# THE INCIDENCE, ECONOMIC IMPORTANCE AND CONTROL OF <u>APHELENCHOIDES</u> <u>BESSEYI</u> CHRISTIE 1942 ON RICE

IN TANZANIA

#### NTOMBANA REGINA GATA

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#### ABSTRACT

The distribution, control and economic importance of <u>Aphelenchoides besseyi</u> Christie 1942 on rice (<u>Oryza sativa</u> L) cultivars grown in mainland Tanzania was studied. Survey results showed that <u>A. besseyi</u> was widely distributed in stored rice seeds.

Hot water treatment (HWT) of dry rice seeds at  $54 - 62^{\circ}$ C for 15 min effectively controlled seed-borne <u>A</u>. <u>besseyi</u>. In dry seed treatment, exposure period rather than treatment temperature was the significant factor for the control of seed-borne <u>A</u>. <u>besseyi</u>. Tested seeds of rice cultivars tolerated dry seed treatment upto 60 ° C for 20 min in respect of viability, vigour and seedling normality.

Seed treatment at 54°C for 15 min following presoaking controlled <u>A</u>. <u>besseyi</u> but also affected viability, vigour and seedling normality of some rice cultivars. The sensitivity of presoaked rice seeds to HWT was genetically related. Seeds treated following presoaking delayed onset, peak, maximum and actual percentage germination. Although HWT of presoaked seeds at 56°C for 15 min completely controlled <u>A. besseyi</u>, it also killed most seeds. However, HWT of presoaked or unsoaked seeds at 48°C for 20 min did not affect seed germination and emergence. Significant control of seed-borne <u>A. besseyi</u> by HWT following presoaking resulted in 10 -27% yield increases and, larger and/or better quality grains.

Booting stage of rice was the critical stage for control of <u>A. besseyi</u> for improvement of yield. The fifth day after the first sign of booting was identified as the most critical stage for infection with <u>A. besseyi</u> to adversely affect yield attributes.

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Control of <u>A.</u> <u>besseyi</u> with carbofuran applied at planting and at 50% booting stage improved rice yield but HWT of <u>A.</u> <u>besseyi</u>-infested rice seed gave better nematode control and better grain yield.

Dry seed treatment to control seed-borne <u>A. besseyi</u> was found to be the most appropriate method for Tanzanian farmers; because it is effective, cheap and safe to the seed and the environment.

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## DECLARATION

I declare that the work presented in the thesis is my own and has not been submitted for a degree in any other University.

27 June 1989

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Mrs N.R. Gata

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GABRIEL MAONDE MUGABE

and

my grandfather,

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You did not live for me to know, love or serve.

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#### 1. INTRODUCTION

Rice (<u>Oryza sativa</u> L.) is one of the most important cereals in the world; being the staple food for about half of the world population especially in South East Asia (Cobley and Steele, 1979). In recent years, it has been gaining increased popularity in Africa, because of its strategic position in food security and for being a crucial element in the staple food economies of several African countries. In 1982, Africa cultivated about 3.5% of the world's total rice area and nearly 2.2% of total production (Zan <u>et al</u>. 1985).

Demand for rice in Sub-Saharan Africa is becoming more 'serious, due to a general dietary shift from conventional foods, and it is anticipated that demand will continue to rise. Major rice producing areas of Africa are concentrated in Western and Eastern parts of the continent. In East Africa, Tanzania is the second largest rice producer and consumer after Madagascar (Chinganga, 1985).

Rice was considered the third most important food crop in Tanzania after maize and sorghum respectively (Monyo, 1973). It has since become more important and is now considered second to maize (FAO, 1978). In Tanzania, rice is a multipurpose crop with the grain being used both as human food and as a cash crop, while the stalks are used as animal feed and thatching, husks as litter in poultry and as mulch. Rice is considered to have the highest digestibility, biological value and protein efficiency ratio of all cereals (Houston, 1972), and provides a substantial proportion of human nutrition.

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Monyo and Kanyeka (1977) estimated that 180 000 metric tons of paddy were produced on 112 000 ha in Tanzania in 1960 and, Chinganga (1985) estimated that 275 600 ha were under rice cultivation annually in Tanzania. Rice in Tanzania is grown under varied ecological conditions, mainly by peasant farmers, mostly in the southern part up to the southern shores of Lake Victoria, but also on the northern shores of Lake Malawi, Pangani, Rufiji and Zanzibar. Rice is grown on large scale at Kilangali, Ruvu, and Mbarali irrigation schemes and on various estates of Morogoro and Tanga regions. A small proportion of upland rice is grown in different parts of the country such as the eastern slopes of the Uluguru Mountains, in East Central Tanzania, the Usambara Mountains and some parts of the Southern Highlands.

Judging from the diversity of the varieties or lines of rice grown in Tanzania (Table 1.1) and their wide geographical distribution which includes the most remote areas, this crop is likely to have had a very long history in the country. It is now the primary staple food for most of the coastal people and is widely accepted by the majority of Tanzanians. The long history and prevalence of rice in Tanzania are factors which are likely to have influenced its acceptability, making it highly competitive with maize as a staple food in urban areas.

