OCCURRENCE OF *APHELENCHOIDES BESSEYI* (Christie, 1942) IN DIFFERENT RICE GROWING REGIONS AND MANAGEMENT OF WHITE TIP DISEASE

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A Thesis submitted to the post-graduate committee of the Yezin Agricultural University in the partial fulfillment of the requirements for the degree of Master of Agricultural Science (Plant Pathology)

Department of Plant Pathology
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The thesis attached hereto, entitled **"Occurrence of *Aphelenchoides besseyi* (Christie, 1942) in Different Rice Growing Regions and Management of White Tip Disease"** was prepared under the direction of the chairperson of the candidate supervisory committee and has been approved by all members of that committee and board of examiners as partial fulfillment of the requirements for the degree of **Master of Agricultural Science (Plant Pathology)**.

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This thesis represents the original work of the author, except where otherwise stated. It has not been submitted previously for a degree at this or any other university.

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DEDICATED TO MY BELOVED PARENTS

U MAUNG THAN AND DAW TA SOPE

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ABSTRACT

One hundred and eleven rice seeds samples of 79 different rice varieties were collected from five different agricultural research farms; Myaungmya, Letpadan, Naungmon, Yezin and Kyaukse in monsoon season to determine the infestation of *Aphelenchoides besseyi.* The observation showed that *A. besseyi* was found in rice seeds samples collected from five agricultural research farms. Fifty three out of 111 rice seeds samples were infested with *A. besseyi* ranged from 1 to 424 nematodes 100 seeds^{-1}. Among study sites, 45% rice varieties in Myaungmya, 10% in Letpadan and 14% in Naungmon were infested with economic threshold level of 30 nematodes 100 seeds^{-1}. Four inoculation methods of introducing nematode suspension; below the leaf sheath (T1), into four holes of soil (T2), spraying nematode suspension to the plant (T3) and dipping the rice seedlings into nematode suspension (T4) were studied to evaluate pathogenicity of *A. besseyi* in rice. Results showed that among tested inoculation methods, higher final nematode population was found in three methods; T4, T1 and T2 with number of 103, 59 and 47 nematodes 100 seeds^{-1}, respectively than that of one nematode 100 seeds^{-1} in T3. The reactions of fifteen rice varieties which were widely cultivated throughout Myanmar to *A. besseyi* were assessed with inoculum level of 500 nematodes plant^{-1} by artificial inoculation of introducing nematode suspension into four holes of soil around the plant. Among them, Paw Hsan Yin, Sin A Kari-3 and Yadana Toe showed moderately resistant reaction while Aye Yar Min, Hnangar, Thee Dat Yin, and Hmawbi-2 were moderately susceptible and Manawthukha, Sin Thu Kha, Shwe War Tun, Sin Thwe Latt, Kyaw Zay Ya, Shwe Bo Paw Hsan, Shwethwe Yin and Shwe Yin Aye were highly susceptible to *A. besseyi*. Effect of different control measures such as soaking infested rice seeds in brine solution (20% NaCl) (T2), seed treated with hot water at 55°C for 30 minutes (T3), application of carbofuran (Furadun 3G with 3% Carbofuran w/w) to soil (T4), combination of seed treated with hot water at 55°C for 30 minutes and brine solution (T5) and combination of seed treated with hot water at 55°C for 30 minutes, brine solution and application of carbofuran to the soil (T6) were conducted to evaluate the effective management of white tip disease in the screen house by using the infested Lone Pu variety. The reduction percent of nematode population were found as 100% in T4, 99.9% in T6, 99.8% in T3 and 99.5% in T5 and these treatments seemed to have good effect in yield and yield components. Accordingly, it could be suggested that nematode infested seeds treated with hot water treatment alone (T3) can be used to minimize the infestation of *A. besseyi* and reduce the loss of grain yield.

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CHAPTER I
INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple foods in the world and over half of the global population was provided as a food source. In Myanmar, rice is widely cultivated in both rainfed and irrigated production systems of different regions. The average yield was 3.92 MT ha^{-1} from total production area of 7.26 million hectares (Ministry of Agriculture, Livestock and Irrigation [MOALI], 2018). Among the rice producers in the world, Myanmar stands at the rank of seventh in total production of rice (Food and Agriculture Organization [FAO], 2018). Therefore, rice production in Myanmar is high enough to fulfill needs for local consumption and the surplus is being exported. To sustain the export amount, it is necessary to increase the rice production and improve the quality of rice seeds for the quarantine aspects. Several factors causing minimal rice productivity includes using low yielding varieties, poor soil fertility and inadequate pest and disease management practices (Department of Agriculture [DoA], 2000).

Nowadays there are many constraints in rice production including abiotic and biotic stress. Among biotic stress, diseases are considered as one of the major constraints in rice production and responsible for yield losses (Wasihun & Flagote, 2016). Of these, nematode infestation can result up to 30% yield losses in rice production (Dobermann & Fairhurst, 2000). On a world-wide basis the nematode species of rice root nematode, root knot nematode, stem nematode, foliar nematode and spiral nematode are of serious concern to rice production (Swastica, 1993). In Myanmar, ufra, root rot, root knot and white tip are the most destructive nematode diseases in rice production (Aung, Yi & Khin, 1993; Mya, 1983).

White tip nematode (*Aphelenchoides besseyi*, Christie 1942) is known as the cause of white tip disease of rice (Fortuner & Williams, 1975; Franklin & Siddiqi, 1972). It feeds ectoparasitically on the meristems of stems, leaves and buds of susceptible plants and has been attributed to cause a considerable amount of loss in rice (Tulek & Cobanoglu, 2010). A reduction in rice yield has been reported as severe as 30-70% in susceptible cultivars (Lin, Ding, Wang, Zho & Lin, 2004; Muthukrishnan, Rajendran & Handrasekaran, 1974; Tikhonova, 1966a). *A. besseyi* is considered to be a major contributor to the seed borne pathogens of rice (Duncan & Moens, 2013) and that distributed widespread in almost all the rice growing areas of

the world (Jamali, Pourjam, Alizadeh & Alinia, 2006). Therefore, it is a potential target from the point of view of quarantine (Gergon & Mew, 1991).

A. besseyi commonly known as foliar nematode of rice and can infect the rice plant in all rice production environments. It is an aboveground ectoparasite affecting leaves and flower primordium inducing characteristic whitening of 2-5 cm of leaf tip (Giudici & Villa, 2003). It can survive in stored seeds for several years, under dry condition (Tiwari & Khare, 2003) and can deteriorate quality of rice seeds (Rajan & Mathur, 1990). In Myanmar, *A. besseyi* is widely distributed throughout the entire country and yield losses can reach up to 50% depending on the nematode population density and the susceptibility of the crop (Swe, 1997).

Many researchers have attempted to control *A. besseyi* using various approaches such as cultural, biological and chemical controls (Islam, Rahman, Farazi, Hossain & Sultan, 2015). The most practical and effective control strategy is early detection and elimination of *A. besseyi* from rice seeds, including the use of nematode free seeds. Although management of *A. besseyi* may exploit the use of resistant cultivars (Popova, Zelenskii & Subbotin, 1994), there is wide range of variation for resistance (El-Shafey, El-Emary & Elamawi, 2010). This nematode is seed borne (Ou, 1985) and, therefore, hot water treatment of rice seeds at 51°C to 53°C for 15 minutes appear to be a sound control strategy in the management of white tip disease (Atkins & Todd, 1959). Several reports of the use of chemical pesticides to manage *A. besseyi* indicated a reduction in nematode infestation (Shigeru & Katsumi, 2000). However, there can be environmental pollution and low germination of treated seeds which serve as serious drawbacks of this approach (Prasad & Varaparasad, 1992). Therefore, proper management of white tip disease is urgently needed to increase the production of rice and to provide seeds without any nematode contamination for quarantine programs.

In Myanmar, 66% of total rice cultivated areas are grown different rice varieties. Among them, 36.5% were high yielding varieties, 12.45% local rice varieties, 1.63% upland rice varieties, 14.0% quality rice varieties, 0.64% others and 1.36% hybrid rice varieties (Department of Agricultural Research [DAR], 2017). These rice varieties are cultivated depending on the climatic and environmental conditions of different rice growing regions. Majority of rice varieties for the farmers were distributed from agricultural research farms under Department of Agricultural Research.

The main source of white tip disease spread is seed transmittion. Thus, there must be potential on the infestation of *A. besseyi* in different rice varieties and different locations because it can survive in the seeds for several years and distributed by seeds (Tulek & Cobanoglu, 2012). The farmers can overlook the presence of *A. besseyi* in rice seeds and faced with constant risk of introducing foliar nematode infected asymptomatic rice seeds into their production whereby rapid spread of the nematodes to adjoining, healthy fields and spread to the other rice growing regions through seed transmission.

In Myanmar, there were very limited researches on white tip disease. Aung et al. (1993) observed that white tip disease has been occurred in rice fields of Myanmar after that War (2009) studied biology of *A. besseyi* in association with rice yield and management. However, the detail information on incidence and distribution of it in rice cultivated areas has not been studied yet. Therefore, the present study was conducted with the following objectives:

1. to determine the infestation of *A. besseyi* in different rice growing regions,
2. to evaluate fifteen widely cultivated rice varieties for resistance to *A. besseyi* and
3. to evaluate the effect of different control measures on white tip disease of rice

CHAPTER II
LITERATURE REVIEW

2.1 White Tip Nematode (*Aphelenchoides besseyi*)

A. besseyi is present all over the rice growing areas of the world. It infests rice plant and the consequent damage symptoms have given rise to its common name (Franklin & Siddiqi, 1972). *A. besseyi* also infests strawberries, where it is the causal agent of "summer dwarf" or "crimp" disease, a disease recorded from the USA, Australia and more latterly Europe. In rice and strawberries, nematode feeds ectoparasitically on the meristem of the stem, leaf and buds of the susceptible plants. The nematode is capable of withstanding desiccation, and may be found in a quiescent state beneath the hulls of rice grains. Thus there is also a risk of spread with the practice of using rice husks as packaging material, which has occurred on some imported consignments of plants.

2.1.1 Economic importance

On susceptible rice varieties, *A. besseyi* feeds ectoparasitically on the meristems of stems, leaves, buds and later on ovary, stamens and the developing embryos. Therefore, diseased plants are reduced in vigor and height, and produce small panicles with reduced number of spikelets. There was variation in growth and yield parameters with progressive increase in the initial inoculums density of nematode and the minimum damaging threshold level of *A. besseyi* was found to be 100 nematodes kg^{-1} soils. Losses caused by *A. besseyi* vary with the initial nematode population level, rice variety, region, environment and management. Yield loss caused by this nematode was 30-50% (Swain, 1987).

The losses of rice caused by *A. besseyi* were estimated in greenhouse and micro plot conditions at Rice Research Institute, Rasht (Guilan province) during 2005-2006. The minimum infestation level leading to symptoms and yield loss was greater with population of 500 nematodes than those of 300 nematodes (Jamali, Pourjam, Safaee & Alizadeh, 2009). Papova (1981) reported that infection of *A. besseyi* reduced 20% of grain yield in Start variety whereas no yield reduction occurred in M 91 variety in Brazil country.

2.1.2 History and distribution

A. besseyi was discovered in a great number of rice growing areas in Asia, tropical America, the USSR, etc. It had not been reported from Africa and measures

were taken to prevent its introduction (Luc, 1960) but a survey carried out between 1965 and 1970 showed that it had probably been in existence there for a long time because it has been found on local varieties (Diatang de Bignona) in Senegal (Barat, Delassus & Vuong, 1969). *A. besseyi* is very widely distributed and now occurs in most rice growing areas (Ou, 1985). Its wide distribution has resulted from dissemination in seed.

The foliar nematode *A. besseyi* causes white tip disease in rice and floral malady in tuberose. This nematode is widely distributed in the rice fields of many states of India, including West Bengal, Andhra Pradesh, Madhya Pradesh and Gujarat (Khan, Handoo, Rao, Rao & Prasad, 2012).

2.1.3 Damage symptoms

The critical symptoms are observed at tillering stage. The diseased plants remain short, the tip of the leaves of infected plants turn yellow or white 3-5 cm and shred. The white tip of leaf is caused due to the obstruction in vessels due to gums and disintegration of phloem cells, the cell growth gets retarded and chloroplasts damaged. In severe cases necrosis occurs. Flag and some upper leaves twist and curl obstructing the panicle emergence.

Symptoms are produced by feeding of the parasitic nematodes that have attacked the tissues of the leaf or panicle head while it was still enveloped (protected) by the sheath or boot resulting injury to the growing point of the shoot and thus, causing a disturbance in the physiology of their rice plant.

Typical symptoms of small grains and erect panicles in Wuyunjing-7 included high sterility, small panicles, distorted glumes, small and chaffy grains, declined grain numbers and stunted plant but without white tip on flag leaf. The nematodes in filled grains were more than those in unfilled grains.

2.1.4 Morphology

The body of female *A. besseyi* is slender, straight to slightly arcuate ventrally when relaxed; annules fine, indistinct, about 0.9 µm wide near mid-body. Lip region rounded, unstriated, slightly offset and wider than body at lip base, about half as wide as mid-body; labial framework hexaradiate, lightly sclerotized. Lateral fields about one-fourth as wide as body, with 4 incisures. Anterior part of spear sharply pointed, about 45% of total spear length, posterior part with slight basal swellings which are 1.75 µm across. Median oesophageal bulb is oval, with a distinct valvular apparatus

slightly behind its center. Oesophageal glands are extending dorsally and subdorsally for 4 to 8 body-widths over intestine. Nerve ring about one body-width behind median oeosphageal bulb.

Excretory pore is usually near anterior edge of nerve ring. Vulva is transverse, with slightly raised lips. Spermatheca is elongate oval (up to 8 times as long as wide when fully distended), usually packed with sperm. Ovary is relatively short and not extending to oesophageal glands, with oocytes in 2-4 rows. Post-vulva uterine sac narrow is inconspicuous, not containing sperm, 2.5-3.5 times anal body width long but less than one-third distance from vulva to anus. Tail is conoid, 3.5-5 times anal body widths long; terminus bearing a mucro of diverse shape with 3-4 pointed processes.

Male *A. besseyi* are about as numerous as females. The posterior end of body is curved to about 180 degrees in relaxed specimens. Lip region, spear and oesophagus are same as described for female; tail conoid, with terminal mucro with 2-4 pointed processes. First pair of ventrosubmedian papillae adanal, second slightly behind middle of tail and third subterminal. Spicules are typical of the genus except that the proximal end lacks a dorsal process (apex) and has only a moderately developed ventral one (rostrum). Testis is single, oustretched.

