraratna, M.F. (1994). Photoperiod Response in Rice (Oryza sativa L.). I. Effects on Inflorescence Initiation and Emergence**.** New Phytologist, 53(3),390-395.

De Datta, S. K. (1981). Principle and practices of rice production. John Wiley&Sons, New York.

Du, L.V., and Tuong, T.P. (2002). Enhancing the performance of dry-seeded rice: Effects of seed priming, seedling rate, and time of seedling. In Pandey, S., Mortimer, M., Wade, L., Tuong, T. P., Lopes, K. and Hardy, B. eds., Direct seeding: Research strategies andopportunities. International Research Institute, Manila, Philippines. 241-256.

Evandro, L.S., Edeltrudes, O.L., Kristerson, R.L., Freire, and Cristina, P.S. (2005). Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Brazilian Achieves of Biology and Technology. 48 (2), 245-250.

Farooq, M., Basra, S.M.A., Hafeez, K., and Warriach, E.A. (2004a). The influence of high and low temperature treatments on the seed germination and seedling vigor of coarse and fine rice. Inter. Rice Res. Notes 29, 69-71.

Huaqi, W., Bouman, B.A.M., Zhao, D., Changgui, W., and Moya, P.F. (2002). Aerobic rice in northern China: opportunities and challenges. Paper presented at the workshop on water-wise rice production, 8-11 April 2002 at IRRI headquarter in Los Banos, Philippines.

Huang, J.W., Chen, J.T., Yu, W.P., Shyur, L.F., Wang, A.Y., Sung, H.Y., Lee, P.D. and Su, J.C. (1996) Complete structures of three rice sucrose synthase isoforms and differential regulation of their expressions. Biosci. Biotech. Biochem. 60, 233-239.

Huang, F.S., Sun, Z.X., Hu, P.S., and Tang, S.Q. (1998). Present situation and prospects for the research on rice grain quality forming. Chin. J. Rice Sci. 12, 172—176.

Imura, O. and Ranendra N. Sinha, R. N. (2006). Principal component analyses of bagged wheat infested with *Sitotroga cerealella* (Lepidoptera: Gelechiidae) and*Sitophilus oryzae* (Coleoptera: Curculionidae). Ecological Research. 199-208.

International Rice Research Institute (IRRI). (2007). Annual Report for 2006. http://www.irri.org/publications/today, visited at 12.05.07.

Ishimaru, T., Matsuda, T., Ohsugi, R. and Yamagishi, T. (2003). Morphological development of rice caryopsis located at the different positions in a panicle from early to middle stage of grain filling. Func. Plant Biol. 30, 1139-1149.

Joanne, S. (2003). Why whole grains are protective: biological mechanisms. Proceedings of the Nutrition Society. 62**,** 129–134

Jones, J.M., Reicks, M., Adams, J., Fulcher, R.G., Weaver, G., Kanter, M., and Marquart, L. (2002a). The importance of promoting a whole grain foods message. Journal of the American College of Nutrition. 21, 293–297.

Krebs, N., Adams, C., Raboy, V. (2002). Calcium absorption is greater in phytic acid reduced maize compared to wild type maize. Federation of American Societies for Experimental Biology (FASEB) Journal, 16, A723.2.

Lee, S.S., Kim, J.H., Hong, S.B., and Yun, S.H. (1998). Effect of humidification and hardening treatments on seed germination of rice. Kor. J. of Crop Sci. 43, 157-160.

Lee, J., Terry, M. B., Micheal, L., Shannon, R. M. P., and Jame H. O. (2006). Proteomic and genetic approaches to identifying defence-related proteins in rice challenged with the fungal pathogen *Rhizoctonia solani*. Molecular Plant Pathology. 7 (5), 405–416.

Liang, J., Zhang, J. and Cao, X. (2001) Grain sink strength may be related to the poor grain filling of *indica-japonica* rice (*Oryza sativa*) hybrids. Physiol. Planta. 112, 470-477.

Loehken, A. (1990). The benefit of seed/early season fungicide application for the management of rice blast (*Pyricularia oryzae*). B.T. Grayson, M.B. Green, L.G. Copping (eds): Pest Management in Rice. Elsevier Applied Sciences, London New York. 71-86.

Mae, T. (1997). Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. Plant and Soil 196, 201–210.

Miller, J.D., Trenholm, H.L. (1994) ed. Mycotoxins in grain: Compounds Other Than Aflatoxin. St. Paul: Eagan Press.

Mosse´, J. (1990). Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. J. Agric. Food Chem. 38, 18–24.

Nishizawa, N., Kitahara, I., Noguchi, T., Hareyama, S., and Honjyo, K. (1977). Protein quality of high protein rice obtained by spraying urea on leaves before harvest. Agric. Biol. Chem. 41, 477-485.

Office of Agricultural Economics. (2006). Area, production, yield, farm price and farm value, 1996-2005. Available at http://www.oae.go.th/ in 4-05-07.

Patrick, W. H., Jr., Mikkelsen, D. S., and Wells, B. R. (1986). Plant nutrient behavior in flooded soils. Pages 197-228. in: Fertilizer Technology and Use. 3 rd ed. O. P. Englested, ed. Soil Science Society of America, Madison, WI.

Preiss, J., and Levi, C. (1980). Starch biosynthesis and degradation. In The biochemistry of plants. Ed. J. Preiss. Academic Press, New York. 371-423.

Reddy, G.R., Reddanna, P., Reddy, P.P., and Curtis, W.R. (1992). 11-Hydroperoxyeicosatetraenoic acid is the major dioxygenation product of lipoxygenase from hairy root of *Solanum tuberosum*. Biochem Biophys Res Commun. 189, 1349-1352.

Ritchie, J.T. and NeSmith, D.S. (1991). Temperature and crop development. pp. 5-29 Hanks, J. and J.T. Ritchie (Eds). 1991. Modeling Plant and Soil Systems. American Society of Agronomy: Madison, Wisconsin.

Ruan, S., Xue, Q., and Tylkowska, K. (2002). Effects of seed priming on germination and health of rice seeds. Seed Science and Technology. 3, 451-458.

Sano, Y. (1984). Differential regulation of waxy gene expression in rice endosperm. Theor. Appl. Genet. 68, 467-473.

Sauer, D.B. (1992). Storage of Cereal Grains and Their Products. Fourth edition. American Association of Cereal Chemists, St. Paul, MN.

Singaravadivel, K., and Anthoniraj S. (1983). Changes in parboiled rough rice caused by improper drying and microbial infection. Journal of Food Biochemistry. 7, 15-21.

Singh, Y.V., Singh, B.V., Pabbi, S., and Singh, P.K. (2007). Impact of organic farming on yield and quality of BASMATI rice and soil properties. Visited at 24 May 2007. available on http://orgprints.org/view/projects/wissenschaftstagung-2007.html.

Slavin, J., Jacobs, D., and Marquart, L. (1997). Whole grain consumption and chronic diseases: Protective mechanisms. Nutrition and Cancer. 27, 14–21.

Shock, C.C., Holmes, Z.A., Stieber, T.D., Eldredge, E.P., and Zhang, P. (1993). The effect of timed water stress and quality, total solids and sugar content of potatoes. J Am of Potato Res. 70, 227-239.

Stanley O., Samonte, P.B., Lloyd, T.W., James C.M., Shannon R.M.P., Anna M. M., and Joveno S.L. (2006). Nitrogen utilization efficiency: relationships with grain yield, grain protein, and yield-related traits in rice. Agronomy Journal. 98, 168-176.

Supradip S., Pandey, A.K., Gopinath, K.A., Bhattacharaya, R., Kundu S., and Gupta, H.S. (2007). Nutritional quality of organic rice grown on organic composts. Agronomy for sustainable development. 263-271.

Suzuki, Y., Sano, Y., Ishikawa, T., Sasaki, T., Matsukura, U., and Hirano, H.Y., (2004). Differences in starch characteristics of rice strains having different sensitivities to maturation temperatures. J. Agron. Crop Sci. 190, 218—221.

Tan, Y., and Corke, H. (2002). Factor analysis of physicochemical properties of 63 rice varieties. J. Sci. Food Agric. 82, 745—752.

Weschke, W., Panitz, R., Sauer, N., Wang, Q., Neubohn, B., Weber, H. and Wobus, U. (2000) Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. Plant J. 21, 455-467.