In view of the important role played by rice as food and its ancillary uses, it is not surprising that the Government of Tanzania mounted a rice development programme as part of its effort to combat food shortage and in pursuit of the country's policy of food selfsufficiency. The programme involved setting up national irrigation schemes, encouraging small-scale farmers to produce more rice

Table 1.1 A list of some of the rice varieties or lines under cultivation in Tanzania Mainland Afaa Kilombero Farmer India Afaa Kilombero 0/906 Fava India Afaa Kilombero ex Mahiwa Faya Mbarali Afaa Morogoro Faya Pemba Afaa Mwanza 0/159 Afaa Mwanza 0/746 Faya Rangi 4 Afaa Mwanza 0/906 Faya Rangi 5 Afaa Mwanza 1/104 Faya Theresa Afaa Pangani Fire Afaa Ruvu Fungu Miji Afaa Tunduru Alupi Gamti Apani Gamti Kigoma Awini Gamti Tunduru Ganda Moja Bagamoyo Basmati 217 Bibi Yampaki 1/324 Bibi m'nyonze Blue Belle Bluu IR 5 Boiwani IR 8 B. 572/A3/472/48/28 IR 20 Chaka Jekundu IR 22 Changuku Cheza IR 42 Chidunari IR 52-18-2 Chikarati Chinise Farmer Demerara Creole CP 231, 1062 Dunduli yamilimani IR 520-1-26 Eal No. 136 SML 242 Eal No. 1322-IR-822-347 Ex Columbia Eal No. 1365 SML IR 532-1-33 Eal No. 1366 SML 140/5 Ex IR Eal No. 1367 SML 140/10/4 IR Eal No. 1391 Accession IR 579-163-2 Eal No. 1488 SML Imerini Ex IR Eal No. 1512-02-0231-Y Eal No. 1513 Shimolata Early Prolific \*LAL No. 1485 SML APWA IR 790-28-6 IR 878-82-62-2 1122 IR 322-347

Faya Rangi (Nene) Gobe Suthimin India (local) introduction IR 1-170-1-3-3 IR 9-60 Ex Mbarali IR 12-178-2-3 IR 24-IR-661-1140-32 IR 127-80-1-10 IR 154-61-1-1 x Sicastin IR 480-5-9-3-3 IR 520-1-26-3-3 Ex Philippines IR 520-1-26-3-3 Philippines IR 532/33 Ex Philippines IR 579-48-1-2 IR 589-66-2-1 IR 661-1-127-3-1 IR 665-24-1 Ex Philippines IR 773-AG 36-2-1

1316 IR 665-1-1-6

Junia

Kailangawa Kajibi Kajungu Long Straw Kiba Cheupe Kibawa Chainzi Kibawa Chekundu Kigunia Kihogo Malini Kihogo Mbarari Kihogo Mbulu Kihogo Red Line Kihogo Red Line 4 Kihogo Red Line 5 Kihogo Red Line 6 Kihogo Red Line 13 Kihogo Red Line 23 Kihogo Red Morogoro Kihogo Selection L No. 2 Kihogo Selection No. 7 Kihogo Selection No. 22 Kihogo 1/146 Kikarati (Chakarati) Kipakapaka (Pakapaka) Kipande Kishingo Kojicho Chamkulima Kolowanali Koroani Labelle L bonnet Kalafulu (Karafuru) Linda Safari Likoto Lingalangila Lukukwi (Rukukwi) Lumoto (Kamoto) Lunungu Manaki Mali Malondila Manda Malawi Mbarali Selection Mbarali Selection-Ringa Mchuzi wakuku Meli Meli Chungua Meli Pangani Meli yamilimani \* Other Melis Meno Meupe .Mambo (Local) Morogoro H1 Morogoro H2 Morogoro H3

Table 1.1 (continued)

Morogoro H4 Morogoro H5 Morogoro H15 Moshi Shinyanga Moshi wasSigara Arusha M'pinga fimbo Mpunga mwepesi Mugubu Mutant - 38 Mwacha-Pande Mwalopa Mwaropa Namilembo Ngoi yamilimani Ngwindimba Nyati Nyuki Nzurikwao Pasani Pijo marali Pishori (Dry Season) Portuguese Radium 601 Runyuki Safari Salama Salama Mgeta Samanini Sena Shingo lamajana Shingo-ya Mjakazi Sifari Mbugani Sindano - Sindano Ex Mbarali Sindano Ex Mia Sindano Kimamba Sindano Nseuyu Sindano 0/606 SL P 70 Soickai Soikete STR 56 Supa Supa Grade Malevy Surinam Surinam K1 Surinam K2 Surinam K4 Surinam K5 Surinam Kahi Surinam V 880 Surtun

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Table 1.1 (continued)
Swamp Rice Mbamba Bay
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Taiwani T - 15
Taiwani 22610 M
Taiwani 22612 Mbarali
Taiwani (22613) Mbarali
Taiwani 22614 (J14)
Tulenabwana
Wamba
YR L1
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through education programmes and subsidising the price of seed and fertilisers as well as the producer price incentive through the years (Table 1.2). As a matter of fact, on average the producer price of paddy has been the highest among all the cereals in Tanzania. The Government also charged researchers with the task of producing high-yielding varieties, resistant to pests and diseases.

Subsequent to the Government efforts, a call for better rice productivity was made by the then Dean of the Faculty of Agriculture, while addressing a National Rice Research Coordinating Committee Meeting on 20 September 1975. He pointed out that Tanzania was spending approximately 1.2 billion shillings in importing cereals (maize, wheat and rice) and appealed to the nation to double its efforts in food production. He charged scientists with the task of producing high-yielding varieties with high nutritional value, under good agro-ecological environments. This was to be achieved, through different disciplines, working together, in what he termed "integrated research with a purpose" to facilitate examination of all aspects of "a good variety, including nutrient requirements, pests, diseases and weeds, time of planting, plant populations per given area". It was further pointed out that a definite national food policy was necessary for Tanzania.

It was observed therefore, that Tanzania was beginning to appreciate the complexity of the dimensions involved in the process of food production. Indeed, food production is a multifactorial system which can be constrained by multiple factors which interact with each other, such that the effect on one factor reflects on the whole system.