2.1.5 Biology

After sowing rice seeds, anabiotic *A. besseyi* get activated on getting water and then they first feed on the tender primordium of sprouting seeds. It feeds endoparasitically in the coleoptile for 7-10 days and ectoparasitically within the innermost leaf sheath during other plant growth stage (Tsay et al., 1998). On tillering, the nematodes reach the growing point and remain on the outer surface of folded young leaves as ectoparasites. They move upward along with the growth of the plant. They feed on the vegetative tissue but do not penetrate them. On completion of tillering the nematodes increase fast and reach the panicle. However, *A. besseyi* is more abundant on the outer surface of the glumes and enters when these separate at anthesis (Yoshii & Yamamoto, 1950b). As grain filling and maturation proceed, reproduction of the nematode ceases, although the development of J3 to adult continues until the hard dough stage (Huang & Huang, 1972) and then anabiosis begins (Nandakumar, Prasad, Rao & Rao, 1975). The population of anabiotic nematodes is predominantly adult females (Huang, Huang & Chiang, 1979). These

nematodes coil and aggregate in the glume axis. More nematodes occur in filled grain than in sterile spikelets (Yoshii &Yamamoto, 1950b) and infected grain tends to occur more towards the middle of the panicle (Goto & Fukatsu, 1952).

2.1.6 Life cycle and reproduction

The duration of the cycle from egg to egg is 6.5 to 7 days. Development of the egg takes 0.5 days, of the second larval stage 0.13 days, of the third 0.65 days and of the fourth 0.9 days; sexual maturation of the adult female takes 4.3 to 4.8 days. Larvae must feed in order to develop: in pure water they become inactive in 2-3 days and development ceases (Sudakova & Stoyakov, 1967). In nature the length of the cycle depends on ecological conditions and lasts 29 days at 14.7°C, 9 days at 20.6°C, 6 days at 25°C and 3 days at 31.8°C (Tikhonova, 1966b).

The rate of multiplication depends on the plant and on its capacity to resist the disease. Reproduction of *A. besseyi* greater after tillering than before and increases after the panicle has started to form (Goto & Fukatsu, 1956). The life-cycle of *A. besseyi* cultured on *Fusarium solani* is 24 days ± 4 at 16°C, 15 days ± 2.9 at 20°C, 9 days ± 2 at 23°C, 10 days ± 2 at 25°C and 8 days ± 2 at 30°C, the last being considered the optimum temperature. At 35°C, egg-laying, hatching and moulting occur, but the population no longer increases (Huang, Huang & Lin, 1972).

2.1.7 Survival and dissemination

A. besseyi can survive 2-3 years in rice grains (Todd & Atkins, 1958; Yoshii & Yamamoto, 1950a), whereas, Zem and Mamteiro (1977) reported their survival even up to 8 years. Recovery of nematodes was higher from seeds stored with low moisture than from seeds having high moisture at most of the temperatures (Chaudhury & Chaudhury, 1996). Nematode inoculated plants produced a greater proportion of light seeds floating on water than non-inoculated plants. Nematode mortality was greater in light seeds than in heavy seeds. Mean degree of seed swelling increased in light seeds as the number of nematodes increased (Togashi & Hoshino, 2003). Nematode lived in the stubbles left in the field which helped in the carryover of the inoculum from season to season. Fungi such as *Fusarium* sp. or *Curvularia* sp. found in the straw were the probable alternate hosts for the nematode in the field (Sivakumar, 1987).

2.1.8 Host range

A. besseyi is known to occur on a rather wide range of hosts including strawberry, onion, soybean, sweet potato, sugarcane, millets, sweet potato, sugarcane,

millets, sweet corn, chrysanthemum, coleus, *Dahlia, Dioscorea, Stylosanthes hamate, Lactyca* etc. *Oryza nivara* is a weed as host of *A. besseyi* (Tiwari & Dave, 1986). Seeds of *Fraxinus americana* infested with *A. besseyi* which was imported from USA (Gokte, Mathur, Lai & Rajan, 1989). *Setaria viridis* (foxtail) may be infected by the nematode and *Panicum sanguinale* (crab grass) and *Cyperus iria* may be slightly infected (Yoshii & Yamamoto, 1950a). Vuong (1969) detected *A. besseyi* on *Cyperus polystachyus* and *Imperata cylindrical*. The nematode also feeds and reproduces on fungi like species of *Alternaria, Curvularia, Fusarium, Nigrospora, Sclerosopora, Phoma* etc. (Fortuner & Williams, 1975).

2.2 Control Methods

Attempts have been made to control white tip disease of rice by cultural operations, use of physical, chemicals, biological means and evolving varieties resistant of *A. besseyi.*

2.2.1 Cultural control

Several cultural practices reduced the population of *A. besseyi* and the disease intensity. If the rice is sown dry and flooded after germination, when it is 7.5 to 10 cm high, white tip appears in 60% of cases. The disease is less serious if the water is brought in at germination. If sown in water, most of the nematodes die within a week and only 0.5 % of the plants are affected. This method reduces the nematode population for several years (Cralley, 1956). The severity of the disease appears to be greater on plants originating from seed beds which have not been flooded and transplanted late and thickly (Yamada, Shiomi & Yamamoto, 1953). High seedling rates in the seed bed (Kobayashi & Sugiyama, 1977) and high numbers of seedlings $hill^{-1}$ (Yamada et al., 1953) tend to increase infection by increasing the number of infection loci in the field. Cralley (1949) recommended that early sowing is better in the USA. The short duration rice varieties come to flower during the rainy season and this helps the nematodes to move towards the panicle (Dastur, 1936). The straws, debris, weeds etc. should be removed and buried or burnt (Vuong, 1969).

2.2.2 Physical control

Hot water treatment has been found useful and various techniques have been developed by numerous researchers time to time. First soaking the seed in cold water for 16 to 20 hours and then treated with hot water for 5 to 10 minutes at 50-52°C

(Yoshii & Yamamoto, 1950c). The germination of rice seeds could be delayed by hot water treatment at 60°C for 20 minutes (Yoshii & Yamamoto, 1951). After studying the various treatments, for large quantities of seed, pre-soaking for 24 hours is recommended if accurate apparatus is not available. For small samples treatment for 10-15 minutes at 55 to 61°C, without pre-soaking, is recommended (Atkins & Todd, 1959). Gamma irradiation of rice seed at doses of 5 to 35 Grey could reduce the infection from 61 to 4% and increased yield (Aleksondrova, 1985).

2.2.3 Chemical control

Numerous chemicals have been tested for seed treatments, soil treatments, spray and fumigation to check the infection of *A. besseyi.* Presoaking rice seeds with 1% potassium chloride or 1% sodium chloride for 20 hours and sun dried at 40-41°C for 6 hours resulted in 95-97.6% disinfestation of *A. besseyi* (Sivakumar, 1987). BMC (Methyl-N2- benzimadazolyl carbamate), thiobendazole, benomyl, carbofuran, fenitrothion, oxamyl etc. have been successfully used as seed treatment (Da Silva, 1992; Fortuner & Williams, 1975; Gregon & Prot, 1993; Tsay et al., 1998).

Soil treatment with fensulphothion (5 kg ha^{-1}) and diazinon (15 kg ha^{-1}) were very effective in reducing the incidence of *A. besseyi*. A 0.5% aqueous concentration of dibetafluoroethyl-p-nitrophenyl phosphate when sprayed on soil was very effective against *A. besseyi* (Sudakova & Stoyakov, 1971).

Four pesticides were tested as spray before inflorescence initiation and found carbandazim to be most effective resulting in nematode free seeds, however fenitrothion resulted in less number of sterilized tillers and phosphomidon gave increased grain weight, yield, nematode free seeds and less number of chaffy grains (Tiwari, 2000). Parathion, etaphos, isophenphos, carbosulfan, monocrotophos were effective when applied at boot leaf stage (Kumar & Shivkumar, 1998). Application of ethoprop and carbofuran after transplanting and on symptom appearance obtained good control with 19% increased yield (Lee, Han & Park, 1976).

The nematode and egg sac of *A. besseyi* get killed when fumigated with methyl bromide at 100 gm^{-3} for 30 minutes (Tsay, Cheng, Cheng, Lin & Wu, 1995). The nematodes in seed of the variety Zenith were destroyed by fumigating with methyl bromide for 6 hours at the rate of 1.25 lb 1000 ft^{-3}, without reducing the germinating power of the seed (Tullis, 1951).

2.2.4 Host plant resistance

Use of resistant cultivars is environment-friendly approach to nematode management in contrast with the currently much disputed use of expensive and hazardous chemicals (Reversat et al., 2003; Sasser, 1989; Starr & Roberts, 2004). Oliveira and Ribeiro (1980) reported blue Bella, Labella, Lebonnet, Belle patna, Dawn and BR-IRGA-409 were resistant to *A. besseyi*. Bonnet 73 have multiple resistant to *A. besseyi* as well as several other diseases. Some other resistant varieties are Fortuna, Nira, Rexoro, Bluebonnet and Arkansan (Zelenskii & Papova, 1991).

Fourteen Iranian rice genotypes including improved and local varieties were evaluated for resistance to *A. besseyi* (Jamali & Mousanejad, 2011). Among the cultivars tested, four varieties showed high resistance, four varieties were moderate resistance, three varieties were moderate susceptible and three varieties were highly susceptible to *A. besseyi*. Susceptible plants expresses also other typical symptoms such as shortening of flag leaf which twisted at apical portion and hinders the emergence of panicle, reduction of panicle length and the grain number, spikelets with distorted glumes and deformed kernels. Sometimes symptomless but infested plants were also found.

An assessment of 1003 rice cultivars from different ecologo-geographic origin for their resistance to *A. besseyi* in the glasshouse resulted that 3 cultivars were immune, 10 highly resistant, 164 moderately resistant and 826 susceptible and highly susceptible to *A. besseyi* (Margarita, Popova, Grigorii & Zelenskii, 1994).

CHAPTER III
MATERIALS AND METHODS

3.1 Occurrence of *A. besseyi* in Different Rice Growing Regions

3.1.1 Collection of rice seeds samples

Rice seeds samples grown in 2017 monsoon season were collected from five agricultural research farms which were located in Myaungmya, Letpadann, Naungmon, Kyaukse and Rice Research Section, Yezin under Department of Agricultural Research (DAR). Total of 111 rice seeds samples were collected from five different agricultural research farms; 22 rice seeds samples from Myaungmya farm, 19 from Letpadann farm, 35 from Naungmon farm, 19 from Rice Research Section, Yezin and 13 from Kyaukse farm, respectively. A total of 79 rice varieties of 111 rice seeds samples obtained from five research farms which are located in different parts of Myanmar (Table 3.1). Hundred grams of rice seeds were collected for each sample.

3.1.2 Examination of *A. besseyi*

From each seed sample, 100 discolored seeds (seeds with distorted glumes and deformed kernels) were carefully separated and pounded with a motor and pestle. The crushed grains were placed on a piece of muslin cloth fitted over sieve (1 mm sieve) and then put in a Petri dish. Tap water was added and amounts were adjusted in the Petri dish to touch the bottom of the sieve and muslin cloth. Lids of Petri dish were used to cover on the top of the assembly to prevent evaporation and drying up quickly (Giudici, Villa, Callegarin & Tamborini, 2003). The Petri dishes were left for 24 hours at room temperature to extract the nematodes into water (Plate 3.1). The sample solution from each extract was collected and put in counting dishes. The presence of nematodes was examined in counting dishes, by using a compound microscope (OLYMPUS, Model: CX22LEDRFS1). The nematodes recovered from rice seeds were killed by boiling water (100°C for 1 minute). Nematode specimens were mounted on anhydrous glycerol on glass slide and sealed by nail polish for examination of morphological characters of *A. besseyi*. The number of *A. besseyi* recovered from 100 rice seeds was recorded. Based on the number of nematode, seeds samples were indexed at different levels of infestation.

Table 3.1 Name of rice variety and number of samples collected from five different agricultural research farms

No.	Collected farms	No. of samples collected	Name of variety
1	Myaungmya	22	Aye Yar Min
2			Aye Yar Padathar
3			Aye Yar Thwe
4			Bay Gyar Gyee
5			Baykyar Yin
6			Hnangar
7			Kauk Kyi Taung Pyan
8			Ma Naw Phyu
9			Mi Kyaung Thwe
10			Myaung Mya May
11			Myo Pwar
12			One Zero
13			Pathein Baykyar
14			Paw Hsan Baykyar
15			Paw Hsan Yin
16			Shwe Bo Paw Hsan
17			Shwe War Yin
18			Sin Thu Kha
19			Sin Thwe Latt
20			Taung Pyan Baykyar
21			Thee Dat Yin
22			Yezin-1
23	Naungmon	35	20060/16
24			Asoya Sabar (early)
25			Asoya Sabar (late)
26			Bu Su-1
27			Bwee Ta Thee
28			CNA-456
29			IR82635-75-2-1
30			IR88614-B-2

Table 3.1 Continued.

No.	Collected farms	No. of samples collected	Name of variety
31			Khao Fine
32			Khao Kan
33			Khao Khan
34			Khao Lai
35			Khao Lane
36			Khao Lee Paw
37			Khao Ma Kaw
38			Khao Ma Phout
39			khao Mon
40			Khao Phi Phan
41			Khao Pu Maw
42			Khao Sann
43			Khao Shan
44			Khao Tan Pu
45			Khao Ywan
46			Khaung Houng
47			Khaung Laung
48			Kone Myint-2
49			Kone Myint-4
50			Lone Phyu
51			Lone Pu
52			Nga Si
53			Yn3274-1-B-B-2-1-3-3UUL
54			Yn3276-4-1-2UUL
55			Yn32773-B-1-4UUL
56			Yn3279-B-B-B-2-B
57			YN3297-1-8-B-B-2-B-5UUL
58	Yezin	22	A Kari Hmwe
59			Hmawbi-2
60			Kyaw Zay Ya
61			Manawthukha

Table 3.1 Continued.

No.	Collected farms	No. of samples collected	Name of variety
62			Paw Hsan Baykyar
63			Pyidaw Yin
64			Sar Ngan Khan Sin Thwe Latt
65			Shwe Pyi Hmwe
66			Shwe Pyi Htay
67			Shwe War Tun
68			Shwe Yin Aye
69			Shwethwe Yin
70			Sin A Kari 3
71			Sin Thu Kha
72			Sin Thwe Latt
73			Thee Dat Yin
74			Yadana Toe
75			Yae Myout Khan 3
76			Yar-8
77			Yay A Nae Lo 7
78			Yet-90
79			Yezin Pale Thwe 3
80	Lepatann	19	A Kari Hmwe
81			Bay Gyar Gyee
82			Kauk Kyi Taung Pyan
83			Pathein Baykyar
84			Paw Hsan Baykyar
85			Pyi Myanmar Sein
86			Pyidaw Yin
87			Shwe Asean
88			Shwe Bo Paw Hsan
89			Shwethwe Yin
90			Sin A Kari 3
91			Sin Thu Kha
92			Sin Thwe Latt

Table 3.1 Continued.