Yamamoto, A., Fujii, Y., Yasumoto, K., and Mitsuda, H. (1980a). Partial purification and study of some properties of rice germ lipoxygenase. Agric. Biol. Chem., 44, 443-445.

Yamamoto, A., Fujii, Y., Yasumoto, K., and Mitsuda, H. (1980b). Products specificity of rice germ lipoxygenase. Lipids 15, 1-5.

Yin, X.Y., and Martin, J.K. (1998). The effect of photoperiod on interval between panicle initiation and flowering in rice. Field Crops Research, 57(3), 301-307.

Zhong, L.J., Cheng, F.M., Wen, X., Sun, Z.X., and Zhang, G.P. (2005). The deterioration of eating and cooking quality caused by high temperature during grain filling in early-season indica rice cultivars. J. Agronomy & Crop Science 191, 218—225.

8. Concluding summary and future study

Botanical fungicides derived from plants are one of the alternative ways to limit the use of synthetic fungicides. The present study was conducted to investigate the following hypothesis: some plant extracts have the potential to act as botanical fungicides and are alternative sources instead of synthetic fungicides for seed coating. These substances are able to control seed borne fungi without seed injuries and are able to ensure high yields as well as the storability of the grain and the final grain quality. Furthermore, a lower contamination with chemical residues in both soil and harvested product is expected.

In the present study, five species of Thai local plants, as *Acorus calamus* Linn., *Eugenia caryophyllus*, *Mammea siamensis* Kost., *Stemona curtisii* Hk.f., and *Stemona tuberosa* L., and an eugenol were screened *in vitro* for their highest antifungal activity. Eugenol is the main active compound of clove (*Eugenia caryophyllus*) which provided the strongest antifungal effectiveness on four species of the most common seed borne fungi: *Alternaria solani*, *Colletotrichum* sp., *Fusarium moniliforme* and *Rhizoctonia solani*.

The inhibitory effect of seed treatment variants against these rice seed borne fungi during 12 months of storage was also compared. From the comparison of all treatments it was concluded that CA had the strongest inhibitory effect on *Alternaria solani*, *Curvularia* sp., *Aspergillus flavus*, and *Aspergillus niger*. The treatment E+CL was most effective against *Bipolaris oryzae* and *Nigrospora oryzae.* However, both CA and E+CL treatments showed no significant different inhibitory effect on *Fusarium moniliforme*.

Interestingly, E+CL was less phytotoxic on the rice seeds than CA did because this treatment affected the seed germinability and viability. Additionally, seedling performances were affected resulting in inhibition of shoot and root development, reduced seedling dry weight, seedling growth rate and delayed germination time, respectively. The seeds treated with CA were susceptible to stress conditions, such as cold, high moisture and temperature, which declined the seed germination potential. Nevertheless, CL and E+CL could maintain the seed storability and the following seed germination and seedling performances were on high levels compared to the other treatments. Subsequently, during storage, the biochemical degradation occurred. It was found that CL and E+CL led to less

phytotoxic effects than CA with following significant effects on the biochemical deterioration.

Also from the field experiments it can be concluded that CL and E+CL have the potential to improve seed germination and seedling establishment. Moreover, these seed coating substances improved plant growth and development, kernel yield and nutrient uptake efficiency. In contrast, CA caused a poor and abnormal establishment of the seedlings, followed by poor plant performance and reduced agronomic yield. The CA treatment caused a low nutrient uptake. Furthermore, the soil and rice grain were contaminated with total organochloride fungicide residues, whereas no contamination was found after CL and E+CL treatments.

Concerning the effects of E+CL on rice grain quality, there were not significant differences among the treatment variants on the protein, lipid, FFA, carbohydrate, amylose and sugar contents, APX and pasting properties but on total antioxidant activity, LOX and SOD. The results suggested that E+CL coating substance did not caused a significant adverse effect on rice grain quality.

From the results of the experiment it could be concluded that the E+CL seed coating substance was the most effective against seed borne fungi, less phytotoxic to seeds and improved crop growth and grain yield. The E+CL coating agent tended to improve also grain quality and decreases grain infection. Moreover, it didn't produce any chemical residues in both soil and harvested grains. Therefore, E+CL seems to be a promising seed coating substance for direct seeded rice production system.

Future study

This experiment has presented the application of biological seed coating technology in rice seeds. Based on the result of this study, the E+CL coating substance enhanced the antifungal activity on rice seed in a short period. Therefore, the formulation of this seed coating substance should be developed to enhance its stability on the seeds for longer control of seed borne fungi. The study of antifungal activity of E+CL on different species of seeds is also necessary.

A multi-layer of coating substances contains several substances that act as insecticide and plant growth promoter and it may contain also essential micronutrients in order to

improve the seedling quality. It should be investigated how biological substances could be included in effective multi-layer coating materials which are suitable for chemical-free crop production systems.

This technology provides a good opportunity for both organic and conventional farming systems because all of agricultural practices demand environmental friendly and safe methods.

Appendix 1: Rice disease, fungi, symptoms, favorable conditions and control application

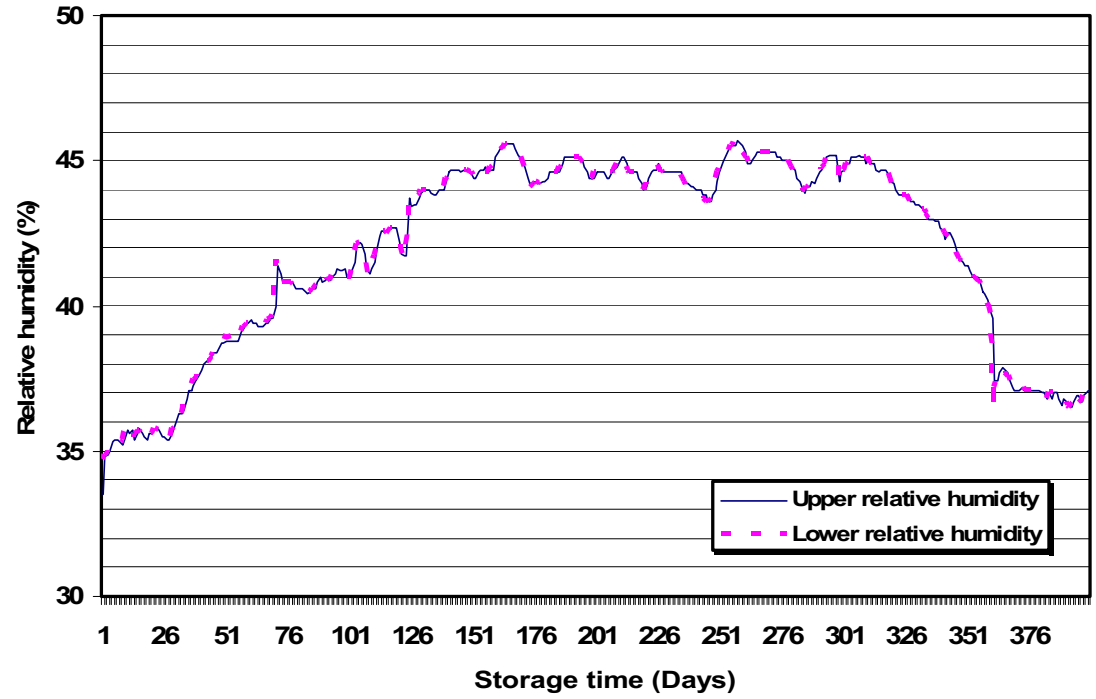
Diseases	Fungi	Symptoms	Favorable conditions	Control application	References
Seedling blight, seed decay	*Bipolaris oryzae*, *Rhizoctonia solani*	Leaf spotty, irregular stands through seed decay, weakened and chlorotic seedling, seedling injure and die back	Damage is most severe on early seeded rice and deeply planted rice	Use of high quality and vigorous seed, fungicide seed treatment, shallow seeding of early planted rice, planting into warm soil	Agrios (1988)
Sheath blight	*Rhizoctonia solani*	Seed or young seedlings and either injure or die back. Initial symptoms usually develop as lesions on sheaths of lower leaves near the water line. The lesions usually develop just below the leaf collar as oval-to-elliptical, green-gray, water-soaked spots about 1/4 inch wide and 1/2 to 1 1/4 inch long. Aged, the lesions expand and the center of the lesions may become bleached with an irregular tan-to-brown border	Damage is most severe on early seeded rice and deeply planted rice. Infection spreads rapidly when humidity exceeds 95% and temperatures are in the range of 29-32^{o}C	Use of high quality and vigorous seed, fungicide seed treatment, shallow seeding of early-planted rice, and planting into warm soil	Webster and Gunnell (1992)
Stem rot	*Fusarium moniliforme*	Stem rot becomes most noticeable in rice fields during the latter stages of maturity. The disease occurs in circular to irregular areas in fields and causes premature death and lodging of the plants. The fungus attacks the rice plant near the water line usually during late tillering or early reproductive stages of growth. It first causes black, rectangular lesions with distinct angular borders on the leaf sheath. Later the lesions become larger, more diffuse, irregular in shape, and penetrate deep into the culm. Plants early infected yield poorly.	Infection spreads rapidly when humidity exceeds 95 % and temperatures are in the range of 29-32^{o}C	Crop rotation, use of early maturing varieties, fluctuating the flood water level, avoiding excessive rates of nitrogen, and rice stubble destruction, fungicides seed treatment	Howard (2003)