In a food production system, there is the farmer in his

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Crop:	71/72	72/73	73/74	74/75	75/76	76/77	77/78	78/79	79/80	80/81	81/82	82/83	83/84	84/85	85/86
ΡΑΌΟΥ	0.52	0.56	0.57	0.65	1.00	1.00	1.20	1.20	1.50	1.75	2.30	3.00	4.00	6.00	8,00
MAIZE	0.24	0.26	0.33	0.50	0.80	0.80	0.85	0.85	1.00	1.00	1.50	1.75	2.20	4.00	5.25
WHEAT	0.57	0.57	0.57	0.77	1.00	1.20	1.25	1.25	1.35	1.65	2.20	2.50	3.00	4.50	6.00
SORGHUM/ BULRUSH MILLET	0.30	0 30	0.50	0.55	0.75	06*0	1.00	1.00	1.00	1.00	1.00	1.60	2.00	3 00	4.00

Source: Marketing Development Bureau, Ministry of Agriculture, Dar es Salaam.

socio- political and economic environment, over which the world socio- economic order is superimposed. There is the land factor, which can range from no land to more than adequate unsuitable land to land of maximum production potential. Climatic factors are important, though generally, they are beyond man's control, for example, temperatures can be too high, optimum, or very low; rainfall can be anything from insufficient, drought to devastating storms and monsoons and, relative humidity, can range from 10 -100%. There are also the biological factors which include pests, diseases and weeds that damage crops and reduce the quantity and quality of food but to some extent can be manipulated to levels below the economic threshold through good management practices.

Therefore, in any given food production system, full cognisance should be taken of these factors to facilitate their maximum manipulation in order to provide an efficient system. In Tanzania, apart from the effects of environmental factors, rice production is known to be affected by pests, diseases, lodging, shattering and low response to fertilizers (Monyo, 1973).

Despite the many known factors that affect rice production in Tanzania (Monyo, 1973), whose quantitative and qualitative effects were little known, the national rice development programme continued to emphasize agronomic and breeding studies at the expense of a wholistic approach. Thus, the other disciplinary work on rice was left to individual scientists not strongly incorporated in the national rice development programme. Hence, the latter approach was taken to study the rice nematode <u>Aphelenchoides besseyi</u> on rice in Tanzania, firstly by East African Agriculture and Forestry Research Organisation (EAAFRO) workers (1970-71) and subsequently through this study.

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Cultivated rice is known to be affected by many pests and diseases (Ou, 1972). Among the rice pests are a wide range of plant parasitic nematodes (Taylor, 1969; Pans, 1976; Babatola, 1984; Prasad <u>et. al.</u> 1987). While most of the nematode pests of rice are soil nematodes affecting rice roots, two rice nematode species; <u>Ditylenchus angustus</u> Filipjev, 1936 and <u>Aphelenchoides besseyi</u> Christie, 1942 are important pests of rice which affect the aerial parts.

<u>A.</u> <u>besseyi</u> is now considered to belong to the order Aphelenchida; superfamily Aphelenchoides, family Aphelenchoididae and genus <u>Aphelenchoides</u> while, <u>Ditylechlus</u> <u>angustus</u> belongs to the order Tylenchida, superfamily Tylenchoidea, family Anguinidae subfamily Anguininae and genus <u>Ditylenchus</u>. Diagramatic representations of the two nematodes is shown on Figure 1.1.

<u>Ditylenchus angustus</u> is an obligate ecto - parasite specific to the species of the genus <u>Oryza</u> (Taylor, 1969; Sheshadri and Dasgupta, 1975). <u>A. besseyi</u> on the other hand has a much wider range of hosts covering many plant species. The list of host range for <u>A</u>. besseyi is given in chapter 2 of the thesis.

Both nematodes feed ectoparastitically on the growing tissues of the stem (Taylor, 1972; Siddiqi 1971, Pans 1976), resulting in white-tip disease of rice by <u>A</u>. <u>besseyi</u> and 'Ufra', Dak pora, Akhet - pet or yad-ngo disease of rice by <u>D</u>. <u>angustus</u>. Huu-Hai-Vuong (1969) reported that in rice fields in Madagascar, it was difficult to distinguish between the symptoms by <u>A</u>. <u>besseyi</u> and <u>D</u>. <u>angustus</u> in ratoon crop, because both nematodes cause malformed panicles, sterility of spikelets and crisping of head sheath and



leaf. However in glasshouse inoculated plants, the symptoms by these two nematodes are different. <u>A</u>. <u>besseyi</u> infected panicles sometimes come out of the sheath with a little scale-like head leaf three or four cm long with distorted tips, covering the peduncle or lower spikelets which are themselves twisted and empty. The panicles infected by <u>D</u>. <u>angustus</u> are generally completely distorted, together with the rachis and the pedicels of the spikelets. The panicles may fail to emerge or partly emerge while under high infestations the panicles are completely destroyed to none existence.

<u>A. besseyi</u> is termed the rice leaf nematode (PANS, 1976), the white tip nematode (Hollis, 1984) while <u>D</u>. angustus is known as the rice stem nematode (Taylor, 1969; PANS, 1976; Hollis 1984). <u>Ditylenchus angustus</u>, when in a state of anabiosis form 'cottony' masses on rice plants and remain on the stubbles when rice is harvested where they serve as inoculum for the next crop. <u>A. besseyi</u> meanwhile is the only seed borne nematode of rice (Hollis, 1984) and is disseminated through the seed, where it is found coiled up in a state of anabiosis inside the rice seed coat. Plate 1 shows the different symptoms exhibited by rice plants infested by <u>D</u>. angustus and <u>A. besseyi</u> respectively. Both nematodes can cause considerable yield losses which vary with countries.

<u>Ditylenchus angustus</u> has been reported in Bangladesh, Burma, India, Madagascar, Malaysia, Phillipines, Thailand, United Arab Republic and, is reported by Taylor (1976) to have been found in Southern Africa at higher elevations which experience temperate climate. <u>D. angustus</u> has not been reported in East Africa, while <u>A</u>. besseyi has been identified in most of the rice growing countries,

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Plate 1.1 (a) Panicles and leaves of rice affected by rice nematode (D. angustus)

(b) White tip symptoms caused by A. besseyi on rice leaves

including Africa, East Africa and Tanzania in particular (see Chapter 2 of the thesis).