No.	Collected farms	No. of samples collected	Name of variety
93			Taung Pyan Baykyar
94			Thee Dat Yin
95			Yadana Toe
96			Yae Myout Khan 3
97			Yay A Nae Lo 4
98			Yay Myout Khan 1
99	Kyaukse	13	Aye Yar Min
100			Bay Gyar Gyee
101			Manawthukha
102			Pathein Baykyar
103			Paw Hsan Baykyar
104			Pyi Myanmar Sein
105			Shwe Bo Paw Hsan
106			Shwethwe Yin
107			Sin A Kari 3
108			Sin Thu Kha
109			Taung Pyan Baykyar
110			Yadana Toe
111			Yet-90

(i) Selected rice seeds sample with distorted glumes and deformed kernels

(ii) Crushing rice seeds with a motor and pestle

(iii) Placing crushed grains on muslin cloth spread over sieve

(iv) Adding water to touch bottom of the sieve and muslin cloth

(v) Incubating assemble for 24 hours

(vi) Counting nematodes under compound microscope

Plate 3.1 Procedure of nematode extraction from rice seeds samples

3.2 Evaluation of Different Inoculation Methods

3.2.1 Preparation of test plants

Manawthukha variety was used as tested variety based on the results of pretest experiment of evaluation of different inoculation methods of *A. besseyi* (Appendix 1). War (2009) also stated that the highest number of *A. besseyi* from rice seeds and typical white tip symptoms were observed in Manawthukha variety. Firstly, rice seeds were treated with hot water at 57°C for 10 minutes to ensure free of *A. besseyi* (Fortuner & Williams, 1975) and then soaked in water for 24 hours and incubated for 24 hours at room temperature for seed germination. The well-germinated rice seeds were sown in polyethylene bags containing sterilized soil then placed in a screen house for raising rice seedlings. Each polythene bag containing 3 kg of sterilized soil was fertilized with triple super phosphate (46% P_2O_5) at the rate of 62 kg ha^{-1} before transplanting. Each 7 days old rice seedling was transplanted into each pot. Urea (46% N) at the rate 125 kg ha^{-1} was equally applied with three split applications (after transplanting, at tillering stage and panicle initiation stage of rice plant). The plants were managed up to harvesting stage (135 days after sowing) with the normal cultivation practices.

3.2.2 Fungal media preparation

A. besseyi is a mycophagous nematode and it can be multiplied on fungal media. The isolate of *Alternaria solani* obtained from Department of Plant Pathology, Yezin Agricultural University, was cultured on Potato Dextrose Agar (PDA) medium and incubated for 7 days at room temperature. Seven days old *A. solani* were used as fungal media for the multiplication of *A. besseyi*.

3.2.3 Inoculum preparation

A. besseyi was extracted from infested rice seeds as described in section 3.1.2. The extracted nematodes were surface sterilized in a 1000 ppm streptomycin sulfate solution for five minutes and then washed with sterilized water for one minute for three times (Moore et al. 1985). Five pairs of surface sterilized nematodes were released onto *A. solani* fungal media. The plates were incubated at room temperature 25-28ºC in darkness (Rao 1985). Four weeks after incubation, *A. besseyi* was harvested by washing the surface of the mycelial mat with water in a container and the remaining agar medium was sliced and processed by the modified Baermann's

funnel technique for 48 hours to extract the nematodes (Hooper 1990). The extracted nematodes were counted in a counting dish under a compound microscope and adjusted aliquot nematode suspension for inoculation to rice plants (Plate 3.2).

3.2.4 Inoculation

Each 20 days old rice seedling was inoculated with the inoculum level of 200 nematodes plant^{-1}. Four different inoculation methods of introducing the nematode suspension; below the leaf sheath by a syringe (T1), into four holes of soil around the plant (T2), spraying the nematode suspension to the plant (T3) and dipping the rice seedlings into nematode suspension for two days (T4) were tested. In spraying inoculation method (T3), nematode suspension was sprayed with hand sprayer at the rate of 10 ml plant^{-1} (containing 200 nematodes). For dipping inoculation method (T4), each 18 days old rice seedling was dipped into the nematode suspension containing 200 nematodes for two days. Four inoculation methods were tested and non-inoculated plants were included as control plants (Plate 3.3).

3.2.5 Experimental design

This experiment was conducted at the Department of Plant Pathology, Yezin Agricultural University during monsoon season from June to November 2018. Completely randomized design (CRD) with three replications (Plate 3.4).

Treatments were as followed;

T1 = nematode suspension introduced below the leaf sheath by a syringe

T2 = nematode suspension introduced into four holes of soil around the plant

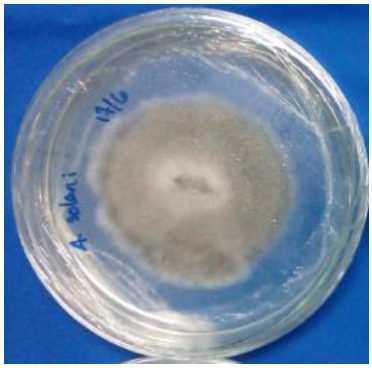
T3 = spraying nematode suspension to the plant

T4 = dipping rice seedlings into nematode suspension for two days

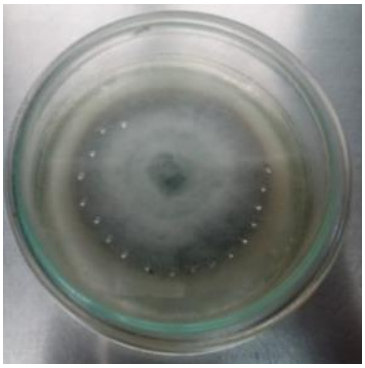
T5 = control (non-inoculated)

3.2.6 Data collection

At the time of harvesting, plant height (cm), number of tillers hill^{-1}, length of panicle (cm), number of filled grains panicle^{-1}, number of unfilled grains panicle^{-1}, number of discolored grains panicle^{-1}, total number of grains panicle^{-1} and 1000 grains weight (g) were measured and recorded. After harvesting, the number of nematodes recovered from 100 discolored seeds was determined as the final population. The extraction of nematode was done as mentioned in section 3.1.2.

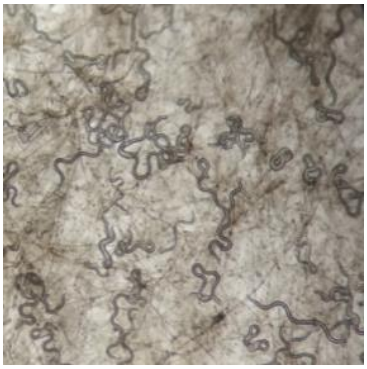


(i) Seven days old colony of *Alternaria solani* on PDA medium



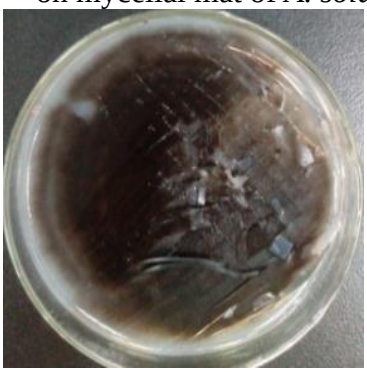
(ii) Adding 5 pairs of *A. besseyi* on mycelial mat

(iii) Incubating Petri dish in darkness



(iv) Multiplication of *A. besseyi* on mycelial mat of *A. solani*

(v) Harvesting nematodes by washing colony surface

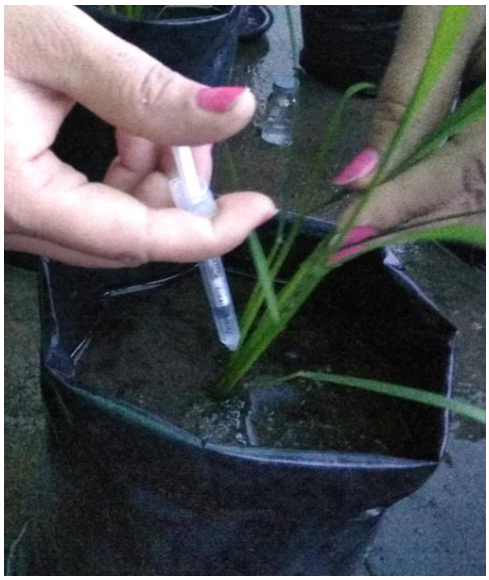


(vi) Slicing agar medium into small pieces

(vii) Extracting nematodes by using Baermann's funnel technique

(viii) Examining viability of *A. besseyi* under compound microscope (5x)

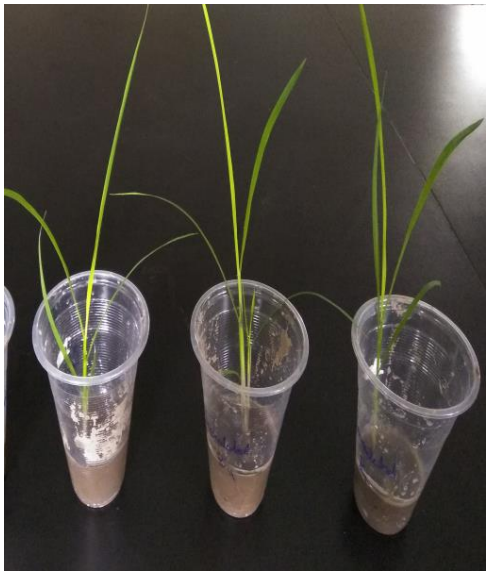
Plate 3.2 Procedure of inoculum preparation for *A. besseyi*



(T1) Introducing nematode suspension below leaf sheath by a syringe

(T2) Introducing nematode suspension into four holes of soil

(T3) Spraying nematode suspension to rice seedling



(T4) Dipping rice seedlings into nematode suspension

Plate 3.3 Different inoculation methods of *A. besseyi* on Manawthukha rice variety at the seedling stage

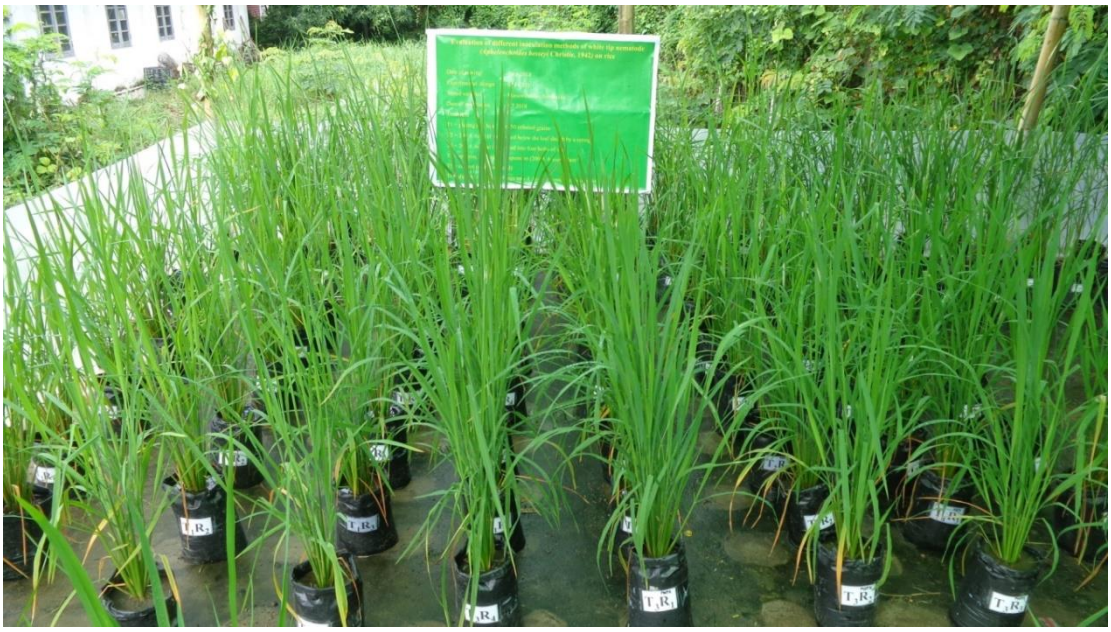


Plate 3.4 Experimental layout for evaluation of different inoculation methods for *A. besseyi* on Manawthukha

3.3 Assessment of Rice Varieties for Resistance to *A. besseyi*

3.3.1 Test varieties and seed treatment

Fifteen widely cultivated rice varieties namely (1) Manawthukha, (2) Sin Thu Kha, (3) Aye Yar Min, (4) Paw Hsan Yin, (5) Shwe War Tun, (6) Sin Thwe Latt, (7) Sinakari-3, (8) Hnangar, (9) Kyaw Zay Ya, (10) Yadana Toe, (11) Thee Dat Yin, (12) Shwe Bo Paw Hsan, (13) Shwethwe Yin, (14) Hmawbi-2 and (15) Shwe Yin Aye obtained from Rice Research Section, Department of Agricultural Research (DAR), were tested (Appendix 2). The seeds of all tested rice varieties were treated with hot water at 57°C for 10 minutes to ensure free of *A. besseyi* (Fortuner & Williams, 1975). About 50 rice seeds treated with hot water from each variety were soaked in water for 24 hours and then incubated for 24 hours at room temperature for seed germination. The test plants were prepared as described in section 3.2.1.

3.3.2 Inoculum preparation and inoculation

The inoculum was prepared as described in section 3.2.3. The aliquot solution was adjusted to obtain the required inoculum levels. According to host responses of fifteen rice varieties to *A. besseyi* by artificial inoculation with 200 and 500 nematodes plant^{-1}, the reactions of tested varieties were more severe in 500 nematodes plant^{-1} (Appendix 3). Therefore, each plant was inoculated with 500 *A. besseyi* by introducing nematode suspension into four holes of soil around the plant at 20 days after sowing. For each variety, non-inoculated plants were included as control plants.

3.3.3 Experimental design

The experiment was conducted at Department of Plant Pathology, Yezin Agricultural University during monsoon season, 2018. The experiment was laid out in a split-plot design with three replications (Plate 3.5). Inoculated and non-inoculated controls were main plot factor and fifteen widely cultivated rice varieties were subplot factor.

3.3.4 Data collection

After inoculation, appearance of white tip symptoms and small grains and erect panicle symptoms were recorded at two weeks intervals throughout the whole season. After harvesting, 100 discolored seeds plant^{-1} were selected and the nematodes were extracted as described in section 3.1.2 then the number of nematodes was determined as the Pf (final population).