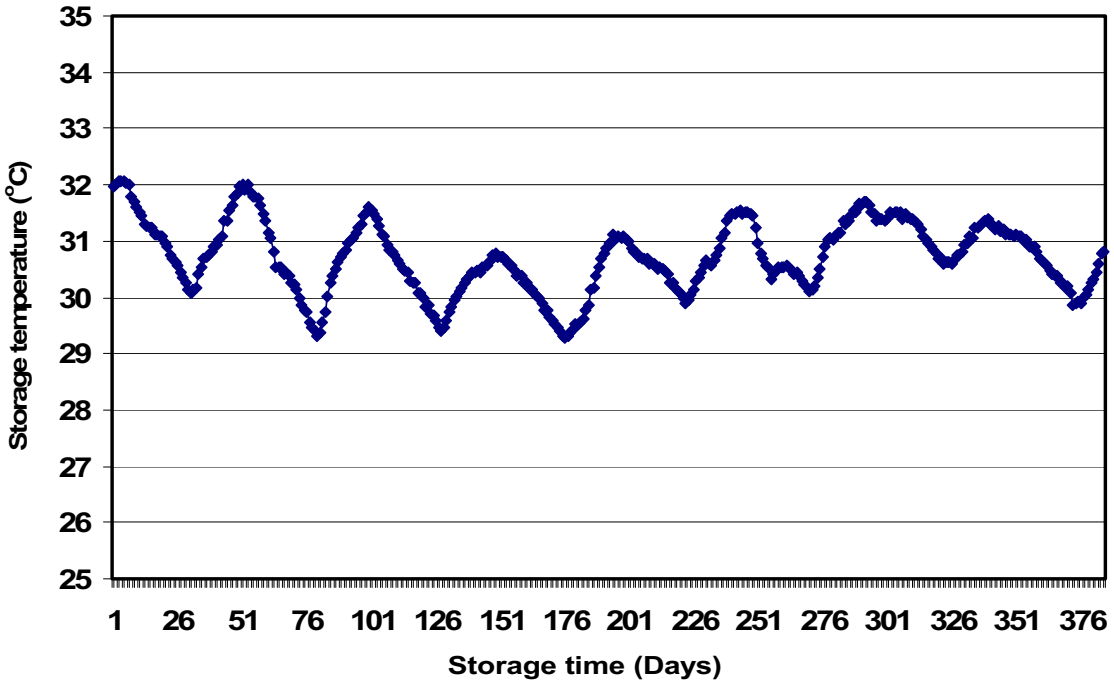
Appendix 1: Rice disease, fungi, symptoms, favorable conditions and control application (continued)

Diseases	Fungi	Symptoms	Favorable conditions	Control application	References
Blast	*Curvularia* sp.	The disease is often called leaf blast, rotten neck, or panicle blast. The fungus produces spots or lesions on leaves, nodes, panicles, and collar of the flag leaves. Leaf lesions range from somewhat diamond shaped to elongated with tapered, pointed ends. The center of the spot is usually gray and the margin brown or reddish-brown. Both the shape and color of the spots may vary and resemble those of the brown leaf spot disease. The blast fungus frequently attacks the node at the base of the panicle and the branches of the panicle. If the panicle is attacked early in its development, the grain on the lower portion of the panicle may be blank giving the head a bleached whitish color, giving the term "blasted" head or rice "blast". If the node at the base of the panicle is infected, the panicle breaks causing the "rotten neck" condition. In addition, the fungus may also attack the nodes or joints of the stem. When a node is infected, the sheath tissue rots and the part of the stem above the point of infection are often killed. In some cases, the node is weakened to the extent that the stem will break causing extensive lodging.	Late planting, frequent showers, overcast skies, and warm weather, high nitrogen fertilization.	Early planting, avoiding excessive or high levels of nitrogen, resistant varieties, and fungicides seed treatment	Farr *et al.* (1989)
Brown leaf Spot	*Bipolaris oryzae*	Most conspicuous symptoms of the disease occur on leaves and glumes of maturing plants. Symptoms also appear on young seedlings and the panicle branches in older plants. Leaf spots may be evident shortly after seedling emergence and continue to develop until maturity. Leaf spots vary in size, typically 1/8 inch in diameter, and are circular to oval in shape. The smaller spots are dark brown to reddish brown, and the larger spots have a dark brown margin and reddish brown to gray centers.	Nutritionally deficient or otherwise unfavorable soil conditions, high moisture and temperature	Balanced soil fertilization, crop rotation, and the use of high quality and vigorous seed, fungicide seed treatment	Reissig *et al.* (1986)

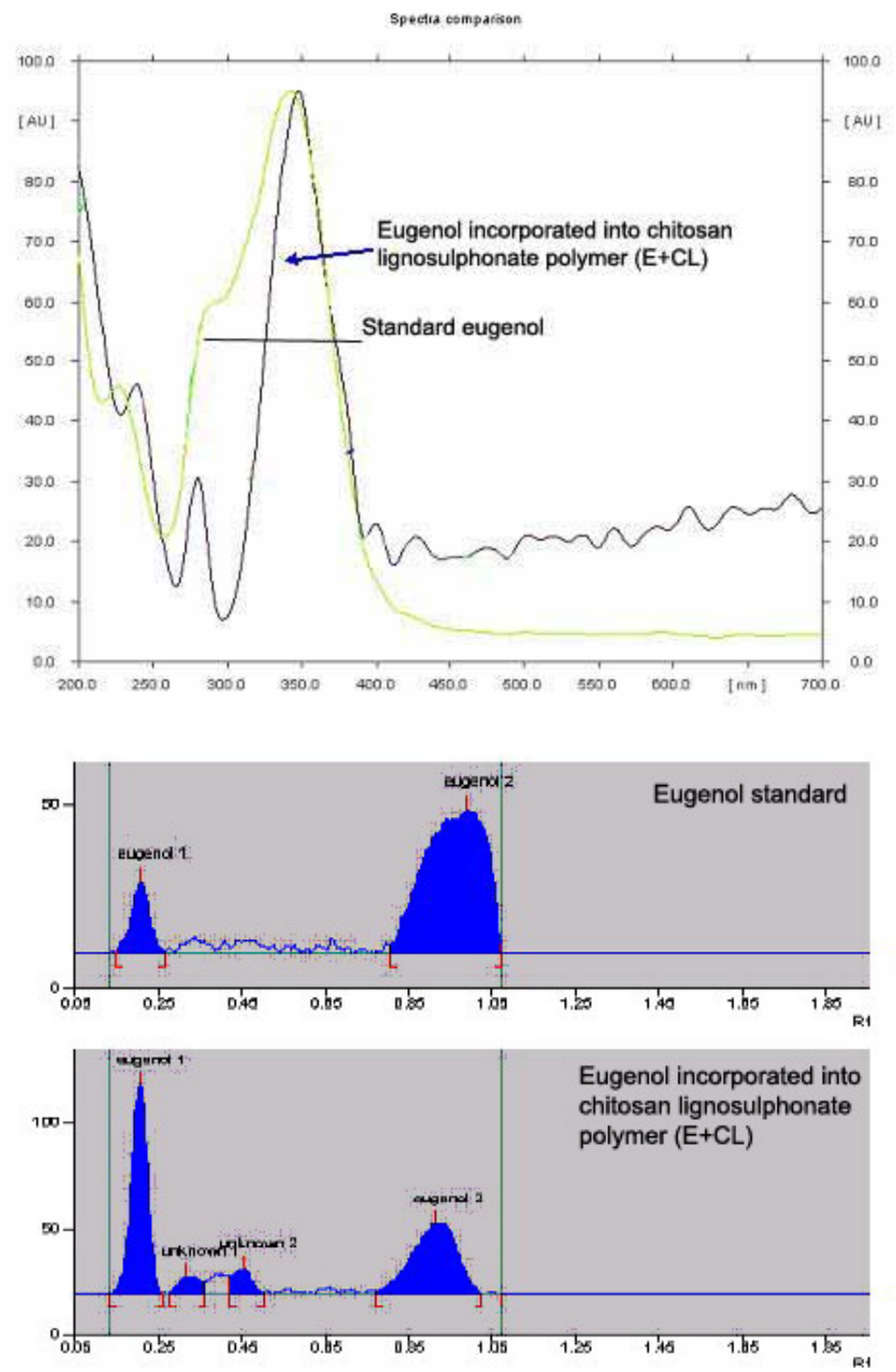
Appendix 1: Rice disease, fungi, symptoms, favorable conditions and control application (continued)