From the results of various workers from many countries, it is clear that the rice nematode <u>A. besseyi</u> causes crop loss, the magnitude of which varies with country, variety, cultural methods and year of crop, and that, it also affects the quality of grain. It is not clear if this qualitative effect on the grain also involves protein except from insinuations by Amano and Nakanishi (1976).

A literature review on the control of <u>A. besseyi</u>, shows that hot water treatment of seeds is considered to be the best method for control of this pest. However, it is also observed that the given treatment regimes vary widely in many aspects of the treatments.

The use of resistant or tolerant varieties can be considered promising but not of immediate practical use to growers of other countries because those resistant varieties may not necessarily, replace locally preferred ones. Screening local material for resistance and tolerance could provide an answer to the problem of <u>A. besseyi</u> on rice through use of locally preferred resistant rice cultivars.

Chemical control has a limitation of cost and practicability. In some cases, most of the recommended chemicals may not be available in some of the developing countries.

The complexity of factors involved in crop losses due to <u>A</u>. <u>besseyi</u> plus the variability in the presented data for its management, necessitates that affected countries study their local problem and where necessary, work out appropriate management methods.

In Tanzania, the wide distribution of A. besseyi coupled

with the heavy seed infestation (EEAFRO, 1971; Taylor <u>et al</u>, 1972) warranted a further survey followed by a study to assess the damage it causes and to work out appropriate management methods. Furthermore in view of the many rice cultivars and lines grown in Tanzania, and the inadequacy of information on some aspects of germination of hot water treated rice seeds, it was considered necessary to study the effects of the latter treatment on germination of different rice cultivars. At the same time, it was also deemed appropriate to test some of the available chemicals for their efficacy in the management of this rice pest.

Therefore, briefly the objectives of this study were to investigate:

- (a) The distribution of <u>A.</u> <u>besseyi</u> in some rice cultivars grown in Tanzania;
- (b) The possible effect of the nematode on rice production in Tanzania;
- (c) Effect of hot water treatment on the management of <u>A. besseyi</u> in seeds of locally grown rice cultivars and on the germination of treated seed; and
- (d) Effect of insecticide/nematicide carbofuran and the soil fumigant dazomet in nematode management and rice yield respectively.

#### 2. LITERATURE REVIEW

#### 2.1 The organism

#### 2.1.1 General

The nematode known to cause "white tip" disease on rice is called <u>Aphelenchoides besseyi</u> in honour of E. A. Bessey who discovered it on strawberry in the southern part of the United States in 1901 (Thorne 1961). It was later described by J. R. Christie in 1942 who named the species after Bessey and gave details of its parasitic habits. This nematode is synonymous with <u>Aphelenchoides oryzae</u> Yokoo, 1948 and <u>Asteroaphelenchoides besseyi</u> Christie, 1942 (Drozdoviski 1967, Fortuner and Orton Williams 1975).

#### 2.1.2 Systematic position

<u>Aphelenchoides besseyi</u> Christie, 1942 belongs to the super family Aphelenchoidea, family Aphelenchoididae, sub family Aphelenchoidinae and genus <u>Aphelenchoides</u> (Baker 1962). Franklin and Siddiqi (1972) and Fortuner and Orton Williams (1975) placed it in the order Tylenchida. However, recently Siddiqi (1980) reassigned this genera to the order Aphelenchida, superfamily Aphelenchoidea of "class" Nematoda based on ecological, ethological and morphological evidence and observations.

#### 2.1.3 General description

<u>A. besseyi</u> are generally slender nematodes with females and males ranging in length between 0.62 - 0.88 mm and 0.44 - 0.72 mm respectively. Fortuner and Orton Williams (1975) in their review reported that it differs from other <u>Aphelenchoides</u> species by: having a short narrow, inconspicuous post vulval sac, a large ovary with several rows of occytes, excretory pore slightly anterior to the nerve ring, labial region slightly larger than the head, four incisures on the lateral field, four processes on the tail in both males and females and spicules lacking a dorsal process.

#### 2.1.4 Biology of A. besseyi

#### 2.1.4.1 Mode of reproduction

Sudakova and Stoyakov (1967) reported that <u>A. besseyi</u> reproduces parthenogenetically but Huang and Huang (1974) found eggs of <u>A. besseyi</u> to invariably carry two nuclei which subsequently fused to form zygotes suggesting an amphimictic mode of reproduction. Cayrol and Dalmasso (1975) and Huang <u>et al</u>. (1979) confirmed that <u>A. besseyi</u> reproduces amphimictically. In a review by Fortuner and Orton Williams (1975), <u>A. besseyi</u> is considered bisexual and that on rice, males and females are found in equal proportions.

#### 2.1.4.2 Sex ratio

The sex ratio of A. besseyi is reported to vary (Fukano

1962; Huang et al. 1972). Work by Huang et al. (1972) using the sex equation for obligate amphimictic nematodes and comparing the estimated ratio, with the experimental results, confirmed the male ratio to be 12,7 %. The same workers also found that the male ratio increased under aging cultural conditions suggesting that either male differentiation was favoured by poor food or that the latter condition shortened the life span of females.

#### 2.1.4.3 Life cycle and duration

A. besseyi develops through four juvenile stages with the Fortuner and Orton Williams second stage attained in the egg. (1975) described the duration for the cycle from egg to egg to be 6.5 - 7 days with embryogenesis taking 0.5 days; second, third and fourth larval stages taking 0.13; 0.65 and 0.9 days respectively while sexual maturation for an adult female takes 4.3 to 4.8 days. The same authors considered feeding a prerequisite for larvae to develop and that in nature, length of generation time and number of generations varied with ecological conditions. A. besseyi life span is reported to be 35 - 50 days (Sudakova and Stoyakov 1967). In rice, the rate of multiplication is said to depend on the rice variety and its capacity to resist the disease (Fortuner and Orton 1975), being greater at tillering and greatest during Williams panicle formation (Goto and Fukatsu 1956).