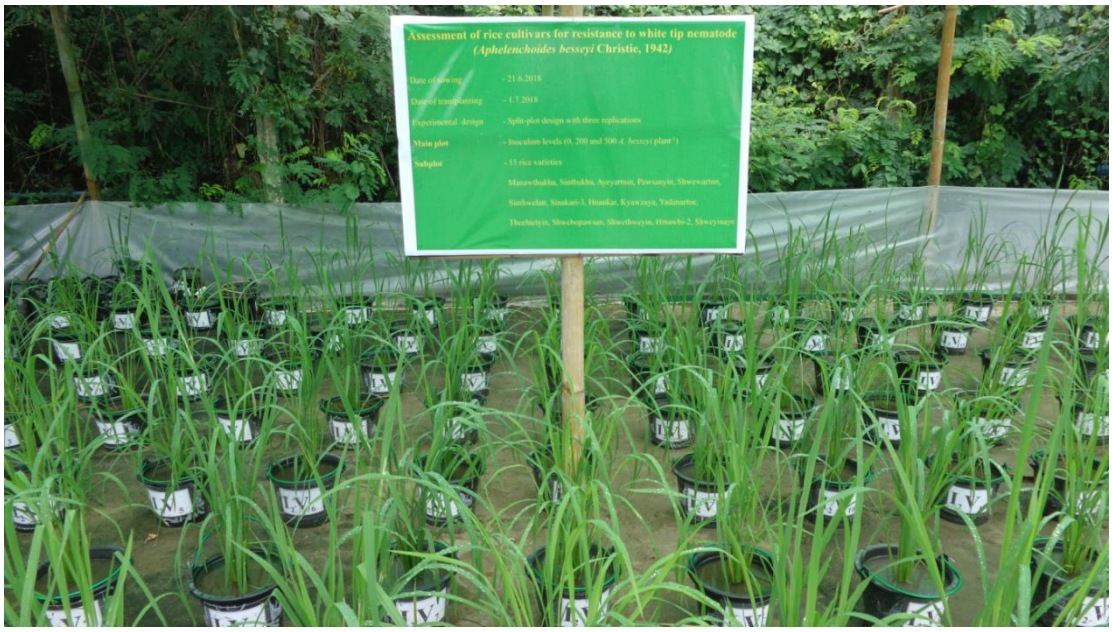


Plate 3.5 Experimental layout for assessment of fifteen rice varieties for resistance to *A. besseyi* in screen house

3.3.5 Method for resistance assessment

Resistance of the rice cultivars to *A. besseyi* were assessed by determining the final nematode population recovered from 100 discolored rice seeds and the appearance of disease symptoms, using a disease index scale (Popova et al., 1994). The following resistance rating was used:

0 = no white tip, no small grains and erect panicles (SGPs), no nematode

1 = white tip absent, SGPs absent; Pf = 1-50 *A. besseyi* 100 seeds $^{-1}$

3 = either white tip or SGPs present; Pf > 50 *A. besseyi* 100 seeds $^{-1}$

5 = white tip present, SGPs present; Pf > 50 *A. besseyi* 100 seeds $^{-1}$

The average index of infection (P) of each variety was estimated by using the formula:

$$P = \frac{\sum (B \times n)}{N}$$

where: Σ (B x n) = sum of the number of plants (n) and corresponding index of infection (B), N = total number of the infected plants.

Disease reactions of tested varieties were categorized according to the disease rating scale by Popova et al. (1994) based on average index of infection (Table 3.2).

3.4 Management of White Tip Disease in Rice

3.4.1 Seeds sample

The most severely *A. besseyi* infested rice variety (Lone Pu) according to the result of experiment 3.1 was used as test variety. The initial population of tested variety was recorded from 100 discolored seeds.

3.4.2 Preparation of treatments and tested plants

Total six treatments; infested rice seed directly sown in soil (T1), infested seeds soaked in brine solution (20% NaCl) (T2), infested seed treated with hot water at 55°C for 30 minutes (T3), application of carbofuran to soil (T4), infested seed treated with hot water at 55°C for 30 minutes and brine solution (20% NaCl) (T5) and seed treated with hot water at 55°C for 30 minutes, brine solution (20% NaCl) and application of carbofuran to soil (T6) were tested in this experiment. Twenty grams of nematode infested rice seeds were selected for hot water treatment at 55ºC for 30 minutes and then taken for treating with brine solution (20% NaCl). Furadun 3G (3% Carbofuran w/w) at the rate of 0.17 g pot^{-1} equivalent to 33 kg ha^{-1} was applied into the soil before sowing rice seeds.

Table 3.2 Disease rating index of rice varieties for resistance against the infection of *A. besseyi*

Average index of infection	Host reaction
0	immune
0.1-1.0	highly resistant
1.1-3.0	moderately resistant
3.1-4.0	moderately susceptible
4.1-5.0	highly susceptible

(Popova et al., 1994)

Each plastic pot (23 cm in diameter and 20 cm in height) was filled with 5 kg of sterilized soil and fertilized with triple super phosphate (46% P_2O_5) at the rate of 62 kg ha^{-1} as the basal application. Urea (46% N) at the rate 125 kg ha^{-1} was equally applied with three split applications (after transplanting, at tillering stage and at panicle initiation stage). The plants were managed up to harvesting stage (150 days after sowing) with the normal cultivation practices.

3.4.3 Experimental design

Pot experiment was conducted at Department of Plant Pathology, YAU, from July to November 2018. The experiment was laid out in a Completely Randomized Design with three replications (Plate 3.6).

3.4.4 Data collection

At the time of harvesting, the data of plant growth and yield components were collected same as described in the section 3.2.6. The nematodes were extracted as mentioned in section 3.1.2 and then the recovered nematodes from 100 discolored seeds were determined as Pf (final population).

3.5 Data Analysis

For all experiments, the collected data were analyzed by Statistix (version 8.0) computer software program. Treatment means were compared by using LSD test at 5% level.

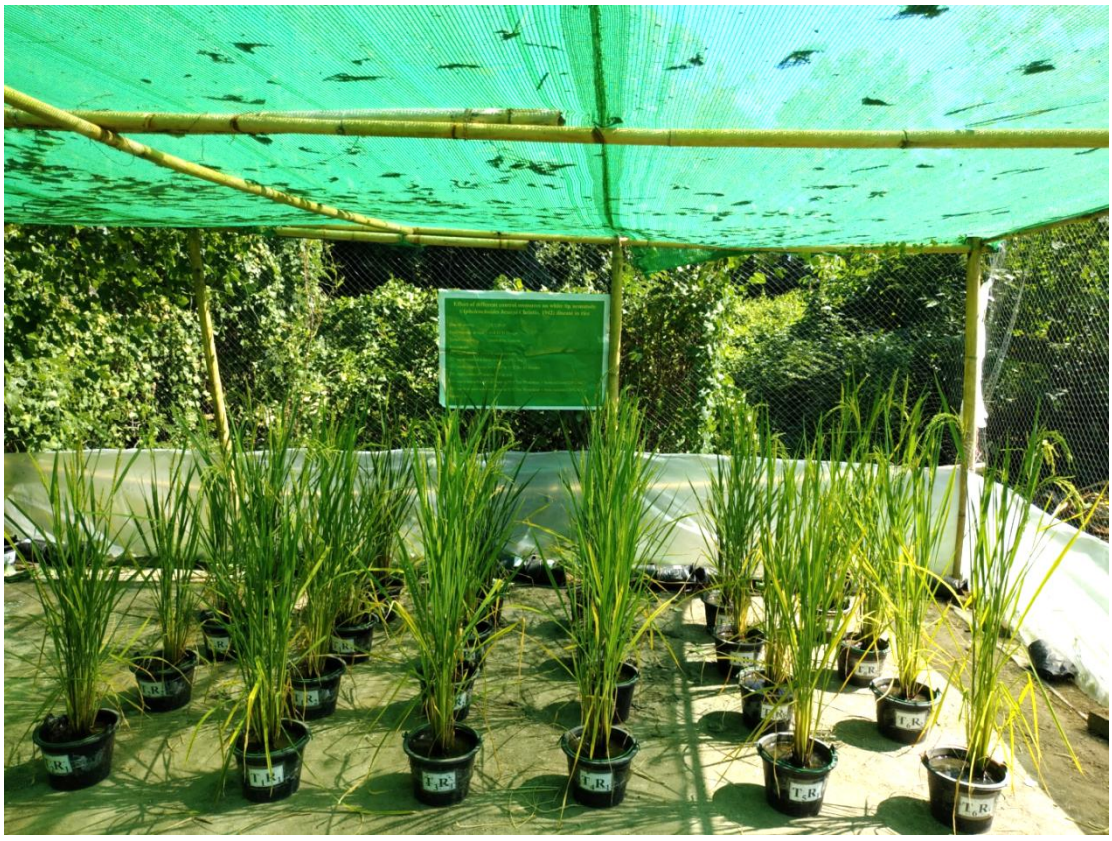


Plate 3.6 Exprimental layout for evaluation of different control measures of *A. besseyi* in Lone Pu variety

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Occurrence of *A. besseyi* in Different Rice Growing Regions

One hundred and eleven rice seed samples (79 different rice varieties) were collected from five different agricultural research farms. *A. besseyi* was found in all the collected farms (Table 4.1). Among 111 rice seed samples, 53 seeds samples (48%) were infested by *A. besseyi* (Plate 4.1 and 4.2) with the population ranging from 1-424 nematodes 100 seeds^{-1}.

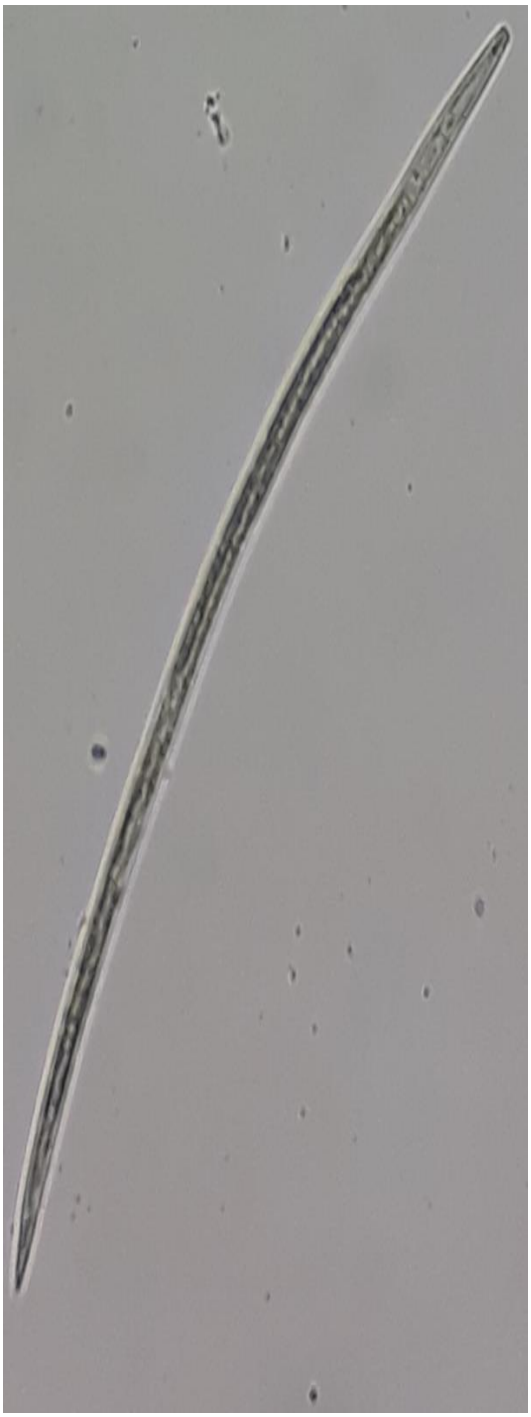
The highest infestation percent of *A. besseyi* was observed as 91% (20 out of 22 seeds samples) in Myaungmya farm, followed by 58% (11 out of 19 seeds samples) in Letpadan farm, 46% (16 out of 35 seeds samples) in Naungmon farm, 23% (5 out of 22 seeds samples) in Rice Research Section, Yezin and 8% (1 out of 13 seeds samples) in Kyaukse farm (Table 4.1 and Figure 4.1). However, the maximum nematode population was found in rice seeds sample collected from Naungmon research farm with the number of 424 nematodes 100 seeds^{-1} and the minimum population was observed in rice seeds sample collected from Kyaukse research farm with number of 2 nematodes 100 seeds^{-1}. The severity of *A. besseyi* infestation was categorized based on the nematode population 100 seeds^{-1} (Shahabi, Kheiri, Rakhshandehroo & Jamali, 2016). Among the infested rice seeds samples 83.0% had the low level of population (<50 nematodes), 7.6% had moderate population (50-100 nematodes) and 9.4% had very high population density (>100 nematodes).

The number of *A. besseyi* in 100 rice seeds was different among different tested varieties. Rahman and Miah (1989) also stated that there were varietal differences in the numbers of *A. besseyi* in rice seeds. Differences in nematode infestation level of rice seeds samples obtained from five different farms were seemed to be different agro-ecological zones with a wide range of climatic conditions in Myanmar. Jamali et al (2006) noted that differences in number of *A. besseyi* in rice seeds samples obtained from rice grown under north and other regions were probably due to climatically differences in the rice environments of Iran.

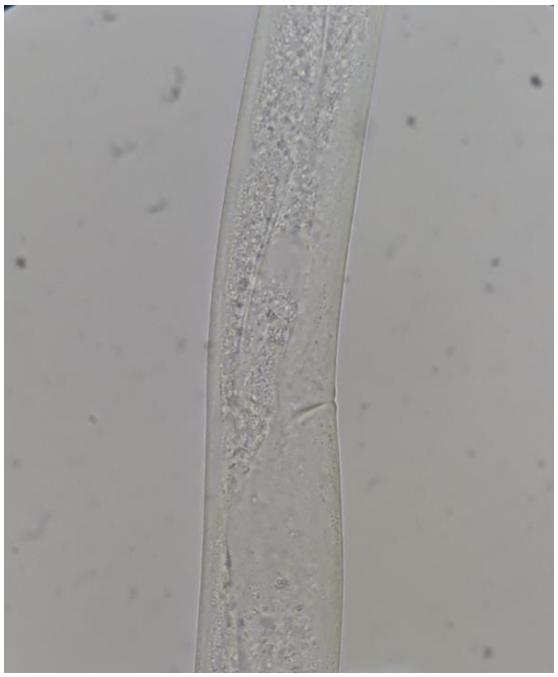
In the present study, 32.07% (17 out of 53 seeds samples) was infested with *A. besseyi* ranged from 30 to 424 nematodes 100 seeds^{-1} and these seeds samples were collected from Naungmon, Myaungmya and Letpadan farms. Fukano (1962) stated that 30 or more live nematodes 100 seeds^{-1} may be the possible economic threshold level in a susceptible cultivar. Therefore, these farms were potential to be important endemic areas for distribution of white tip disease if the rice cultivars were susceptible to *A. besseyi*.

