CHAPTER 8

Conclusion

This research focused on selecting and developing the biological fungicide for decreasing the damage of root-knot nematode (*Meloidogyne incognita*) causing galled disease of various vegetables in Thailand. The acquired outputs would be applied to replace the pesticides which can deleteriously affect farmers, consumers and the environment. Much research including this study showed that biological fungicide can induce plant growth so that a farmer might be saved on the cost of fertilizers. Principally, an efficient biological agent composed of nematophagous fungi or nematode-trapping fungi must carry on various processes following the hypotheses stating that "the fungal isolates are virulent and viable for long periods of storage are a suitable biological control agents; moreover, the best production of biological pesticides are an important factor in their efficiency and survival for field application to reduce damage from root-knot nematodes (Brand *et al*., 2010)". According to the aforementioned hypotheses, the study would be divided into five steps which related to the procedure. The main points were as follows:

The first step of biological fungicide production was a collection and selection of efficient nematophagous fungi against root-knot nematode. A number of highly virulent fungi damaged second stage juveniles (J2) of root-knot nematodes using special infection structures including *Arthrobotrys* sp. isolate JDI1-001, *Arthrobotrys* sp. isolate MTI2-001, *Arthrobotrys* sp. isolate MSO1-001, *Arthrobotrys* sp. isolate MPI1-003 and *Arthrobotrys* sp. isolate API3-001 infected eggs by means of hyphal

tips as *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003 were selected from planted soil samplings of northern Thailand.

The second step was a study and species classification of selected fungi using morphology characteristics and molecular techniques. In this study, the results indicated that isolate DLO1-001 (old stocked isolate) and isolate MTI2-001 were identified as *Arthrobotrys oligospora*. Isolate API3-001 characterized as *A. conoides* whereas isolate MSO1-001 showed a concordant relationship with *A. musiformis*. The morphological and molecular data of isolate JDI1-001 and MPI1-003 were less clear cut; however, they were identified as *A. thaumasia*. Isolate WJI1-003 and KJO1-003 were morphologically categorized as *Paecilomyces* sp. and *Pochonia* sp.

The third step was a study of some abiotic factors related to growth and sporulation of fungi including their survival tendency in the rhizosphere. The statistical data summarized that all factors including media, temperature, light, pH and pesticides had an action on fungal vegetative and reproductive stages. Therefore, *in vitro* culture of selected fungi before development to assigned biological pesticide started by inoculating the fungi to corn meal agar media (CMA) or potato carrot agar (PCA) which adjusted to pH 8 (alkaline state) or pH 7 (neutral state). An inoculative media was incubated at 30^{o}C under the regime of 24 hour dark followed by 24 hour light, calling stock culture. In addition, the application of bio-controls should consider the effects of pesticides because almost all those studied inhibited fungal growth and sporulation especially insecticides including dazomet, carbaryl and chlorpyrifos. Similar suppressive effects on the bio-controls were seen with fungicides such as metalaxyl mixed with mancozeb, fosetyl aluminium and quintozene mixed with

etridiazole. The most suppressive herbicides were paraquat dichloride, oxyfluorfen and glyphosate-isopropyl ammonium, respectively.

The fourth step was a biomass culture preparation of competent fungi for controlling root-knot nematodes. A stock culture of fungi was transferred to solid substrates such as corn (14 baht per kilogram) or rice (25 baht per kilogram) grain in polypropylene bags which contained 125 g per bag and incubated under optimized condition which referred from the third step for inducing sporulation approximately 14 days by shaking every 5 days. On the other hand, a large amount of conidia could be prepared by biomass culture in a liquid medium such as enteromophthoraceae medium (64.26 baht per liter) and incubated in a growth chamber at 30±1ºC together with vertical shaking at 180 rpm for 14 days followed by pouring biomass to sterilized trays which were covered with straining cloth and incubating in clean room at 30±1ºC, 75% RH under adequate ventilation for 7 days. Pouring biomass to sterilized trays and incubating process in clean room could be applied for solid substrate to get more increasing of conidial production.

The fifth step was an evaluation of biological agent applications in the greenhouse and field. Many trials in this study indicated that selected nematophagous fungi supported plant growth parameters including height, root length, fresh weight of shoot and dry weight of shoot and could decrease the number of galls per root system based on the characteristic of each isolate. Moreover, some treatments showed a greater efficiency than chemical fumigation especially biological agents isolate MTI2-001, isolate API3-001 and isolate WJI1-003.

Therefore, the usability of nematophagous fungi as biological fungicide to reduce the population of root-knot nematodes should be performed as follows:

(1) Preparation of competent fungal biomass referring the fourth step.

(2) Amending biomass with seedling medium which had high volume of cow dung manure.

(3) Should be avoided those pesticides that may have a strong suppressive effect on bio-controls agent

(4) Expanding a number of biological agents by scattering five percent of each fungal biomass ($\times 10^6$ spore/ml) on multiplied ingredient which consist of 40% well decomposed cow dung manure (CDM), 30% paddy husk, 20% powder of ash and 5% corn bran and combining all ingredients followed by moderate watering. The mixture was incubated in an approximately 75% RH and obscure light at 21 days and well combined again before using.

(5) Before transplantation for 7 days, grinding and fermenting the complete biological agents from subtopic (4) to root-knot infested areas which continually obtained a low humidity condition. In some case, the fermented method may not be necessary.

(6) Transplanting the seedlings to target areas and carrying on normal practices as applying of water and fertilizer or spraying pesticides (if necessary). Avoid the application of soil chemical because they could decrease the ability of biological fungicides referring from results of the third step.

In this study, application of bio-fungicide formulation No.3 (*Paecilomyces* sp. isolate WJI1-003) was optimal methods to induce growth and reduce a number of galls per root on head lettuce while bio-fungicide formulation No.1-2-3 (*A. oligospora* isolate MTI2-001, *A. conoides* isolate API3-001 and *Paecilomyces* sp. isolate WJI1-003), bio-fungicide formulation No.2 (*A. conoides* isolate API3-001) amended with

No.3 (*Paecilomyces* sp. isolate WJI1-003) and bio-fungicide formulation No.1 (*A. oligospora* isolate MTI2-001) amended with No.2 (*A. conoides* isolate API3-001) were optimal methods to reduce gall of root knot nematodes but showed mediumry on growth. Bio-fungicide formulation No.1 (*A. oligospora* isolate MTI2-001) amended with No.3 (*Paecilomyces* sp. isolate WJI1-003) was a good inducing plant height and fresh weight of shoot and could reduce the galled formation of baby cos lettuce to scale 0.

However, farmers should investigate the severity of galled disease in areas before selecting biological agent formula. In case of a severe level, applying a bio-fungicide amended with No.1 (*A. oligospora* isolate MTI2-001) No.2 (*A. conoides* isolate API3-001) and No. 3 (*Paecilomyces* sp. isolate WJI1-003) at ratio 1:1:1 or bio-fungicide formulation No.2 (*A. conoides* isolate API3-001) amended with No.3 (*Paecilomyces* sp. isolate WJI1-003) should be done on the first time to significantly decrease the population of root-knot nematodes following either application of bio-fungicide formulation No.3 (*Paecilomyces* sp. isolate WJI1-003) for 2-3 times.

Future research should be focused on the formulation of biological agent as a commercial product. For example, adding an activated substance which has the special property to induce fungal growth and sporulation such as corn bran, powder of botanical extraction, carbon powder and organic powder to product ingredients. On the other hand, examination of *in vitro* procedures to induce resting spore formation which could increase survival through stressful times and extend shelf-life should be an ongoing effort as well as the study of conidial preservation to reduce the effect of hazards.