### 2.1.5 Effect of temperature on activity and life cycle of A. besseyi

While optimum temperature for activity and for completion of life cycle of <u>A besseyi</u> is 31.8° C, the nematode can stay active

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between  $13^{\circ} - 42^{\circ}$  C (Tikhonova 1966c). It remains motionless and dies after one month at  $10^{\circ}$  C (Hashioka 1964) whereas, at  $42^{\circ}$  C and  $44^{\circ}$  C, the nematode dies in 16 and 4 hours respectively.

# 2.1.6 Effect of atmospheric humidity and grain moisture on activity of A. besseyi

<u>A. besseyi</u> is able to move only over a film of water (Tikhonova 1966c). Thorne (1961) considered intra- plant migration of <u>A. besseyi</u> to be restricted to periods when a film of moisture is present, such as following rain, dew or high humidity. It is able to feed when atmospheric humidity is above 70 % (Tikhonova 1966c). Activity of the nematode is reduced at grain moisture of 40 - 35 % or less, when the nematode goes into quiescence or dies (Huang <u>et al. 1972</u>). The same authors reported that grain moisture of 27 % or less results in progressively less revivals from anabiosis.

#### 2.1.7 Physiological strains

Literature on the existence of physiological strains of <u>A. besseyi</u> is not explicit. Ectoparasitism of <u>A. besseyi</u> on Vanda and its endoparasitism on <u>Saintpaulia</u> is attributed to the host differences rather than dissimilarity of the nematodes themselves (Allen 1952). Noegel and Perry (1963) however noticed that strawberry plants grown among diseased chrysanthemums were not attacked by the nematode. Fortuner and Orton Williams (1975), suggested that on rice there may be two or more physiological strains of the <u>A. besseyi</u> species.

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#### 2.1.8 Host range

Rice is considered to be the most important host of <u>A. besseyi</u> on which it causes "white tip" disease, followed by strawberry on which it causes "summer dwarf" or "crimp" (Franklin and Siddiqi 1972). However <u>A. besseyi</u> has numerous other hosts ranging from herbs, vegetables, millets, corn, legumes, grasses, ornamental plants to many other wild plants. A list of host range is presented in Table 2.1. It is surprising that, in spite of the known wide host range for this nematode species, assessment of plant damage caused by <u>A. besseyi</u> has only been done for rice and strawberry. Consequently, the economic importance of <u>A. besseyi</u> is not fully known.

#### 2.1.9 Geographical distribution

The distribution of <u>A.</u> <u>besseyi</u> on rice is world-wide with records from many countries in all the continents (Franklin and Siddiqi 1972; Fortuner and Orton Williams 1975). The distribution of <u>A.</u> <u>besseyi</u> on rice in Africa and in Tanzania is given in Figures 2.1 and 2.2 respectively.

#### 2.1.10 A. besseyi the cause of "white tip" disease of rice

The rice disease now known as "white tip" was at first known by different names in different countries and the causal organism for the white tip disease of rice was not known for a long time.

In the United States, Jodon in 1935, is considered to be the

LATIN NAME OF HOST PLANT	COMMON NAME	AUTHOR/COUNTRY
Allium cepa L.	Onion	Timm, 1965 (Thailand)
Boehmeria nivea Gaudich	Ramie	Fortuner, 1970 (Philippines)
Brassica pekinensis (Lour.) Rupr.	Chinese cabbage	Sher, 1954 (Philippines)
Chrysanthemum maximum Ram	Shasta daisy	Sher, 1954 (Hawaii)
Chrysanthemum morifolium Ram	Florist's chrysanthemum	Sher, 1954 (Hawaii)
<u>Cyperus iria</u> L.	Sedge	Yoshiii and Yamamoto, (1950b) (Japan)
Cyperus spp	Sedge	Vuong Huu Hai and Rabarifoela, 1958 (Comoro Islands)
Dahlia viriabilis Desf.	Dahlia	Sher, 1954 (Hawaii)
<u>Digitaria ciliaris</u> (Retz.) Koel ( <u>D. adscendens (HB</u> K) Henrard)	Summergrass	Ino, 1971 (Japan)
Digitaria sanguinalis (L.) Scop.	Hairy fingergrass	Yoshii and Yamamoto, 1950b (Japan)
Dioscorea trifida L.	Yam	Kermarrec and Anais, 1973 (Guadeloupe)
<u>Echinochloa</u> <u>frumentacea</u> Link ( <u>Panicum cruys-galli</u> var. <u>frumentaceum</u> )	Barnyard grass, Japanese millet Billion dollar grass	Ino, 1971 (Japan)
Erecgtutes oraeakta Raf.	1	Sher, 1954 (Hawaii)
Ficus elastica Roxb (C.V. decora)	Rubber plant	Marlatt, 1966 (U.S.A.)