Table 4.1 Infestation of *A. besseyi* in five different agricultural research farms

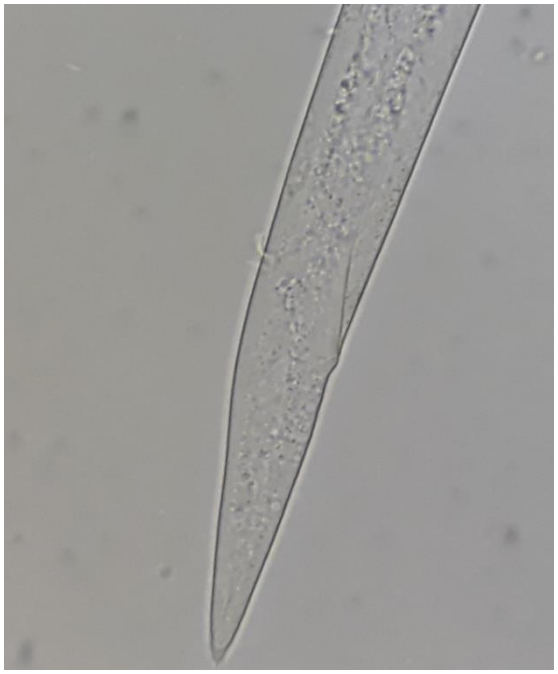
Name of farm	No. of samples collected	No. of samples infested	Nematode population 100 seeds^{-1} (min-max)	Percent of infestation
Myaungmya	22	20	2-181	91
Letpadan	19	11	2-31	58
Naungmon	35	16	1-424	46
Yezin	22	5	2-13	23
Kyaukse	13	1	2	8
Total	**111**	**53**		**48**



Whole body of female (10x)

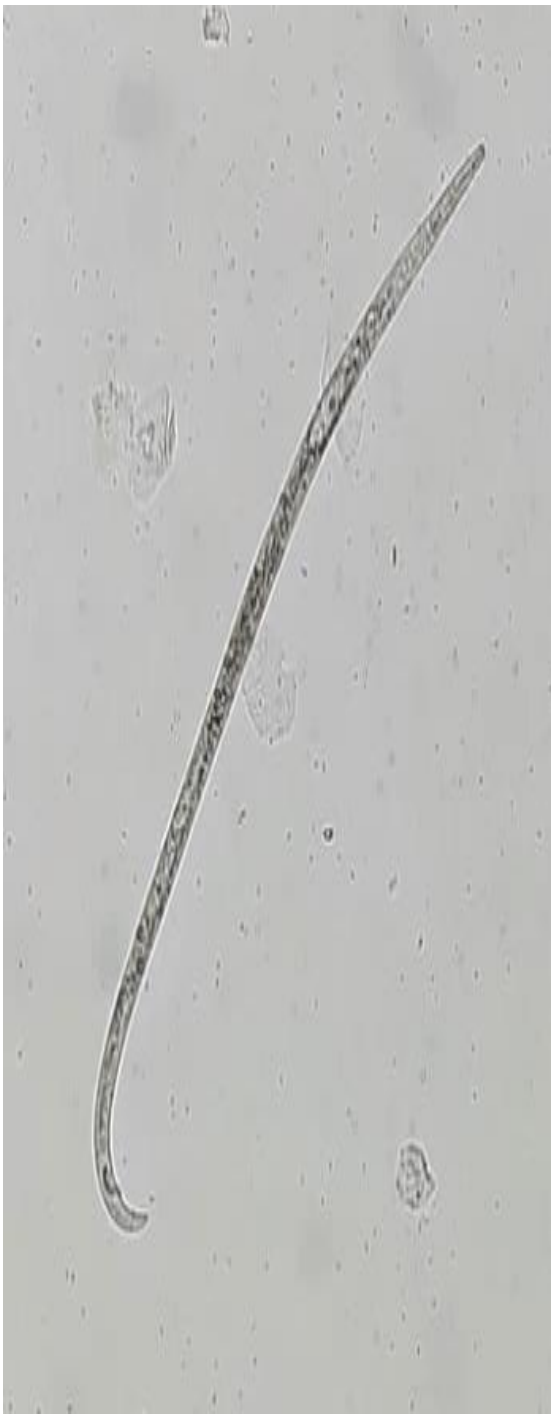


Slightly posterior vulva (100x)



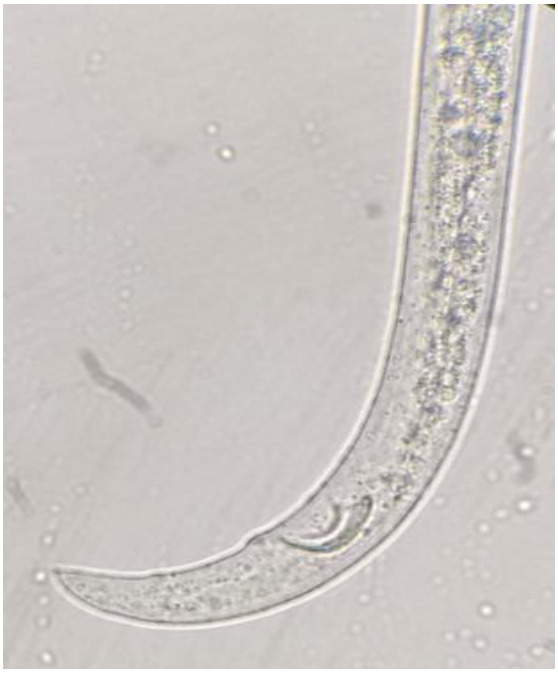
Tail region of female (100x)

Plate 4.1 Female *Aphelenchoides besseyi* from infested rice seeds samples



Whole body of male (10x)

Head region of male (100x)



Tail region of male (100x)

Plate 4.2 Male *Aphelenchoides besseyi* from infested rice seeds samples

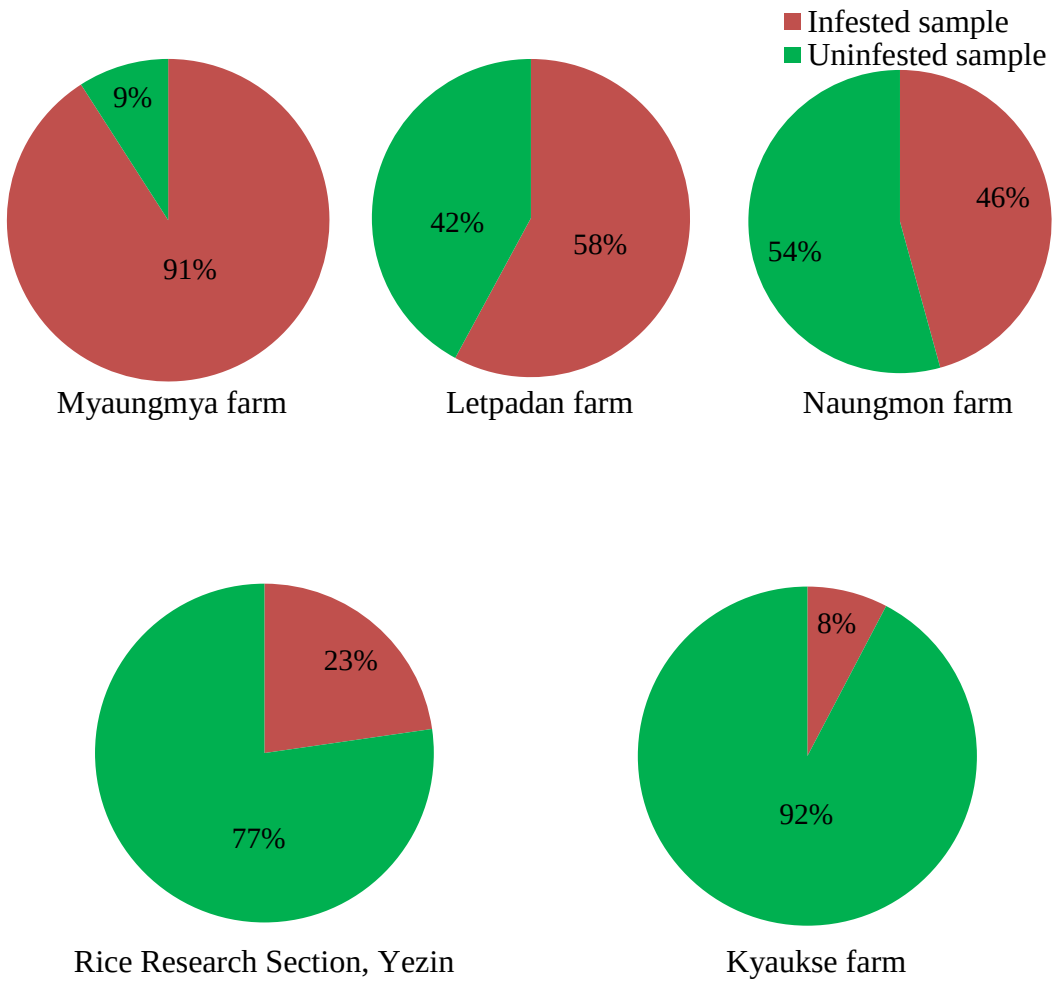


Figure 4.1 Infestation percent of *A. besseyi* in five different agricultural research farms

Among 79 different rice varieties, 42 varieties (53.16%) were infested with *A. besseyi* ranged from 1-424 nematodes 100 seeds^{-1} (Table 4.2). Fifteen varieties out of 42 infested varieties (19%) were infested with more than 30 nematodes 100 seeds^{-1}. The fifteen varieties namely; Bay Gyar Gyee, CNA-456, Kyauk Kyi Taung Pyan, Khao Kan, Khao Lane, Khao Shan, Lone Pu, Mi Kyaung Thwe, Myaung Mya May, One Zero, Pathein Baykyar, Paw Hsan Baykyar, Sin Thwe Latt, Shwe Bo Paw Hsan and Sin Thu Kha have high risk of *A. besseyi* damage. However, the number of nematode in fifteen rice varieties varied with different farms (Table 4.3). Among fifteen tested varieties, 4 varieties namely Bay Gyar Gyee, Paw Hsan Baykyar, Shwe Bo Paw Hsan and Sin Thu Kha were infested with *A. besseyi* ranged from 4-181 nematodes 100 seeds^{-1} in Myaungmya and Latpantann frams but these varieties were not infested in Kyaukse farm. This might be due to different regions with different climatic conditions of research farms and different farming practices used in these farms such as field sanitation, irrigating system etc. Five varieties of CNA-456, Khao Kan, Khao Lane, Khao Shan and Lone Pu which were grown in Naungmon farm were infested with *A. besseyi* ranged from 30-424 nematodes 100 seeds^{-1}. Jamali et al. (2006) also stated that *A. besseyi* is widely distributed because of its dissemination in seed, but its importance varies between regions, countries and localities.

4.2 Evaluation of Different Inoculation Methods

All Manawthukha test plants inoculated with four different inoculation methods exhibited typical symptoms of white tip disease (Plate 4.3). Therefore, all inoculation methods could establish the infection of rice plants by *A. besseyi*.

4.2.1 Final population of *A. besseyi*

Final nematode population of *A. besseyi* from rice seeds was varied depend on different inoculation methods (Figure 4.2). Significantly higher final nematode population was observed in T4 (103 nematodes 100 seeds^{-1}), T1 (59 nematodes 100 seeds^{-1}), T2 (47 nematodes 100 seeds^{-1}) than that of T3 (1 nematode 100 seeds^{-1}). There was no nematode observed in rice grains of non-inoculated plants (T5).

According to Fukano (1962), 30 viable nematodes 100 seeds^{-1} are essential as inoculum density for successful invasion. In this study, initial inoculum level of 200 nematodes plant^{-1} was used in each inoculation method. Therefore, initial inoculum level was supposed to be sufficient to infect the rice plants and showing typical symptom of white tip disease.

Table 4.2 Infestation of *A. besseyi* in collected rice varieties from five agricultural research farms

No.	Variety	Total no. of samples collected	Percent of infestation	No. of samples infested	No. of *A. besseyi* 100 seeds^{-1}
1	A Kari Hmwe	2	0	0	0
2	20060/16	1	100	1	7
3	Asoya Sabar (early)	1	0	0	0
4	Asoya Sabar (late)	1	0	0	0
5	Aye Yar Min	2	50	1	2
6	Aye Yar Padathar	1	0	0	0
7	Aye Yar Thwe	1	100	1	2
8	Bay Gyar Gyee	3	67	2	31-149
9	Baykyar Yin	1	100	1	5
10	Bu Su-1	1	0	0	0
11	Bwee Ta Thee	1	100	1	2
12	CNA-456	1	100	1	40
13	Hmawbi-2	1	0	0	0
14	Hnangar	1	100	1	3
15	IR82635-75-2-1	1	0	0	0
16	IR88614-B-2	1	100	1	1
17	Kauk Kyi Taung Pyan	2	100	2	17-69
18	Khao Fine	1	100	1	3
19	Khao Kan	1	100	1	32
20	Khao Khan	1	0	0	0
21	Khao Lai	1	100	1	3
22	Khao Lane	1	100	1	40
23	Khao Lee Paw	1	100	1	6
24	Khao Ma Kaw	1	0	0	0
25	Khao Ma Phout	1	100	1	15
26	khao Mon	1	0	0	0
27	Khao Phi Phan	1	0	0	0
28	Khao Pu Maw	1	100	1	10

Table 4.2 Continued.

No.	Variety	Total no. of samples collected	Percent of infestation	No. of samples infested	No. of *A. besseyi* 100 seeds^{-1}
29	Khao Sann	1	0	0	0
30	Khao Shan	1	100	1	30
31	Khao Tan Pu	1	0	0	0
32	Khao Ywan	1	0	0	0
33	Khaung Houng	1	0	0	0
34	Khaung Laung	1	100	1	6
35	Kone Myint-2	1	100	1	11
36	Kone Myint-4	1	0	0	0
37	Kyaw Zay Ya	1	0	0	0
38	Lone Phyu	1	100	1	5
39	Lone Pu	1	100	1	424
40	Ma Naw Phyu	1	100	1	11
41	Manawthukha	2	0	0	0
42	Mi Kyaung Thwe	1	100	1	30
43	Myaung Mya May	1	100	1	65
44	Myo Pwar	1	100	1	15
45	Nga Si	1	0	0	0
46	One Zero	1	100	1	51
47	Pathein Baykyar	3	100	3	139
48	Paw Hsan Baykyar	4	50	2	123
49	Paw Hsan Yin	1	0	0	0
50	Pyi Myanmar Sein	2	0	0	0
51	Pyidaw Yin	2	50	1	13
52	Sar Ngan Khan Sin Thwe Latt	1	100	1	13
53	Shwe Asean	1	0	0	0
54	Shwe Bo Paw Hsan	3	67	2	17-58
55	Shwe Pyi Hmwe	1	0	0	0
56	Shwe Pyi Htay	1	0	0	0
57	Shwe War Tun	1	0	0	0

Table 4.2 Continued.