Diseases	Fungi	Symptoms	Favorable conditions	Control application	References
Kernel smut	*Nigrospora* spp.	This disease causes losses in both yield and quality. The endosperm of the grain is attacked by the fungus causing either part or all of the starchy material to be replaced by a black mass of smut spores. Release of the smut spores from within the kernel will cause a discoloration of hulls. Milled rice has a dull or grayish appearance when smutted grains are present in the sample.	High moisture	Use of semi-dwarf varieties in fields with a history of smut, reduce nitrogen rates and floodwater depths in fields where very susceptible varieties must be grown, fungicide seed treatment	Nathan *et al.* (2004)
Sheath spot	*Rhizoctonia* sp.	Small sclerotia initiate the disease on the outermost leaf sheath. A reddish-brown lesion develops on the plant near the waterline. During internode elongation, the sheath spot may be pushed up above the waterline because of this new growth. With age, the lesion enlarges slightly to assume an eliptical to irregular shape (1/2 to 1 inch long) with a distinct purple-brown border and a tan-to-straw colored center. Sometimes the fungus will cause a yellowing of the leaf attached to the sheath it has infected. Significantly reduction in yield may occur in tillers that are infected	Warm weather and high humidity, after raining	Use the high quality and vigorous seed, fungicide seed treatment	Pascual *et al* .(2000)
Bakanae disease	*Fusarium* sp.	Infected seedlings are thin, chlorotic, may die before or after transplanting. In the field, infected plants have few tillers and leaves die in short time. Live plants have empty panicles. Some infected plants may be stunted instead of elongated, while the abnormal elongation of these (infected) plants in seed bed or field is the most common symptom of this disease.	Infection spreads rapidly when humidity exceeds 95 % and temperatures are in the range of 29-32^{o}C	Use the high quality and vigorous seed, fungicide seed treatment, resistance variety	Smith *et al.* (1988)

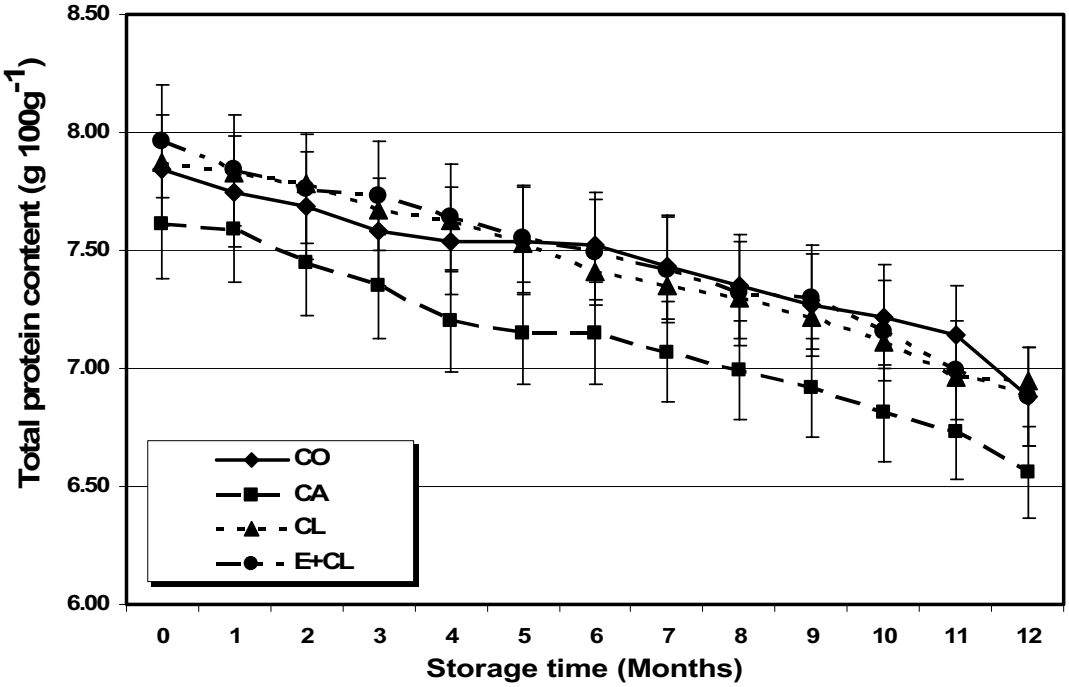




Appendix 2: Relative humidity and storage temperature during seed storage for one year

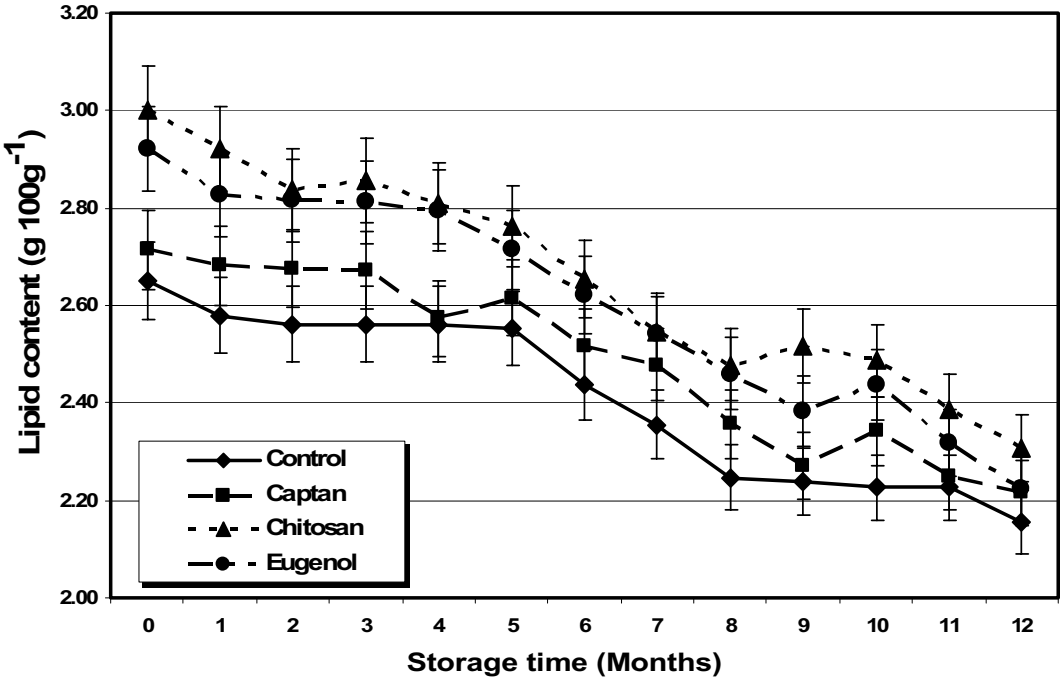


Appendix 3: The persistence of eugenol active compound on the E+CL coated seeds compared with standard eugenol, established by HPTLC with the solvent system of C_7H_8-EtO