Table 2.1 Host range of <u>Aphelenchoides</u> besseyi, Christie 1942

tinued)	
2.1 (con	
Table 2	

	LATIN NAME OF HOST PLANT	COMMON NAME	AUTHOR/ COUNTRY
	Setaria italica (L.) P. Beauv.	Italian Millet	Yoshii and Yamamoto, 1950a (Japan)
	<u>Setaria viridis</u> (L.) P. Beauv.	Green bristle grass, Bristle	Yoshii and Yamamoto 1950a (Japan)
50	Solenostemon scutellariodes (L.) Codd ( <u>Coleus blumei Benthi.</u> )	Coleus, painted nettle-leaf	Sher, 1954 (Hawaii)
00316	Sporobolus poiretii (Roem. & Schult) Hitch	Smut grass	Marlatta, 1970 (U.S.A.)
	Tagetes spp	African marigold	Sher, 1954 (Hawaii)
	Tithonia diversifolia A. Gray	Red sunflower	Sher, 1954 (Hawaii)
	<u>Torenia fournieri</u> Linden	Bluewings	Sher, 1954 (Hawaii)
	Vanda x Miss Joaquim	Orchid	Allen, 1952 (Hawaii)
48	Vanda x Rose Marie	Orchid	Sher, 1954 (Hawaii)
072	<u>Vanda</u> x Miss Deum	Orchid	Sher, 1954 (Hawaii)
	<u>Veda</u> x Trimeril	Orchid	Sher, 1954 (Hawaii)
	<u>Vanda</u> x Luma	Orchid	Sher, 1954 (Hawaii)
	<u>Vanda</u> x Miss Joaquim x Kapoho	Orchid	Sher, 1954 (Hawaii)
	Zea mays L.	Maize, Sweet corn	Timm, 1965 (Hawaii)
	Zinnia elegans Jacq.	Zinnia	Sher, 1954 (Hawaii)

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 2.1 (	continued)
N	

LATIN NAME OF HOST PLANT	COMMON NAME	AUTHOR/COUNTRY
<u>Fragaria ananassa</u> Duchsne ( <u>F. chiloensis</u> Duch. var. <u>ananassa</u> )	Strawberry	Allen, 1952 (Hawaii)
Fragaria chiloensis Duch.	Strawberry	Christie, 1942 (U.S.A.)
<u>Glycine max</u> (L.) Merr ( <u>G. hispida</u> Max)	Soyabean	Barat <u>et. al.</u> 1966a (U.S.A.)
Hibiscus brackenridgei Gray	Hibiscus	Raabe and Holtzmann, 1966 (Hawaii)
Hydrangae macrophylla Ser.	Hydrangae	Sher, 1954 (Hawaii)
Impatiens balsamina L.	Balsam	Sher, 1954 (Hawaii)
Imperata cylindrica Beauv.	Silverspike	Vuong Huu Hai and Rabarijoela, 1968 (Comoro Islands)
Ipomoea batatas Lam.	Sweet Potato	Tim and Ameen, 1960 (Pakistan)
Panicum bisulcatum Thumb.	Chaff Panic	Ino, 1971 (Japan)
<u>Pennisetum</u> glaucum (L.) R. Br. <u>Pennisetum</u> <u>typhoides</u> (Burm.) Stapf & Hubbard	Bulrush Millet	Hashiok, 1964
Pluchea odorata Cass.	I	Sher, 1954 (Hawaii)
Polianthes tuberosa L.	tuberose	Holtzmann, 1968 (Hawaii)
Pycreus polystanchyos (Rollb.) Beauv.		Vuong Huu Hai and Rabarifoela, 1968 (Comoro Islands)
Saccharum officinarum L.	Sugarcane	Fernandez and Diaz Silveira, 1967 (Cuba)
Saintpaulia ionantha Wendl.	African violet	Allen 1952 (U.S.A.)







first to mention the rice disease now known as "white tip" (Todd and Atkins 1958). Up until 1949, several pathologists in the United States considered white tip to be a physiological disease. Tullis and Cralley (1936) reported about a chlorosis of rice supposed to have been caused by iron deficiency; symptoms of which were similar to those of white tip. This resulted into a theory that white tip was caused by iron deficiency, and the latter theory was supported by Jones <u>et al.(1938)</u> and Tullis (1940).

However, Martin (1939) and Martin and Alsatt (1940) concluded that the disease was due to magnesium deficiency. Later, Takimoto (1943) in Japan, reported that the cause of the white tip disease of rice in Kyushu area might be identical to that of the American white tip reported by Martin (1939). Yoshii (1946) was unable to confirm the work of Martin (1939) that it was due to magnesium deficiency. He found that nematodes were associated with the diseased plants, and named the disease "Senchu Shingare Byo" (Heart blight caused by a nematode) and later in 1946 he reported the effects of hot-water seed treatment tests. Though Yoshii (1946) reported the causal nematode as Aphelenchoides oryzae Yokoo, 1946; Yokoo did not publish the description until 1948. Cralley (1949) confirmed that white tip of rice was caused by a nematode and became the first in the United States of America to confirm the findings of Yoshii (1946) and Yokoo (1948).

The fact that <u>Aphelenchoides</u> <u>oryzae</u> is only a synonym of <u>A. besseyi</u> was established by Allen (1952) who found no morphological differences between <u>Aphelenchoides</u> <u>besseyi</u> Christie 1942 from strawberries and <u>A. oryzae</u> Yokoo 1946 from rice. Yoshii and Yamamoto (1950c) concluded that the nematodes are seed borne, and that the soil did not seem to serve as a source of inoculum. Yoshii and Yamamoto (1950b) also found that the nematodes could persist in stored rice for 3 years.

Todd and Atkins (1958, 1959) experimentally proved that <u>A. besseyi</u> is capable of causing "white tip" symptoms on rice. Other pathogens capable of causing "white tip" symptom on rice are two insects, namely <u>Chlorops oryzae</u> Matsumura and <u>Eusarcoris ventralis</u> Westwood (Yoshii and Yamamoto, 1950a) but the symptoms in these cases are accompanied by physical injury. In Madagascar lack of fertilizer and the effect of parasitic fungi were found to produce symptoms similar to "white tip" of rice caused by <u>A. besseyi</u> (Vuong Huu Hai and Rabarijoela 1968).