No.	Variety	Total no. of samples collected	Percent of infestation	No. of samples infested	No. of *A. besseyi* 100 seeds^{-1}
58	Shwe War Yin	1	100	1	8
59	Shwe Yin Aye	1	0	0	0
60	Shwethwe Yin	3	0	0	0
61	Sin A Kari 3	3	33	1	3
62	Sin Thu Kha	4	50	2	4-181
63	Sin Thwe Latt	3	100	3	5-48
64	Taung Pyan Baykyar	3	33	1	23
65	Thee Dat Yin	3	67	2	11-28
66	Yadana Toe	3	67	2	2-9
67	Yae Myout Khan 1	1	0	0	0
68	Yae Myout Khan 3	2	50	1	2
69	Yar-8	1	0	0	0
70	Yay A Nae Lo 4	1	0	0	0
71	Yay A Nae Lo 7	1	100	1	2
72	Yet-90	2	0	0	0
73	Yezin-1	1	100	1	11
74	Yezin Pale Thwe 3	1	0	0	0
75	Yn3274-1-B-B-2-1-3-3UUL	1	0	0	0
76	Yn3276-4-1-2UUL	1	0	0	0
77	Yn32773-B-1-4UUL	1	0	0	0
78	Yn3279-B-B-B-2-B	1	0	0	0
79	YN3297-1-8-B-B-2-B-5UUL	1	0	0	0

Table 4.3 Number of *A. besseyi* in fifteen rice varieties from five agricultural research farms

No.	Variety	Name of collected farms				
		Myaungmya	Latpantann	Naungmon	Yezin	Kyaukse
1	Bay Gyar Gyee	150[x]	31	-[y]	-	0
2	CNA-456	-	-	40	-	-
3	Kauk Kyi Taung Pyan	69	17	-	-	-
4	Khao Kan	-	-	32	-	-
5	Khao Lane	-	-	40	-	-
6	Khao Shan	-	-	30	-	-
7	Lone Pu	-	-	424	-	-
8	Mi Kyaung Thwe	30	-	-	-	-
9	Myaung Mya May	65	-	-	-	-
10	One Zero	51	-	-	-	-
11	Pathein Baykyar	139	21	-	-	2
12	Paw Hsan Baykyar	123	25	-	0	0
13	Sin Thwe Latt	48	31	-	5	-
14	Shwe Bo Paw Hsan	58	17	-	-	0
15	Sin Thu Kha	181	4	-	0	0

[x] means of three replications.

[y] means no sample.

Thirty or more live nematodes 100 seeds^{-1} may be the possible economic threshold level in a susceptible cultivar (Fukano 1962).

Whitening or chlorosis of leaf tip in tillering stage

Not or partially emerged panicle in heading stage

Deformed or discolored spikelets in maturity stage

Plate 4.3 Symptoms of white tip disease on Manawthukha variety with different inoculation methods of *A. besseyi*

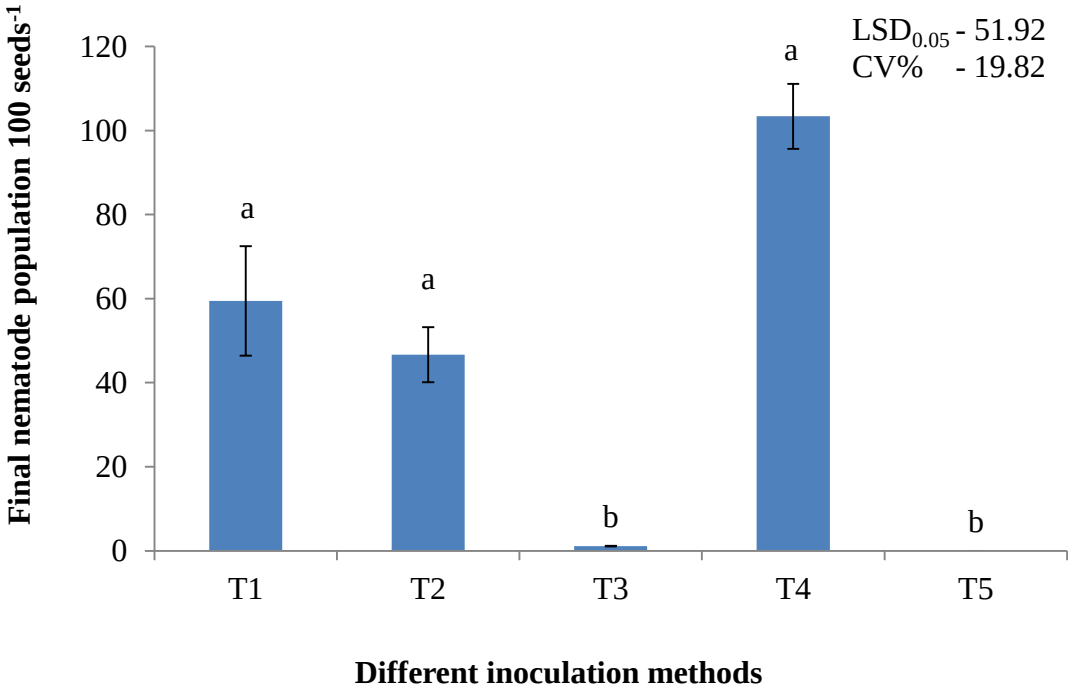


Figure 4.2 Final population of *A. besseyi* in different inoculation methods

T1 = introducing nematode suspension below leaf sheath by a syringe

T2 = introducing nematode suspension into four holes of soil

T3 = spraying nematode suspension

T4 = dipping the rice seedlings into nematode suspension for two days

T5 = control (non-inoculated)

The nematode population of rice seeds from three inoculation methods of T1, T2 and T4 were seemed to be effective to inoculate *A. besseyi* with showing typical symptoms of white tip disease. The results were in accordance with the findings of Swascita (1993) who inoculated *A. besseyi* to pots just near the plant surface of 7 days old seedlings by slowly releasing the nematodes with the help of a micropipette and established successful infection and exhibited typical symptoms of white tip disease. Latif, Rahman and Rahman (1997) also stated that three inoculation methods of *A. besseyi*; releasing nematodes in water containing sprouted seeds, introducing nematodes in water at the base of seedlings and placing nematodes inside leaf sheaths with inoculum level of 100 nematodes plant^{-1} were equally effective regarding nematode multiplication in plants at different growth stages and in grains.

Although the higher number of nematode was found in the method of dipping rice seedlings into the nematode solution (T4), this method takes time to infect the rice seedlings and it can lead to excessive development of fungi, bacteria and protozoa etc. in the nematode suspension during the process. In case of introducing nematode suspension into leaf sheath by a syringe (T1), higher number of nematode was observed but it is laborious for inoculation of large plant population. Latif et al. (1997) mentioned that sheath inoculation method was found laborious and time consuming while nematode released at the base of seedling and released of nematodes in water containing sprouted seeds were found easy and convenient.

The inoculation method of *A. besseyi* should be simplified and convenient for the inoculation of large plant population. In the present study, introducing nematode suspension into four holes of soil around the plant (T2) might be very simple and close to natural infection of *A. besseyi*. Todd (1954) also observed that sheath culm inoculation with nematode suspension applied with a hypodermic syringe and flask culture contents incorporated into the soil were both quite effective as means of inoculation. While the former technique proved to be more effective, the latter method more closely approached natural infestation.

4.2.2 Plant growth and yield components

The effect of infection with different inoculation methods of *A. besseyi* on plant growth and yield parameters were shown in Table 4.4 and 4.5. The plant height was not significantly different among the inoculation methods. Numerically, the shortest plant height of 76.3 cm was recorded in T3.

Table 4.4 Effect of infection of *A. besseyi* with different inoculation methods on growth and yield components

Treatment	Plant height (cm)[x]	Tiller (no.)[x]	Panicle length (cm)[x]	1000 grains weight (g)[x]	Grain yield plant^{-1} (g)[x]	Grain yield reduction over control (%)
T1	81.5	17.3	19.4	15.5	5.83 b[y]	50.6
T2	78.3	16.0	18.9	14.1	7.18 b	39.2
T3	76.3	17.3	18.7	15.2	10.30 a	12.7
T4	79.2	15.0	16.6	12.4	5.34 b	54.7
T5	77.7	18.3	18.9	13.2	11.80 a	-
$LSD_{0.05}$	13.41	7.23	3.18	2.21	2.81	
Pr>F	0.9295	0.8564	0.3792	0.0516	0.0016	
CV%	9.38	23.66	9.43	8.62	19.12	

[x] Means of three replications

[y] Means followed by the same letter in the same column were not significantly different at 5% level

T1 = nematode suspension introduced below the leaf sheath by a syringe

T2 = nematode suspension introduced into four holes of soil around the plant

T3 = spraying nematode suspension to the plant

T4 = dipping rice seedlings into nematode suspension for two days

T5 = control (non-inoculated)

Table 4.5 Effect of infection of *A. besseyi* with different inoculation methods on yield components

Treatment	Filled grains panicle^{-1} (no.)^{x}	Unfilled grains panicle^{-1} (no.)^{x}	Discolored grains panicle^{-1} (no.)^{x}	Total grains panicle^{-1} (no.)^{x}
T1	92 (70)^{y} b^{z}	38 (30)	114 (87) a	131 ab
T2	73 (67) bc	35 (32)	86 (79) ab	112 b
T3	89 (73) b	33 (26)	82 (67) b	122 ab
T4	61 (72) c	24 (28)	67 (78) b	84 c
T5	117 (84) a	23 (16)	55 (39) b	141 a
LSD $_{0.05}$	21.02	15.71	31.72	21.84
Pr > F	0.0015	0.1866	0.0181	0.0017
CV%	13.36	27.98	21.58	10.17

^{x} Means of three replications

^{y} Mean numbers in parentheses are percent data.

^{z} Means followed by the same letter in the same column were not significantly different at 5% level

T1 = nematode suspension introduced below the leaf sheath by a syringe

T2 = nematode suspension introduced into four holes of soil around the plant

T3 = spraying nematode suspension to the plant

T4 = dipping rice seedlings into nematode suspension for two days

T5 = control (non-inoculated)

In tiller number, there was no significantly different among treatments. Numerically, lower number of tillers was produced in different inoculation methods ranged from 15-17.3 in compared with those of 18.3 in non-inoculated plants. The panicle length of inoculated plants ranged from 16.6-19.4 cm was not statistically different from non-inoculated plants. The shortest panicle length of 16.6 cm was found in T4. There was no significantly difference in thousand grains weight of rice plants among different inoculation methods. The lowest thousand grains weight, 12.4 g was found in T4. Therefore, the infection of white tip disease caused by different inoculation methods was not severely affected plant height, tiller number, panicle length and 1000 grains weight of Manawthukha variety.

Significant lower numbers ranged from 84-131 spikelets panicle^{-1} were produced in inoculated plants than that of 141 in non-inoculated plants. This means that the infection of *A. besseyi* with different inoculation methods can reduce number of spikelets panicle^{-1}. Swastica (1993) also found that plants infected with *A. besseyi* yielded less number of grains as compared to control and increasing the level of inoculum could progressively reduce the number of grains panicle^{-1}.

The number of filled grains was significantly reduced at 1% level of significance in all inoculation methods ranged from 61-92 compared with those of 117 in the non-inoculated plants. The lowest number of filled grains 61 was observed in T4 and it was not statistically different from T2 with the number of 73. The reason of fewer numbers of filled grains panicle^{-1} of rice plants among different inoculation methods might be due to *A. besseyi* entered into the developing spikelets at the time of panicle emergence and then, fed in spikelets resulted in the reduction of filled grains. El-Shafeey, EL-Shafey, Abdel-Hadi and Sehly (2014) reported that *A. besseyi* infected panicles showed shorter length and reduced in weight.

The plants inoculated with different inoculation methods produced comparatively lower grain yield ranged from 5.34 g to 10.3 g than non-inoculated plants, 11.8 g. The significantly lower grain yield was observed in T4 (5.34 g), T1 (5.83 g) and T2 (7.18 g). Final nematode population of rice grains can reduce the grain yield of rice plants ranged from 12.7-54.7% over non-inoculated plants. It was observed that there was positive relationship ($r = 0.93$) between final population from rice grains and reduction in grain yield (Figure 4.3). Wang, Yang and Ji (2006) proved that the yield and quality of rice were strongly influenced by white tip disease.

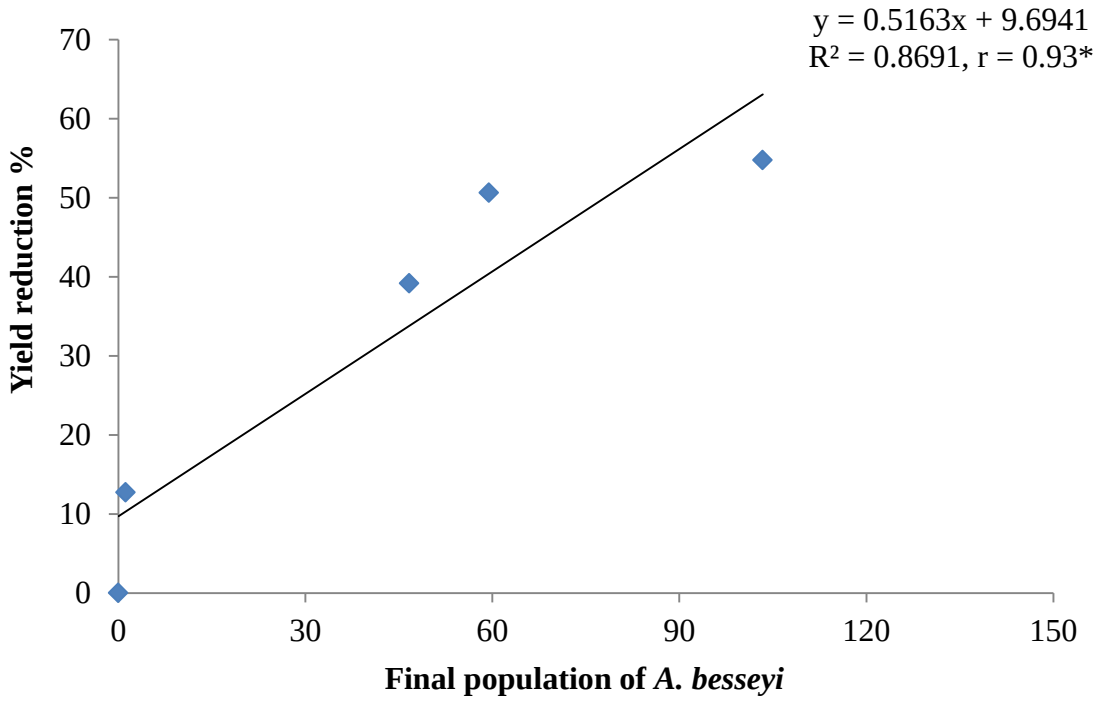


Figure 4.3 Relationship between final nematode population of different inoculation methods and yield reduction percent

In the number of unfilled grains, there was no statistically different among the inoculation methods. However, numerically higher number of 38 and 35 unfilled grains panicle^{-1} were found in T1 and T2, respectively. It was noticed that the number of unfilled grains was increased due to the infection of *A. besseyi* in the rice grains resulting chaffy spikelets. The result was agreed with the finding of Rao, Bhavani, Rao and Reddy (2000) who stated that spikelets sterility or grain discoloration of rice was due to *A. besseyi* infestation.