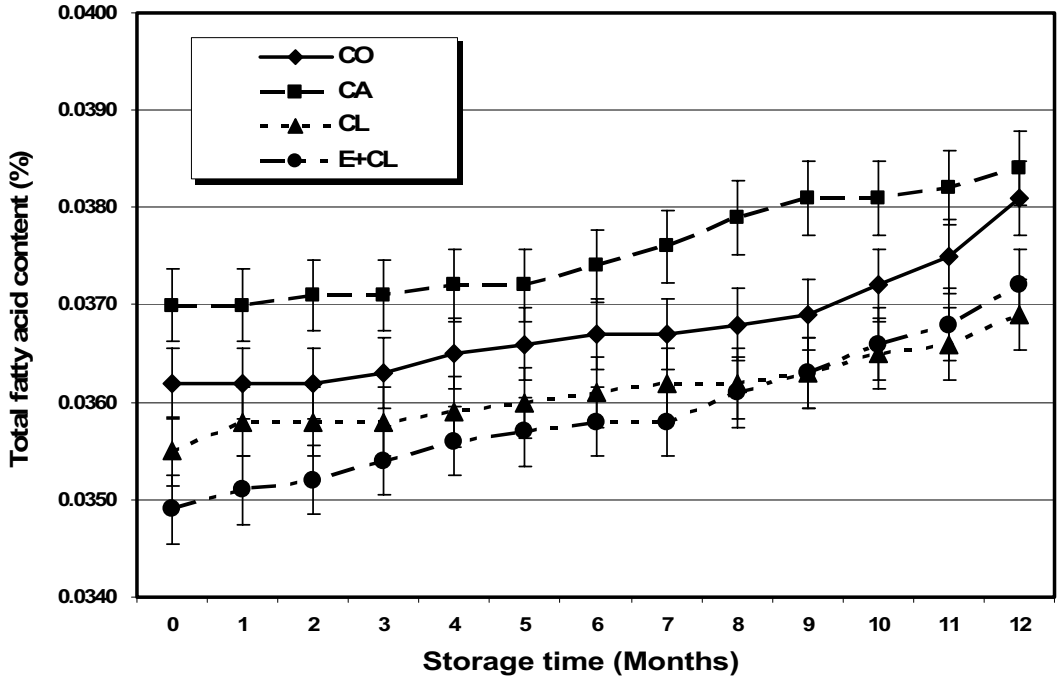
Appendix 4: The effect of seed treatment variants and storage duration on protein content of rice seed during storage for 12 months*

*Values reported as means±SD of 4 replications of storage samples



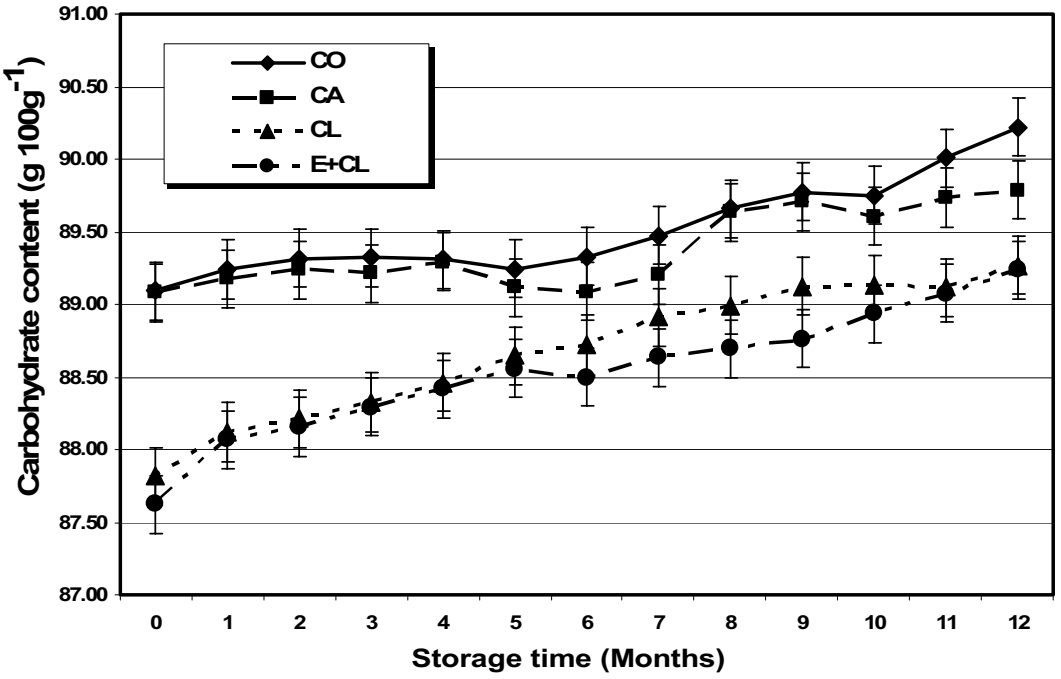
Appendix 5: The effect of seed treatment variants and storage duration on lipid content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



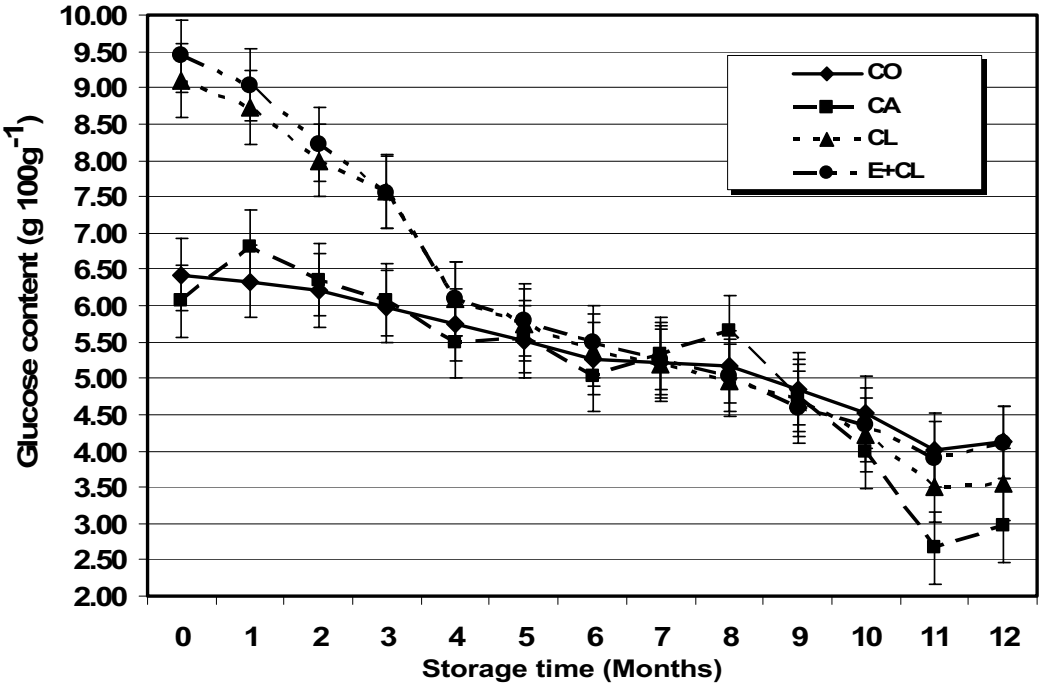
Appendix 6: The effect of seed treatment variants and storage duration on free fatty acid content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