#### 2.1.11 Symptomatology

Studies by several workers (Tanaka and Uchida 1941; Todd and Atkins 1958: Tikhonova Yoshii and Yamamoto 1950a: 1966a; Ou 1972; Ueda and Matsumo 1975) revealed that parasitism of rice plants by A. besseyi can result in abnormal growth of (1950a) described the affected plants. Yoshii and Yamamoto symptoms of the disease as being discolouration of leaf tips for a the typical "white distance of 3-5cm (generally considered as tip" symptoms) plus short whorled and twisted flag leaves from which abnormally deformed ears emerge. Parasitism of rice plants by A. besseyi is also reported to result in obstruction of xylem by gums followed by phloem disintegration (Fortuner and Orton Williams 1975). The latter authors reported that cell growth in some leaves of affected plants is retarded and chloroplasts are scarce or absent. Formation of necrotic areas in badly affected plants (Yoshii and Yamamoto, 1950a), retarded growth (Tanaka and Uchida 1941; Ueda and Matsumo 1975) stunted growth, lack of vigour and production of small panicles (Ou 1972), development of abnormal tillers from third and fifth nodes up the main tillers (Yoshii 1946) are some of the symptoms observed for the growth phase of the rice plant affected by A. besseyi.

Uebayashi et al. (1971) showed that injury by <u>A. besseyi</u> to rice during early milk stage resulted in longitudinally cracked and blackened kernels. Fortuner and Orton Williams (1975) summed up the symptoms of affected plants as having shorter panicles, often atrophied at the tips with glumeless spikelets at the tips, some sterile flowers producing empty spikelets with twisted husks and fertile flowers that sometimes produce morphologically abnormal grain with low germination potential and delayed date of maximum germination. The abnormal growth of earheads and spikelets in rice infested with <u>A. besseyi</u> was considered to be the result of the reaction of the host tissue to the secretions of the nematode (Rao 1976).

The typical "white tip" symptoms are not invariably observed in infested fields nor are they exhibited by all infested plants (Yoshii and Yamamoto 1951). In Tamilnadu (India) local varieties did not show these symptoms (Muthukrishnan <u>et al</u>. 1974) and in Bangladesh infected plants did not exhibit "white tip" symptoms (Fortuner and Orton Williams 1975). EAAFRO workers (1971) could not detect any "white tip" symptoms in infested fields in East Africa. Todd and Atkins (1959) reported that the most resistant varieties rarely showed foliar symptoms even though their yield was reduced by this pest. Babatola (1984) reported that white tip symptoms had not been observed anywhere in Nigeria even though rice seeds may be infested by A besseyi.

#### 2.1.12 Etiology

Todd and Atkins (1958, 1959) experimentally demonstrated that <u>A. besseyi</u> was one of the causal agents of "white tip" disease of rice. The same authors and Yoshii and Yamamoto (1950) also demonstrated that the nematode fed ectoparasitically on rice. <u>A. besseyi</u> is known to parasitise upland and lowland rice and is disseminated by infested rice seeds. Fukano (1962) considered infested seeds to be the primary source of infestation by this nematode while Cralley (1952) considered them to be the sole source of infestation. Vuong and Rodriguez (1970) however, were able to demonstrate that a ratoon crop was a possible inoculum source for the subsequent crop. Yoshii and Yamamoto (1950c) proposed that a possible infestation could arise from debris and weeds.

<u>A. besseyi</u> is reported to move from plant to plant and in dry seeds, the nematodes are found between the grain and its husks coiled in a state of anabiosis from which it is reactivated when the seed is hydrated. In an anabiotic state in stored grain, these nematodes are known to survive for considerable lengths of time, up to 3 years (Yoshii and Yamamoto 1950b; Todd and Atkins 1958).

On being hydrated, the quiescent A. besseyi becomes

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reactivated, swim away from the seeds and eventually return to enter seed buds (Tamura and Kegasawa, 1957). Movement of A. besseyi from infested seeds was found to be related to temperature and budding of seed soaked in water while percentage survival and chances to infest the host were related to the time following movement of the nematodes from the seed (Tamura and Kegasawa 1958). Higher temperatures during swimming away results in low percentage survival, similarly the longer the time after moving from the seed, the less chances for survival and successful infestation. Optimum temperature for swimming away was put at 20° C and rates of mortality during swimming from budding seed at 20° C, 25° C and 30° C and 35° C were 53.3; 66.7; 90.9 and 100 % respectively (Tamura and Kegasawa 1957). Tamura and Kegasawa (1958) demonstrated that the migrating nematodes could attack any part of the rice seedlings except roots and they showed that the nematodes could also move from one part of a plant to another independent of geotaxis and that their migratory behaviour was related to their overall biology while their vitality was related to the cultural environment of rice plants in the paddy fields.

Interplant migration of <u>A. besseyi</u> was reported to be maximum at first leaf stage under double dose nitrogen fertilizer, while under normal nitrogen fertilizer, maximum movement is during third and fourth leaf stages (Tamura and Kegasawa 1958). Goto and Fukatsu (1956) demonstrated that a chemical attraction to certain cultivars of young growing rice plants was involved and Fortuner and Orton Williams (1975) reported that susceptible varieties were more attractive than resistant ones.

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The nematodes are said to swim away from the germinating seed and invade seedlings within the first 10 days after sowing and are said to prefer young growing parts. At six leaf stage, the whereabouts of the nematodes on the plants is not known (Yoshii and Yamamoto 1950a). However, at tillering stage, it is generally believed that <u>A. besseyi</u> are found in the cavity above growing points of the rudimentary culm and/or on the young leaf surrounded by the innermost leaf sheath. The nematodes move up with the growing plant feeding on the tender growing tissues.

The population increases rapidly from the late tillering stage of rice onwards and the nematodes migrate to the developing panicle where they either puncture the inflorescence or enter the glumes through the apical tunnel at the apices of lemma and palea. The nematodes multiply abundantly before anthesis, feeding on fleshy tissues of the stamens, lodicules and embryos.