Mean number of discolored grains was significantly higher at 5% level of significance in different inoculation methods ranged from 67-114 compared with that of 55 in non-inoculated plants. The highest number of discolored grains 114 was observed in T1. However, it was not statistically different from T2 with number of 86 discolored grains panicle^{-1}.

Among tested inoculation methods, three methods of T1, T2 and T4 were seemed to be produced not only fewer numbers of filled grains and grain yield but also greater number of unfilled grains and discolored grains in compered with uninoculated plants. Therefore, these three inoculation methods established successful infection of *A. besseyi* and reduced the grian yield of Manawthukha rice variety.

4.3 Assessment of Rice Varieties for Resistance to *A. besseyi*

4.3.1 Reactions of fifteen rice varieties to *A. besseyi*

Fifteen widely cultivated rice varieties were evaluated for their resistance to *A. besseyi* with inoculum level of 500 nematodes plant^{-1}. Final nematode population of *A. besseyi* from rice seeds after artificial inoculation was significantly different among tested varieties (Table 4.6). The highest final nematode population 100 seeds^{-1} was observed in Sin Thu Kha (829 nematodes) and followed by Kyaw Zay Ya (512 nematodes), Manawthukha (376 nematodes), Sin Thwe Latt (374 nematodes), Shwethwe Yin (343 nematodes), Shwe War Tun (299 nematodes) and Shwe Bo Paw Hsan (296 nematodes). The lowest final nematode population was found in Yadana Toe (53 nematodes) and followed by Thee Dat Yin (57 nematodes), Paw Hsan Yin (88 nematodes), Hmawbi-2 (80 nematodes), Shwe Yin Aye (100 nematodes), Aye Yar Min (113 nematodes), Hnangar (116 nematodes) and Sin A Kari-3 (122 nematodes), respectively.

Table 4.6 Host responses of fifteen rice varieties to *A. besseyi* by artificial inoculation with 500 nematodes plant^{-1}

No.	Rice varieties	Nematode population 100 seeds^{-1x}	Symptoms reaction	Average index of infection[x]	Host Response [y]
1	Paw Hsan Yin	88 (1.86) de [z]	-	2.3	MR
2	Sin A Kari-3	122 (2.25) cd	-	3.0	MR
3	Yadana Toe	53 (1.67) e	-	3.0	MR
4	Aye Yar Min	113 (2.02) de	SPGs	3.7	MS
5	Hnangar	115 (2.02) de	WT & SPGs	3.7	MS
6	Thee Dat Yin	56 (1.72) e	SPGs	3.7	MS
7	Hmawbi-2	80 (1.82) e	SPGs	3.7	MS
8	Manawthukha	367 (2.78) a-c	WT & SPGs	5.0	HS
9	Sin Thu Kha	829 (2.90) a	WT & SPGs	4.3	HS
10	Shwe War Tun	298 (2.44) bc	WT & SPGs	4.3	HS
11	Sin Thwe Latt	374 (2.49) bc	WT & SPGs	4.3	HS
12	Kyaw Zay Ya	512 (2.70) ab	WT & SPGs	4.3	HS
13	Shwe Bo Paw Hsan	295 (2.47) bc	WT & SPGs	5.0	HS
14	Shwethwe Yin	343 (2.53) a-c	WT & SPGs	4.3	HS
15	Shwe Yin Aye	100 (1.99) de	WT & SPGs	4.3	HS
	$LSD_{0.05}$	0.40			
	Pr > F	<0.0001			
	CV %	10.80			

[x] Means of three replications.

[y] Based on Popova et al. (1994) rating scale, the average index of infection is 0 = Immune (I), 0.1-1.0 = Highly resistant (HR), 1.1-3.0 = Moderately resistant (MR), 3.1-4.0 = Moderately susceptible (MS), 4.1-5.0 = Highly susceptible (HS)

[z] Means followed by the same letter in the same column were not significantly different at 5% level

WT = white tip symptoms, SPGs = small grains and erect panicles symptoms, "-" = no symptom

Means numbers in parentheses are log transformation.

According to Popova et al. (1994) rating scale, fifteen tested varieties were categorized on the basis of symptom expression and final nematode population 100 seeds^{-1}, three varieties Paw Hsan Yin, Sin A Kari-3 and Yadana Toe were ranked as moderately resistant reaction because of lack of typical white tip symptoms and small grain and erect panicle symptoms. Four varieties; Aye Yar Min, Hnangar, Thee Dat Yin, and Hmawbi-2 were moderately susceptible and Manawthukha, Sin Thu Kha, Shwe War Tun, Sin Thwe Latt, Kyaw Zay Ya, Shwe Bo Paw Hsan, Shwethwe Yin and Shwe Yin Aye were highly susceptible to white tip disease because of presence of typical white tip symptoms and small grain and erect panicle symptoms.

According to the results, it was found that the reaction to white tip disease was varied among different tested varieties. These findings were similar to El-Shafey, Anis & Elmoghazy (2016) who stated that responses of tested varieties were significantly differed according to the varieties and nematode infestation among varieties. Nishizawa (1953) already mentioned that differences in resistance to *A. besseyi* could be varied according to genetically differences among rice varieties.

In the present study, rice varieties which showed moderately susceptible and highly susceptible reactions exhibited the typical white tip symptoms of leaves after one month inoculation and small grain and erect panicle symptoms were observed at the later reproductive stage of rice plants (Plate 4.4). The diseased symptoms expression was varied with different varieties. These results were in accordance with those of El-shafey (2007) who reported that some varieties were white tip symptomless and their grains harvested more buildup of nematodes. Jamali and Mousanejad (2011) stated that although reproduction is important in determining resistance but it is not the major criterion to use in determining nematode pathogenicity. Plant response in terms of symptom development should also be evaluated.

4.4 Management of White Tip Disease in Rice

4.4.1 Effect of different control measures on plant growth, yield components and final nematode population of *A. besseyi*

Final nematode population of *A. besseyi* of rice plants treated with different control measures was highly significant different at 1% level of significance (Table 4.7).

White tip symptoms on leaf

Small grains and erect panicle symptoms

Plate 4.4 Symptoms of white tip disease exhibited on different susceptible varieties

Table 4.7 Effect of different control measures on plant growth, yield characters and final nematode population of *A. besseyi*

Treatment	Plant height (cm)[x]	Tiller (no.)[x]	Panicle length (cm)[x]	1000 grains weight (g)[x]	No. of *A. besseyi* 100 seeds^{-1}
T1	113.9 b[z]	5.7 b	15.3 c	20.2 b	164.0 (2.22)[y]a
T2	117.3 ab	10.0 a	17.9 ab	23.2 ab	156.7 (2.01) a
T3	130.0 ab	7.3 b	19.4 a	25.6 a	0.3 (0.12) b
T4	118.5 ab	11.0 a	17.4 b	23.7 ab	0.0 (0.00) b
T5	134.0 a	7.0 b	19.5 a	26.1 a	0.8 (0.22) b
T6	120.8 ab	10.3 a	18.1 ab	24.5 ab	0.2 (0.06) b
$LSD_{0.05}$	17.21	2.12	1.93	4.31	0.47
Pr>F	0.1548	0.0010	0.0068	0.1166	<0.0001
CV%	7.73	13.61	5.92	9.92	34.13

[x] Means of three replications.

[y] Means numbers in parentheses are log transformation.

[z] Means followed by the same letter in the same column were not significantly different at 5% level.

T1 = seeds directly sown in soil (control)

T2 = seeds soaked in brine solution (20% NaCl)

T3 = seeds treated with hot water at 55°C for 30 minutes

T4 = application of carbofuran (Furandun 3G with 3% carbofuran w/w) in soil

T5 = seeds treated with hot water at 55°C for 30 minutes + brine solution (20% NaCl)

T6 = seeds treated with hot water at 55°C for 30 minutes + brine solution (20% NaCl) + carbofuran

There was no nematode observed in treatment of application of carbofuran into the soil (T4) and lower final nematode population was found in the combined treatment of seeds treated with hot water and then soaked in 20% NaCl solution and application carbofuran into the soil (T6), hot water at 55°C for 30 minutes (T3) and combined treatment of hot water and 20% NaCl solution (T5) with the number of 0, 0.2, 0.3 and 0.8 nematode 100 seeds^{-1}, respectively. Seeds soaked in 20% NaCl solution (T2) gave the higher number of 157 nematodes 100 seeds^{-1} and it was not significantly different from untreated check (T1) with the number of 164 nematodes 100 seeds^{-1}.

All control measures T3, T4, T5 and T6 were found to be effective in controlling white tip disease with reduction of final nematode population. Among different control measures, application of carbofuran inhibited the growth and activity of the nematodes and that no *A. besseyi* was observed in rice seeds of carbofuran treated plants. Cho, Han and Yang (1987) also reported that effective chemical control for *A. besseyi* was seed disinfection before seeding and carbofuran 3G treatments on the day before transplanting also effective.

In present study, hot water treatment alone and combined treatments with hot water were found to be effective in controlling *A. besseyi*. These results were in accordance with the findings of Atkins and Todd (1959) who also stated that hot water treatment of seeds can be used to destroy white tip disease as *A. besseyi* was not recovered from rice seeds which treated with hot water at 55°C for 15 minutes but it was observed in seeds subjected to hot water treatment at 50°C. In present study, there was seemed to have no effect on seed germination of Lone Pu rice variety after treatment with hot water at 55°C for 30 minutes. Yoshii and Yamamoto (1951) reported that injury to seeds occurred only at 60°C applied for more than 20 minutes.

The effect of different control measures on plant height was not significantly different among treatments. In tiller numbers, there was significantly different among control treatments. The higher number of tillers was observed in all control measure treated plants ranged from 7-11 than those of 5.7 in untreated plants (T1). The highest tiller number was observed in T4 and it was not statistically different from T6 and T2 with tiller number of 11, 10.3 and 10, respectively.

Plants treated different control measures showed significant longer panicle length ranged from 17.4-19.5 cm than those of 15.3 cm in untreated check. The longest panicle length was found in T5 but it was not statistically different from T3, T6 and T4 with the length of 19.5, 19.4, 18.1 and 17.9 cm, respectively. There was not significantly different in 1000 grains weight among the different control measure treatments. Numerically, the maximum thousand grains weight (26.1 g) was observed in T5 whereas minimum thousand grains weight (20.2 g) was produced by untreated plants (T1).

Effect of different disease control measures and its impact on yield and yield components were described in Table 4.8. According to the results, grain yield was significantly different between the plants treated with different control measures and untreated plants at 1% level of significance (Figure 4.4). The plants treated with different control measures gave comparatively higher grain yield ranged from 8.47-13.47 g. The highest grain yield was produced in T6, followed by T4, T5 and T3 with 13.47, 13.10, 11.51 and 11.31 g, respectively.

The percent increase in yield over untreated plants ranged from 38-61% in all treatments. The maximum yield increased percent of 61% was produced by T6. These findings was similar to that of Islam et al. (2015) who stated that hot water treatment of seeds was found to exert good effect in respect of plant growth and yield characters.

Significantly higher number of filled grains panicle^{-1} was observed ranged from 50-63 in different control treatments in compared with 31 in untreated check. The higher filled grains number was found in T5, T3 and T6 with the number of 63, 61 and 53 grains panicle^{-1}, respectively. The effect of different control measures was not significantly different in the number of unfilled grains and discolored grains of all control measure treatments. However, number of unfilled grains and discolored grains were numerically lower in all control measures than those of untreated plants.

According to the results, different control measures was found to be have good effect in respect of plant growth and yield characters with lower number of final nematode population except the treatment of seeds soaked in 20% NaCl solution (T2). These results were similar to findings of Islam et al. (2015) who described that farmers saved seeds soaked in brine solution and hot water treatment had comparatively better response in plant growth and yield components.

Table 4.8 Effect of different control measures on grain yield and yield components of Lone Pu variety

Treatment	Filled grains panicle^{-1} (no.) [x]	Unfilled grains panicle^{-1} (no.) [x]	Discolored grains panicle^{-1} (no.) [x]	Grain yield per plant (g)	Yield increased percent over control
T1	31 (48)[y] c[z]	33 (52)	59 (92)	5.35 c	
T2	51 (63) b	30 (37)	48 (59)	8.47 b	38
T3	61 (71) a	25 (29)	50 (58)	11.31 ab	53
T4	50 (65) b	26 (34)	57 (75)	13.10 a	59
T5	63 (70) a	28 (30)	58 (64)	11.51 a	53
T6	54 (67) ab	27 (33)	47 (58)	13.47 a	61
LSD $_{0.05}$	10.58	20.50	17.60	2.90	
Pr > F	0.0020	0.9403	0.4898	0.0007	
CV%	11.26	39.78	18.09	15.12	

[x] Means of three replications

[y] Means numbers in parentheses are percent data.