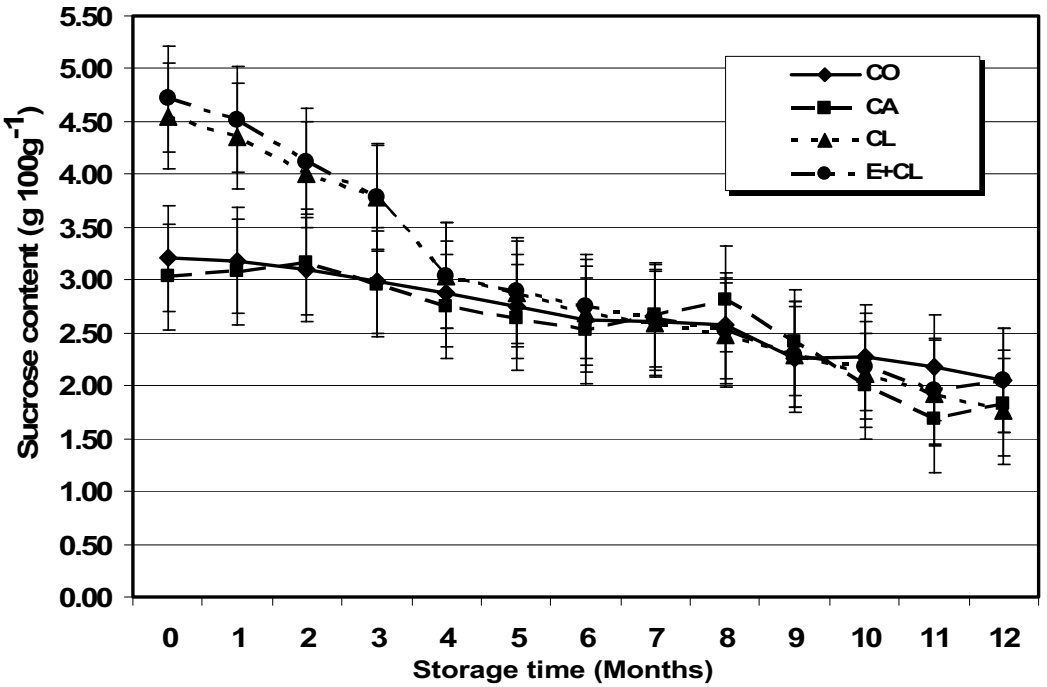
Appendix 7: The effect of seed treatment variants and storage duration on carbohydrate content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



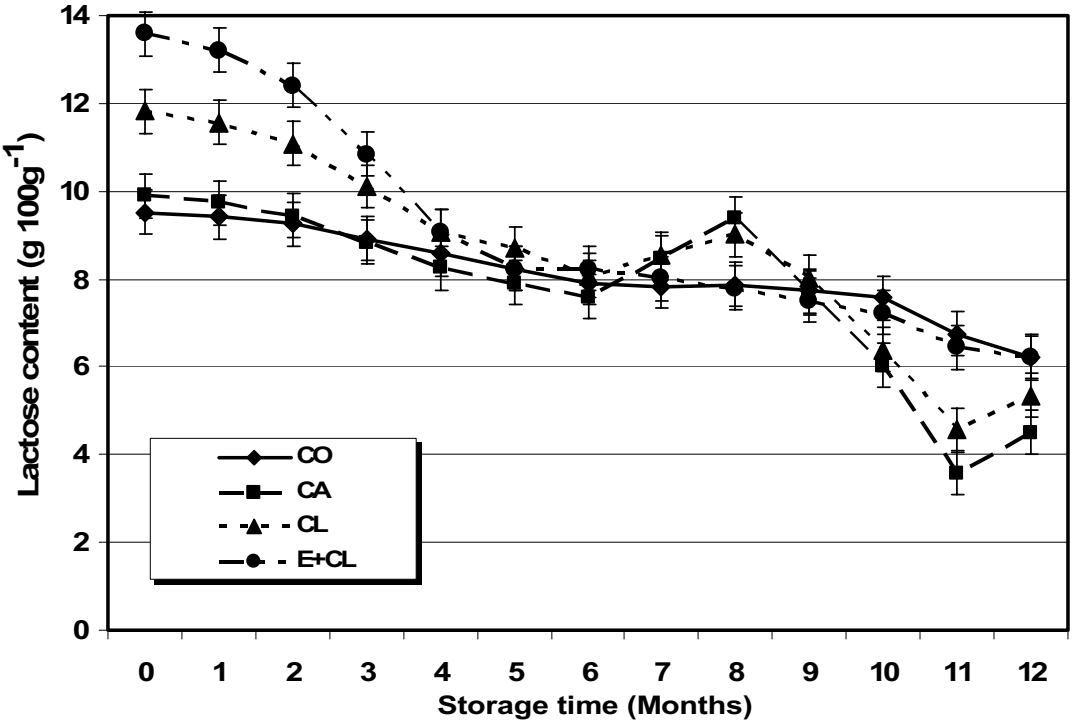
Appendix 8: The effect of seed treatment variants and storage duration on glucose content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



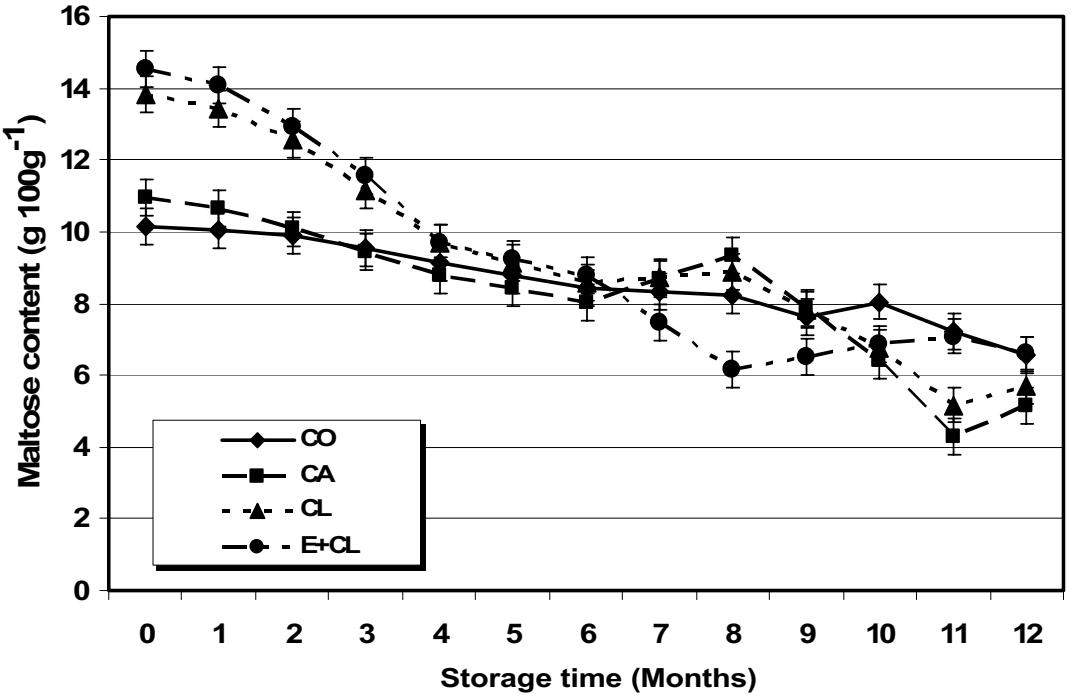
Appendix 9: The effect of seed treatment variants and storage duration on sucrose content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



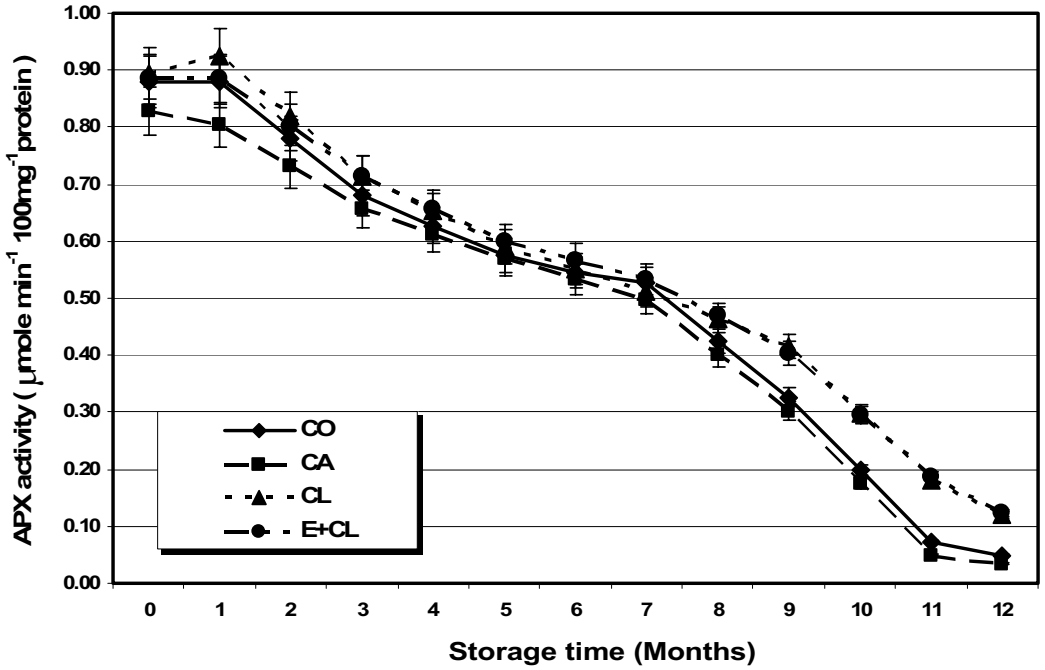
Appendix 10: The effect of seed treatment variants and storage duration on lactose content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



