CHAPTER 1

Introduction

1.1 Research Rationale

Microorganisms and theirs activities in the rhizosphere considerably influence plant growth by effecting decomposition, absorption, storage, and cycling of nutrients, suppression of pathogens and development of soil structure. Protecting the functional biodiversity of rhizosphere organisms through proper management practices is essential to maintaining ecosystem symmetry and flexibility (Coleman *et al.*, 1984; Mariam, 2008). Nematode (nema = thread; oides = resembling) or un-segmented roundworm is a type of microorganism usually found in the soil and plant roots, but a few species may attack above-ground parts of the plant. Plant-parasitic and free-living nematodes comprise a principal group of nematodes. Almost all plant species are attacked by specific species of nematode and many species have very wide host ranges (Koenning *et al*., 1999; Béatrice *et al*., 2011).

Plant-parasitic nematodes are important pests in numerous plant production systems such as agriculture, horticulture, ornamentals and turf. Root-knot nematodes *Meloidogyne* species, are the most common and wide spread plant-parasitic nematodes in vegetable production. Gall or knot formations on roots from *Meloidogyne* species can cause significant decreases in the quantity and quality of yields which, in turn, causes considerable economic losses. An average of 10% loss in yield due to root-knot nematodes is frequently cited for vegetables (Koenning *et al*., 1999; Béatrice *et al*., 2011). However, highly susceptible vegetable crops such as

Solanaceae, *Cucurbitaceae* and *Cruciferae* have over 30% yield losses. Four species; *Meloidogyne arenaria*, *M. javanica*, *M. incognita* and *M. hapla* are mainly challenges to vegetable production and the first three species are found worldwide.

Selecting control methods should be predicated on reduction of damaging levels of nematode populations instead of trying to eradicate these organisms (Mariam, 2008). In agricultural fields, widely-used management strategies for root-knot nematodes include soil fumigation, application of non-fumigant nematicides, heat-based methods (steam treatment and soil solarization), rotation with non-host crops, use of resistant varieties, organic amendments, fertilization, biological control and integration of these practices (Becker, 2002). Nevertheless, designing sustainable management methods based on plant resistance (Thakur, 2007; Béatrice *et al*., 2011) and use of antagonistic microorganisms remains a challenge.

Nematicides are expensive and highly toxic to both humans and the environment (Abawi & Widmer, 2000). In addition, nematicides do not eliminate all the nematodes from fields. Moreover, the most commonly-used fumigant nematicide, methyl bromide, is being progressively banned, highly restricted or totally banned for use in vegetable production in developed countries due to environmental and public health concerns. Thus the development of alternative control strategies and long-term integrative approaches is urgently needed in order to replace chemical nematicides for sustainable farming systems (Martin, 2003). Biological control agents (BCAs), such as fungi, offer great scope for field application, but the development of a viable bioprocess for their commercial production is not a simple matter.

Field applications of biological control agents are mainly accomplished by means of virulent and fungal conidiospores viable after long periods of storage. Solid state fermentation (SSF) has many advantages for effective, large scale production of conidiospores. Two considerations for controlling root-knot nematodes in agriculture are discussed with emphasis on biological control research and practices using virulent spores and their appropriate application (Brand *et al*., 2010).

According to the aforementioned principles, theory and rationale, this study will cover the following hypotheses:

1. Filamentous, nematophagous fungi or nematode-trapping fungi are virulent and viable for long periods of storage and suitable biological control agents for field application to reduce damage from root-knot nematodes.

2. The growth physiology of filamentous fungi is an important factor in their production and survival as nematophagous fungi.

1.2 The objectives of this Study

The main objectives were as follows:-

1) To screen and classify efficient nematophagous fungi from vegetable crop production sites
2) To study the essential growth factors of selected nematophagous fungi for mass culture
3) To compare capacity between selected nematophagous fungi and a commercial bio-product against root-knot nematodes
4) To produce bio-pesticides for controlling root-knot nematode in vegetable crop production

1.3 Scope of this thesis

This thesis is divided into three parts. Part 1 (Chapters 3 and 4) describes selection and identification of efficient nematophagous fungi against root-knot nematode from agricultural areas. Part 2 (Chapter 5) describes essential growth and sporulation factors of these fungi involving rhizosphere colonization and survival. Part 3 (Chapters 6 and 7) describes development of formulation and field-application techniques for fungal bio-control agents in vegetable crops.

Part 1: Selection and identification of efficient nematophagous fungi against root-knot nematode

Randomized samples of infected root-knot nematode soil from infected root-knot nematode plantations and adjacent areas rich in organic material were collected and processed for isolation of nematophagous fungi by soil sprinkling technique and adding root-knot nematodes suspension as bait. The fungi were purified and preliminarily examined for their *in vitro* parasitic ability, i.e. endoparasite, predacity and egg-parasite, on egg and second stage juvenile (J2) of root-knot nematodes. The fungal isolates, which were vigorous and showed high capability to trap nematodes or attack nematode eggs were selected for subsequent experiments (Chapter 3). The morphological characteristics of efficient nematophagous fungi were studied in order to identify which correlated with molecular methods, internal transcribed spacer (ITS) primers (Chapter 4).

Part 2: Essential survival factors of the fungi for successful establishment in soil environments

This part (Chapter 5) focuses on the *in vitro* study of five important growth and sporulation factors of nematophagous fungi. The factors examined were media, temperature, light, pH and pesticide (herbicide, insecticide and fungicide) tolerance. Each factor was divided into particular treatments for comparison. Diameters of the resulting colony were measured including sporulation before statistical analysis. The suitable treatments which were conducive to high fungal growth and sporulation, were utilized in fungal mass production experiments. In addition, morphological interactions between selected nematophagous fungi and other antagonistic fungi for soil-borne disease management were observed. Ecological influences were assessed using dual culture techniques, which consider percentage of fungal growth inhibition.

Part 3: Development of formulating and applying fungal bio-control agents

This part covers two topics. First, chapter 6 describes the selection of fungal culture preparation methods. The selected nematophagous fungi were inoculated into solid media and liquid media in order to analyze conidial mass production. Second, Chapter 7 presents greenhouse screening of the fungi against root-knot nematodes. This research was divided into seedling and post-transplantation periods. Mass culture of each fungus growing on grain utilized techniques developed from previous experiments. Collected data included plant height, root length, fresh weight of shoot, dry weight of shoot, number of galls per plant and J2 of *Meloidogyne incognita* per root system were analyzed and compared for selecting perfect fungi. The capacity of selected fungal biomass formulations to control root-knot nematodes in vegetable crops was further compared with application of fumigant nematicides. Data obtained included plant height, root length, fresh weight of shoot, dry weight of shoot, number of galls per plant on a scale of 0–10 (Barker, 1978) and % galled reduction.