[z] Means followed by the same letter in the same column were not significantly different at 5% level

T1 = seeds directly sown in soil (control)

T2 = seeds soaked in brine solution (20% NaCl)

T3 = seeds treated with hot water at 55°C for 30 minutes

T4 = application of carbofuran (Furandun 3G with 3% carbofuran w/w) in soil

T5 = seeds treated with hot water at 55°C for 30 minutes + brine solution (20% NaCl)

T6 = seeds treated with hot water at 55°C for 30 minutes + brine solution (20% NaCl) + carbofuran

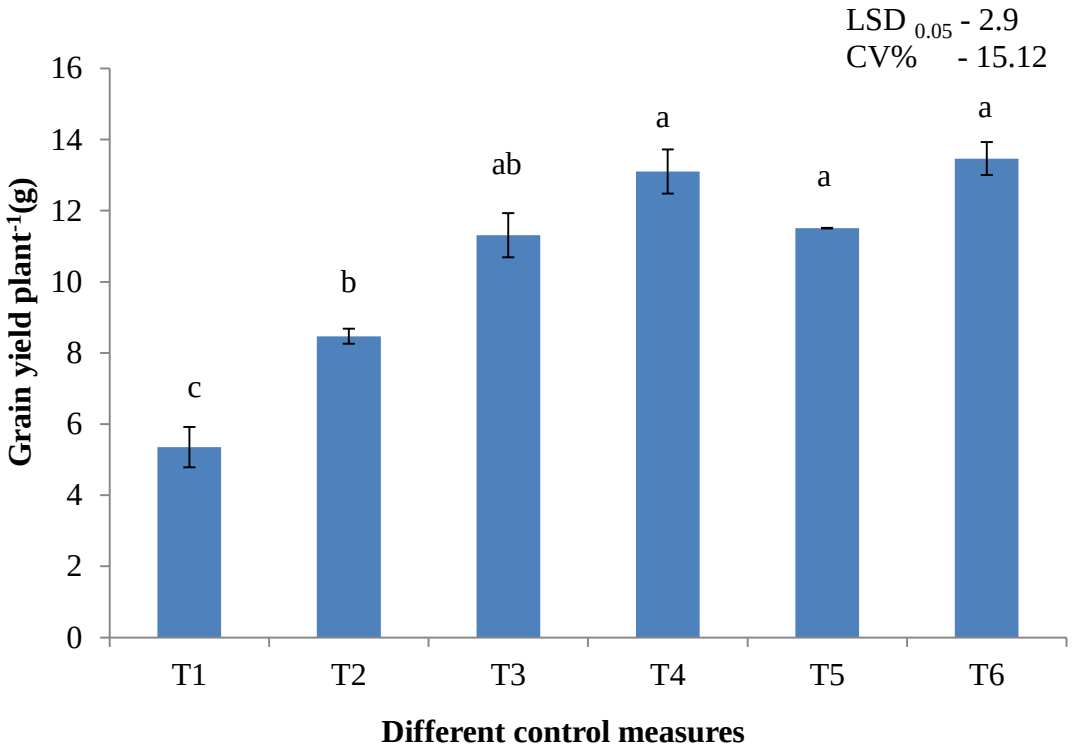


Figure 4.4 Effect of different control measures on grain yield of Lone Pu variety associated with *A. besseyi*

T1 = seeds directly sown in soil (control)

T2 = seeds soaked in brine solution (20% NaCl)

T3 = seeds treated with hot water at 55°C for 30 minutes

T4 = application of carbofuran (Furandun 3G with 3% carbofuran w/w) in soil

T5 = seeds treated with hot water at 55°C for 30 minutes + brine solution (20% NaCl)

T6 = seeds treated with hot water at 55°C for 30 minutes + brine solution (20% NaCl) + carbofuran

CHAPTER V
CONCLUSION

The examination of rice seeds samples collected from five different agricultural research farms showed the presence of *A. besseyi* in seeds samples of all collected farms. Among the collected farms, rice seeds samples collected from three agricultural research farms located in Naungmon, Myaungmya and Letpadan were highly infested and it could be potential for damage caused by *A. besseyi* if the infested seeds were sown. Among 111 rice seeds samples collected from differern farms, 48% of seeds samples were infested with *A. besseyi*. Fifteen rice varieties out of 79 varieties were infested over 30 number of *A. besseyi* 100 seeds^{-1} and these varieties could be potential risk to reduce grain yield.

The results of evaluation of different inoculation methods of *A. besseyi* indicated that all inoculation methods were able to cause infection and exhibited typical white tip symptoms after inoculation of *A. besseyi*. It was found that the final nematode population in three methods of introducing nematode suspension; below the leaf sheath by a syringe (T1), into four holes of soil around the plant (T2) and dipping the rice seedlings into nematode suspension (T4) showed higher nematode number, it was suggested that these three methods can cause infection and build up the population of *A. besseyi*. However, among different inoculation methods, introducing the nematode solution into four holes of soil around the plants (T3) can easily perform and very closely related with natural infestation of *A. besseyi*. Therefore, this inoculation method can be used as effective inoculation method for varietal screening of large plant population.

Responses of fifteen widely cultivated rice varieties to *A. besseyi* infection were observed as different reactions. Three varieties namely Sin A Kari-3, Yadana Toe and Paw Hsan Yin were rated as resistant reactions, while Aye Yar Min, Hnangar, Thee Dat Yin and Hmawbi-2 were moderately susceptible and Manawthukha, Sin Thu Kha, Shwe War Tun, Sin Thwe Latt, Kyaw Zay Ya, Shwe Bo Paw Hsan, Shwethwe Yin and Shwe Yin Aye were highly susceptible to white tip disease. It could be concluded that the resistant reaction of rice varieties to white tip disease would depend on the level of nematode population in rice seeds. In this experiment, seeds samples of Sin Thu Kha, Sin Thwe Latt and Shwe Bo Paw Hsan collected from different farms showed higher nematode population which corresponds

to susceptible reactions shown in the screen house experiment. According to the results, it can be suggested that susceptible varieties should not be grown due to high risk for infestation of *A. besseyi*. The varieties that exhibited resistant reactions namely Sin A Kari-3, Yadana Toe and Paw Hsan Yin could be recommended to grow in disease prevalent areas. However, integrated management with other control measures should be attempted to get effective disease management system.

In the present study, different control measures of seed treated with hot water at 55°C for 30 minutes (T3), application of carbofuran in soil (T4), combination of seed treated with hot water at 55°C for 30 minutes and brine solution (20% NaCl) (T5) and combination of seed treated with hot water at 55°C for 30 minutes, brine solution (20% NaCl) and application of carbofuran (T6) were effective in managing of *A. besseyi* by producing few number of final nematode population. These control measures produced good responses in growth and yield components such as plant height, tiller number, panicle length, number of filled grains, total number of spikelets panicle^{-1} and grain yield with lower number of unfilled grains and discolored grains panicle^{-1}. Therefore, all control measures tested except seeds soaked in 20% NaCl solution (T2) can be used to minimize the infestation of *A. besseyi* and reduce the loss of grain yield in Lone Pu rice variety. Although application of carbofuran can reduce the nematode population, use of chemical can cause the environmental pollution. Therefore, it could be suggested that among different control measures, seeds treated with hot water treatment at 55°C for 30 minutes alone (T3) can be used to minimize the infestation of of *A. besseyi* and reduce the loss of grain yield without any interference of seed germination. However, further investigations should be carried out for evaluation of efficiency of different control measures against white tip disease of rice in field condition.

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APPENDICES

Appendix 1 Effect of infection of *A. besseyi* with different inoculation methods on growth and yield components and final nematode population in Manawthukha

Treatment	Plant height (cm)	Tillers hill^{-1}	Total grains panicle^{-1}	No. of filled grains panicle^{-1}	No. of unfilled grains panicle^{-1}	No. of discolored grains panicle^{-1}	1000 grains weight (g)	Grain yield plant^{-1} (g)	No. of *A. besseyi* 100 seeds^{-1}
T1	90.8 a	9.0 a	90.7 c	78.3 c	12.3 a	20.0 b	17.5 a	12.4 c	1195 a
T2	93.0 a	8.7 a	103.7 bc	94.7 ab	9.0 a	35.0 a	17.3 a	14.3 bc	1569 a
T3	91.5 a	8.3 a	110.0 ab	91.0 bc	19.0 a	32.0 a	17.3 a	13.1 bc	1303 a
T4	95.7 a	9.7 a	109.0 ab	95.7 ab	13.3 a	31.7 a	17.8 a	16.4 ab	893 a
T5	98.0 a	9.3 a	123.7 a	109.0 a	14.6 a	32.0 a	17.9 a	18.1 a	0 b
LSD $_{0.05}$	7.66	1.88	15.26	14.75	10.19	10.56	1.35	3.65	800.32
Pr>F	0.2698	0.5624	0.0097	0.0132	0.3447	0.0710	0.7486	0.0310	0.0162
CV%	4.49	11.48	7.81	8.65	41.00	19.26	4.22	13.51	44.34

***** Means of three replications

Means followed by the same letter in the same column were not significantly different at 5% level

T1 = placing rice husk from 50 infested grains, T2 = introducing 200 *A. besseyi* suspension below the leaf sheath by a syringe

T3 = introducing 200 *A. besseyi* suspension into four holes of soil, T4 = introducing 200 *A. besseyi* suspension into soil

T5 = control (uninoculated)

Appendix 2 Characteristics of fifteen rice varieties included in assessment of rice cultivars for resistance to *A. besseyi*

No.	Characteristics	Manawthukha	Sin Thu Kha	Aye Yar Min	Paw Hsan Yin	Shwe War Tun
1.	Origin	India	Myanmar	Malaysia		Myanmar
2.	Parent	Taichung 65/*2 Mayang Ebos 80	Manawthukha/ IRBB21	Machando		Yarkyaw-2 (mutant line)
3.	Breeding number	Mashuri mutant 3628	IR Yn 1068-7-1	Machando		IR5 Mutant
4.	Released year	1977	2009	1977	1946	1972
5.	High quality/ high yielding variety	HYV	Rainfed lowland rice	Rainfed lowland rice	HQV	Rainfed lowland rice
6.	Grain type	Let Ywe Zin (medium)	Emahta	Emahta	Meedon	Emahta
7.	Day to maturity	135 -140	138-140	140-145	Nov. (Flowering)	140-145
8.	Plant height (cm)	105	107	135	160	120
9.	Panicles per hill	10 - 12	10-12	10-12	13	10-12
10.	1000 grain weight (g)	19.0	20.6	24.0	28.0	24.3
11.	Grain appearance	Translucent	Translucent	Translucent	Not translucent	Translucent
12.	Grain no. per panicle	188	220	160	115	150
13.	Amylose content (%)	26.5	23.7	26.23	24.2	30.4
14.	Yield (bsk/ac)	100-120	90-130	70-90	40-60	60-80

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Appendix 2 Characteristics of fifteen rice varieties included in assessment of rice cultivars for resistance to *A. besseyi* (Continued)

No.	Characteristics	Sin Thwe Latt	Sin A Kari-3	Hnangar	Kyaw Zay Ya	Yadana Toe
1.	Origin	IRRI	Thailand		Myanmar	Thailand
2.	Parent	IR 52912/IR29723//IR28224	RD 23B		Kyawzaya/Yarkyaw-2	Thai 1
3.	Breeding number	IR 53936-60-3	RD 23B	B 34-1	X70-18-38	Thai 1-9-3E
4.	Released year	2004	1985	1969	1980	2009
5.	High quality/ high yielding variety	Rainfed lowland rice	Rainfed lowland rice	HQV	Rainfed lowland rice	Irrigated lowland rice
6.	Grain type	Emahta	Emahta	Letywezin	Emahta	Emahta
7.	Day to maturity	135-140	135-140	152-158	140-145	120-125
8.	Plant height (cm)	120	120	153	120	125
9.	Panicles per hill	9-11	10-12	5-7	10-12	10
10.	1000 grain weight (g)	27.9	27.9	21.0	25.0	27.3
11.	Grain appearance	Translucent	Translucent	Large white belly present	Translucent	Translucent
12.	Grain no. per panicle	246	158	155	186	190
13.	Amylose content (%)	20.4	17.9	25.7	26.8	29.6
14.	Yield (bsk/ac)	90-120	100-120	40-60	80-100	100-140

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Appendix 2 Characteristics of fifteen rice varieties included in assessment of rice cultivars for resistance to *A. besseyi* (Continued)

No.	Characteristics	Thee Dat Yin	Shwe Bo Paw Hsan	Shwethwe Yin	Hmawbi-2	Shwe Yin Aye
1.	Origin	IRRI		IRRI	IRRI	
2.	Parent	IR 305 / BABAWEE // IR 36		IR 2153 / IR 28 // IR36	IR 4227-28 / KDML // IR 4570-124-3	
3.	Breeding number	IR 13240-108		IR 50	IR 21863-90-3	
4.	Released year	1991	1945	1985	1985	
5.	High quality/ high yielding variety	Irrigated lowland rice	HQV	Irrigated lowland rice	Rainfed lowland rice	
6.	Grain type	Emahta	Meedon	Emahta	Emahta	
7.	Day to maturity	110-115	Nov. (Flowering)	105-110	135-140	135
8.	Plant height (cm)	90	165	100	105	120
9.	Panicles per hill	8-10	8-10	10-12	10-12	9
10.	1000 grain weight (g)	24.0	30.0	19.5	25.5	29.0
11.	Grain appearance	Translucent / Trace white belly present	Not translucent	Translucent	Translucent	Not translucent
12.	Grain no. per panicle	105	180	130	140	
13.	Amylose content (%)	30.4	21.0	30.6	18.2	
14.	Yield (bsk/ac)	100-120	40-60	100-120	60-80	115

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Appendix 3 Host responses of fifteen rice varieties to *A. besseyi* by artificial inoculation with different inoculum levels

No.	Variety	I2 (200 *A. besseyi* plant^{-1})				I3 (500 *A. besseyi* plant^{-1})			
		Nematode population 100 seeds^{-1x}	Average index of infection	Symptoms reaction	Host Response[y]	Nematode population 100 seeds^{-1}	Average index of infection	Symptoms reaction	Host Response[y]
1	Paw Hsan Yin	19.2 e[z]	1.00	-	HR	88.0 ef	2.33	-	MR
2	Sin A Kari-3	54.3 c-e	1.67	-	MR	221.2 c-f	3.00	-	MR
3	Yadana Toe	24.8 de	1.00	-	HR	53.7 f	3.00	-	MR
4	Aye Yar Min	25.8 de	1.00	-	HR	113.0 ef	3.67	SPGs	MS
5	Hnangar	34.0 de	1.00	-	HR	115.8 d-f	4.33	WT & SPGs	HS
6	Thee Dat Yin	44.0 de	2.33	-	MR	56.7 f	3.67	SPGs	MS
7	Hmawbi-2	78.0 c-e	3.67	SPGs	MS	80.0 ef	3.67	SPGs	MS
8	Manawthukha	181.7 a-c	3.00	WT	MR	376.2 bc	5.00	WT & SPGs	HS
9	Sin Thu Kha	307.0 a	3.67	WT & SPGs	MS	829.0 a	4.33	WT & SPGs	HS
10	Shwe War Tun	158.7 b-d	4.33	WT & SPGs	HS	298.7 b-e	3.67	WT & SPGs	MS
11	Sin Thwe Latt	123.2 c-e	3.00	WT	MR	374.5 bc	4.33	WT & SPGs	HS
12	Kyaw Zay Ya	273.7 ab	3.67	WT	MS	512.5 b	4.33	WT & SPGs	HS
13	Shwe Bo Paw Hsan	185.0 a-c	5.00	WT & SPGs	HS	295.5 b-e	5.00	WT & SPGs	HS
14	Shwethwe Yin	36.8 de	1.67	WT	MR	343.8 b-d	4.33	WT & SPGs	HS
15	Shwe Yin Aye	48.5 c-e	2.33	WT	MR	100.7 ef	4.33	WT & SPGs	HS
	CV %	77.27				53.16			
	Pr ≥ F	0.0008				<0.0001			
	$LSD_{0.05}$	136.98				228.07			

[x] Means of three replications.

[y] Based on Popova et al. (1994) rating scale, the average index of infection is 0 = Immune (I), 0.1-1.0 = Highly resistant (HR), 1.1-3.0 = Moderately resistant (MR), 3.1-4.0 = Moderately susceptible (MS), 4.1-5.0 = Highly susceptible (HS).

[z] Means followed by the same letter in the same column were not significantly different at 5% level.

WT = white tip symptoms, SPGs = small grains and erect panicles symptoms, "-" = no symptom.