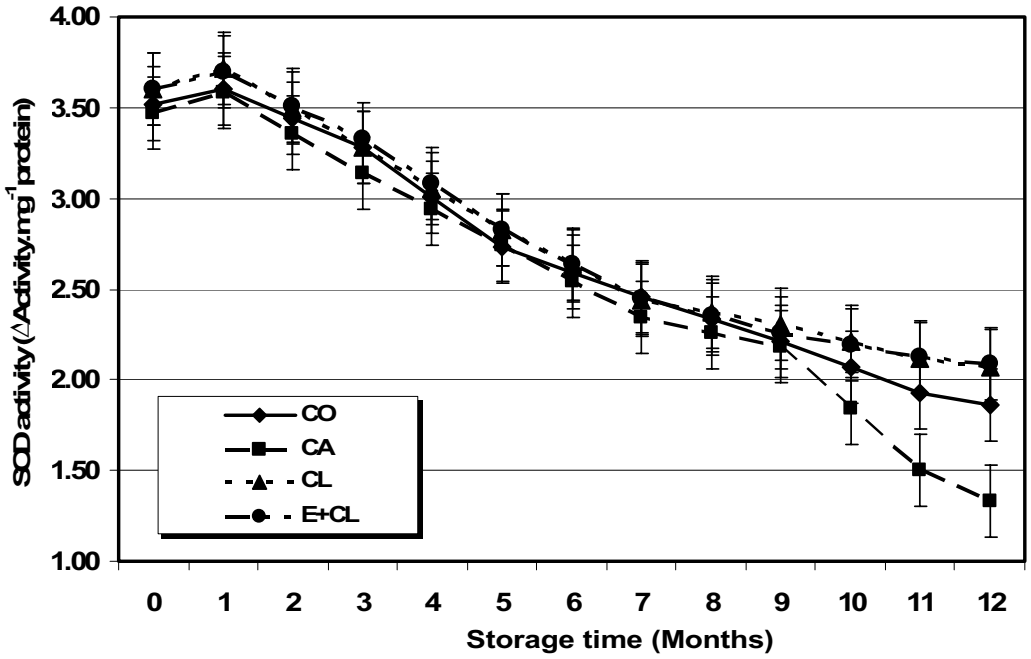
Appendix 11: The effect of seed treatment variants and storage duration on maltose content of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



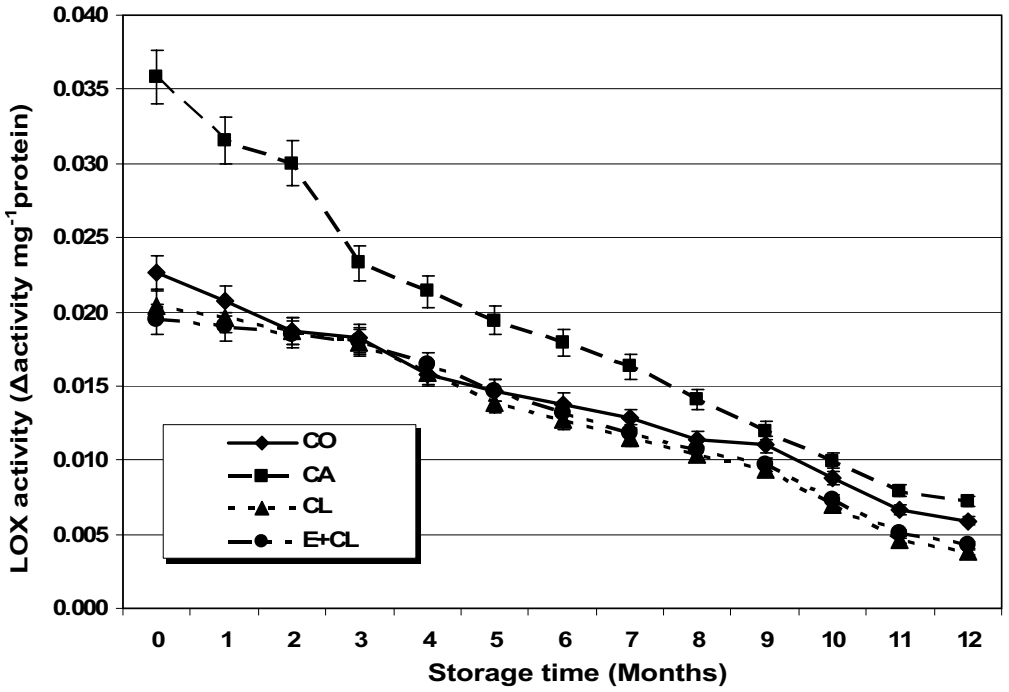
Appendix 12: The effect of seed treatment variants and storage duration on the activity of ascorbate peroxidase of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



Appendix 13: The effect of seed treatment variants and storage duration on the activity of superoxide dismutase of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples



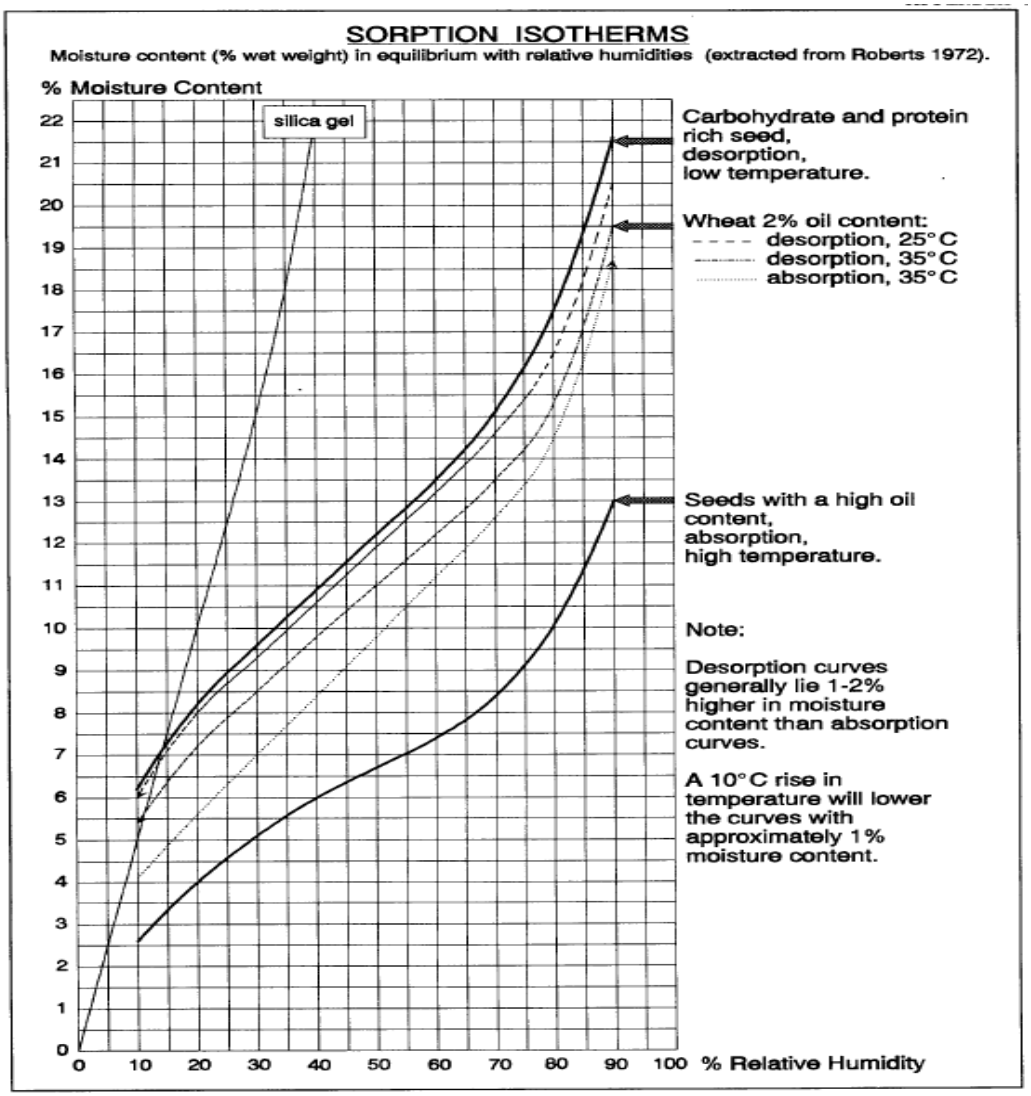
Appendix 14: The effect of seed treatment variants and storage duration on the activity of lipoxygenase of rice seed during storage for 12 months*

*Values reported as a means ± SD of 4 replications of storage samples

Appendix 15: The effect of seed treatment variants and storage duration on the total antioxidant activity of rice seed during storage for 12 months*

	Antioxidant Activity (IC50 to scavaging DPPH free radical)													
	Storage time (month)													
Treatment	0	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CO	0.118	0.141	0.146	0.162	0.189	0.205	0.232	0.256	0.316	0.391	0.484	0.700	0.966	0.331
CA	0.126	0.127	0.154	0.174	0.195	0.201	0.245	0.265	0.338	0.407	0.559	0.740	0.893	0.340
CL	0.115	0.127	0.139	0.154	0.177	0.197	0.217	0.243	0.291	0.355	0.439	0.629	0.809	0.299
E+CL	0.117	0.132	0.137	0.154	0.180	0.195	0.215	0.241	0.295	0.364	0.453	0.623	0.826	0.303

*Values reported as a means ± SD of 4 replications of storage samples



Appendix 16: Sorption isotherm (Roberts, 1980)

Appendix 17: The weather condition during growing season 2005 and 2006 at Agriculture Research Station, Chiang Mai University, Thailand

Parameters	August		September		October		November		December	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Pressure (hPa)										
Mean	1005.05	1006.39	1009.54	1007.96	1011.23	1011.23	1011.93	1011.93	1013.86	1014.22
Mean max.	1006.99	1002.09	1004.45	1010.22	1013.37	1013.56	1014.33	1014.40	1016.21	1016.94
Mean min.	1002.55	1004.54	1007.29	1005.01	1008.13	1008.16	1008.64	1008.63	1010.89	1010.93
Temteratur(°C)										
Mean	26.2	26.0	26.7	26.0	26.0	25.8	24.6	24.0	21.9	21.8
Mean max.	30.5	30.7	32.2	30.9	31.7	31.6	31.6	30.6	29.1	27.8
Mean min.	23.6	23.4	23.4	23.0	22.1	22.0	20.5	18.2	17.5	16.1
Relative Humidity (%)										
Mean	90.6	87.9	88.4	85.8	84.9	84.1	83.7	77.9	79.3	73.2
Mean max.	98.6	97.8	98.5	98.0	98.4	97.9	98.1	97.7	95.8	95.4
Mean min.	75.0	71.4	70.8	64.4	63.3	62.5	61.7	50.3	57.5	46.5
Rainfall (mm.)										
Total	341.4	155.2	436.3	194.8	192.0	69.9	22.8	0	27.9	0
Mean	11.0	5.2	17.5	6.5	6.2	2.3	0.8	0	0.9	0
Sunshine Duration (hr.)										
Total	56.9	92.5	118.4	151.1	192.9	205.7	215.0	256.8	170.6	256.7
Mean	1.8	3.0	3.9	5.0	6.2	6.6	7.2	8.6	5.5	8.3
Evaporation (mm.)										
Total	78.7	110.2	100.4	128.1	112.7	121.6	103.3	114.6	87.8	101.7
Mean	2.5	3.7	3.5	4.4	3.9	3.9	3.4	3.8	2.8	3.3

(**Source:** Northern Meteorological Center, Thai Meteorological Department, Ministry of Information and Communication Technology)

Appendix 18: The properties and mineral contents of soil before the field experiments in seasons 2005 and 2006

Treatment	Sand (%)	Silt (%)	Clay (%)	Texture	pH 2005	pH 2006	OM (%) 2005	OM (%) 2006	CEC (cmol(+)/Kg) 2005	CEC (cmol(+)/Kg) 2006
CO	54.4	29.0	16.6	Sandy loam	5.61	5.33	2.49	2.61	3.81	4.00
CA	54.4	30.0	16.0		5.48	5.21	2.07	2.17	3.60	3.78
CL	54.4	30.0	15.6		5.72	5.43	2.32	2.44	3.74	3.93
E+CL	54.0	31.5	15.6		5.60	5.32	2.74	2.88	3.70	3.89

Treatment	Total N (g 100g^{-1})		P (mg.kg^{-1})		K (mg.kg^{-1})		Ca (mg.kg^{-1})		Mg (mg.kg^{-1})		Fe (mg.kg^{-1})		Mn (mg.kg^{-1})	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
CO	0.07	0.08	69.20	72.7	18.90	19.85	516.00	541.80	37.00	38.85	145.20	152.46	18.20	19.1
CA	0.06	0.07	53.20	55.9	17.40	18.27	541.00	568.1	37.90	39.8	156.60	164.43	14.50	15.2
CL	0.06	0.07	56.70	59.5	17.70	18.59	622.00	653.1	43.10	45.26	132.70	139.34	17.40	18.3
E+CL	0.07	0.08	53.80	56.5	17.60	18.48	545.00	572.3	40.30	42.32	143.20	150.36	15.00	15.8

Treatment	Zn (mg.kg^{-1})		Cu (mg.kg^{-1})		Al (mg.kg^{-1})		Na (mg.kg^{-1})		Cl (mg.kg^{-1})		S (mg.kg^{-1})		B (mg.kg^{-1})	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
CO	1.48	1.55	1.20	1.26	8.05	8.45	109.50	115	21.80	22.89	57.30	60.17	0.162	0.170
CA	1.02	1.07	1.25	1.31	8.25	8.66	102.90	108.1	19.40	20.37	52.00	54.6	0.153	0.161
CL	1.05	1.1	1.20	1.26	8.00	8.40	104.60	109.8	23.00	24.15	54.20	56.19	0.154	0.162
E+CL	1.17	1.23	1.19	1.25	7.75	8.14	109.80	115.3	24.90	26.15	57.80	60.69	0.156	0.164

CURRICULUM VITAE

Personal particular

Name	: Pitipong Thobunluepop
Nationality	: Thai
Date of birth	: 18 January 1978
Place of birth	: Lampang, Thailand
Sex	: Male

Address : **Office**: Faculty of Technology, Department of Plant Production, Mahasarakham University, Khantaravichai, Mahasarakham, 44150

: **Home**: 235/7 M.11 Yinglak, Prachinthanee Rd., Thambon Dhongphraram, Mouang, Prachinburi, Thailand, 25000

E-mail : pitipongtho@yahoo.com

School education

1984 – 1989	: Prachawit Primary School, Lampang
1990 – 1992	: Matayom Witthaya Secondary School, Lampang
1993 – 1995	: Bunyawat Witthayalai High School, Lampang

University education

1996 – 1999	: Faculty of Agriculture, Department of Soil Science Technology, Section of Plant Fertility, Chiang Mai University, Thailand: B. Sc. (Agriculture)
2000 – 2003	: Faculty of Agriculture, Department of Agronomy, Section of Seed Science and Technology, Chiang-Mai University, Thailand: M. Sc. (Agriculture)
Since 2004	: Post graduate program (PhD), Section Quality of Plant Products, Georg-August University of Göttingen, Germany

Professional employment

2000 – 2001	: Seed quality assurance staff and plant biotechnology researcher, Bangkok Seed Industry Co, Ltd., (Charoen Pokphand Group), Lopburi, Thailand
2001 – 2004	: Seed Agronomist Supervisor, Syngenta Seed (Thailand) Co, Ltd., Bangkok, Thailand
Since 2005	: Lecture at Faculty of Technology, Department of Plant production, Mahasarakham University, Mahasarakham, Thailand

……..

(Pitipong Thobunluepop)