After anthesis, nematode multiplication declines with the age and moisture content of the grain. Development of the second to third stage larvae continue up to the eighteenth day after anthesis and from the latter stage to adult it takes up to 30 days by which time the grain is also ripe. However, grain ripening is achieved before some larvae are mature and reduction of grain moisture from 18 - 15 % induces anabiosis to all stages except second stage larvae.

Nematodes in a state of anabiosis can be found in all parts of the rice plant, including straw, this being the physiological state through which this pest survives unfavourable conditions. In this state the nematode's food reserves of polysaccharides are

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hydrolysed to simple sugars.

In the grain the nematodes are said to be mainly distributed in the middle followed by the lower part, and the terminal part. On the other hand Uebayashi <u>et al</u>. (1971) observed that the nematodes live in the space between the hull and the ventral side of the developing endosperm. Nandakumar <u>et al</u>. (1975) showed that in dry seeds, the nematodes are found coiled inside the palea and on the surface of the lodicules. Ichinohe (1964) also reported presence of <u>A. besseyi</u> inside the palea.

(1970) and Todd and Atkins (1958) observed chaffiness Rao and abnormal elongation of glumes in some spikelets, rachii and rachillae. Cell damage to the outer wall of the ovary, caused partial filling of kernels and, damage to the lodicules prevented closure of flowers after anthesis, exposing the embryo to environmental stress like dessication, infection by Trichoconis padwickii and spikelet sterility (Rao and Rao 1979). Dew deposition or high humidity (above 90% R.H.) was found to be essential for the nematodes movement and migration to the reproductive fillers, without which few if at all any reached developing grains for cryptobiosis (Nandakumar et al. 1975). A range of 21 - 23°C air temperature is said to be suitable for A. besseyi activity and expression of damage symptom in crops (Rao et al.1983)

### 2.1.13 Nature of crop loss due to <u>A. besseyi</u> and its economic importance

Crop losses due to this nematode have been found to vary

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from country to country depending on environmental factors, rice varieties and cultural practices. In Taiwan, Huang (1959) estimated 20 - 50 % loss. In India, loss of grain yield due to chaffiness of ear heads was reported to be 20.4 % (Muthukrishnan <u>et al</u>. 1974) and earhead damage due to partially filled grains ranged from 21 - 46.3% (Nandakumar <u>et al</u>. 1975). In America Atkins and Todd (1959) recorded 17 % crop loss over a period of 3 years and, 41 - 71 % crop loss was reported in the USSR (Tikhonova 1966). Tamura and Kegasawa (1959) noted that the ratio of damaged stems was not correlated with yield components during abnormal weather conditions, suggesting that ecological conditions of rice and nematodes in bad weather differed from those existing during normal weather.

Ueda and Matsumo (1975) pointed out that "white tip" disease caused yield reduction through lowering of grain weight caused by lowered stem and plant height and withered white tips of leaves, which resulted in increased numbers of immature rice grains, lowered grain weight and reduction in actual numbers of grains. Muthukrishnan <u>et al.</u> (1974) reported that nematodes in the spikelets caused damage to lodicules and the ovary. Atkins (1974) stated that "white tip" disease reduces yield and quality of grain, panicle size, fertile florets and rice grains and resulted in grains frequently abnormal in shape.

#### 2.1.14 Management of A. besseyi

Numerous investigators have worked on various methods of managing <u>A.</u> <u>besseyi</u> on rice namely cultural, chemical and physical as well as the use of resistant varieties (Fortuner and Orton

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Williams, 1975; Prassad <u>et al</u>. 1986). Considering the cultural management method, sowing under water (Cralley 1956) and flooding at germination were considered effective in managing "white tip" disease. Early planting (Cralley 1949) and advancing planting by 60 days (Yoshii and Yamamoto 1951) were also recommended for management of <u>A. besseyi</u>. A special oil cover on seed beds was recommended by Tamura and Kegasawa (1959), rotation by Tikhonova (1966a), burying of straw, debris, and weeds by Vuong Huu Hai (1969), and spreading 200 to 300 kg/ha of magnesium sulphate by Martin and Alstatt (1940).

The use of resistant varieties to manage "white tip" disease was investigated by several workers including Cralley (1949), Cralley and Adair (1949), Dastur (1936), Goto and Fukatsu (1956), Komori et al. (1963), Nishizawa (1953a), Orsenigo (1954, 1955) and Todd and Atkins (1959). These researchers working in various countries came up with a number of rice varieties whose response ranged from very tolerant to very susceptible. Fukano and Yokoyama (1955) found that "Tsurugiba" variety did not exhibit symptoms even when infected and considered it resistant., Goto and Fukatsu (1956) identified tolerant varieties as those which do not easily express "white tip" symptoms even if they supported a certain number of nematodes in their panicles. Atkins (1974) considered resistance to "white tip" by commercial varieties to vary greatly. He considered long grain varieties, as generally resistant, while medium and short grain were considered susceptible. He recommended growing of resistant varieties as the simple solution to "white tip" disease. Resistance in "Asa-Hi" variety was thought to be hereditary and

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innate (Goto and Fukatsu 1956; Nishizawa 1953).

Numerous workers in several countries have tested a large number of chemicals ranging from fungicides, insecticides and nematicides. Fortuner and Orton Williams (1975), reviewing the work on chemical control concluded that the experiments with various chemicals undertaken up to the 1960s did not produce any true solution to this disease problem but that, the discovery of systemic crop protection chemicals produced interesting results. In the line of systemic nematicides Locascio <u>et al</u>. (1967) found thionazin, carbofuran, dazomet and diazinon to be effective in controlling <u>A. besseyi</u> and that dazomet at 10 - 20 kg/ha achieved complete destruction of the nematodes and produced excellent plant growth. Several other chemicals including phosphamidon, thiabendazole, fensulfothion, diazinon, disulfoton and phorate were found to give good results (Vuong and Rodriguez 1970).

According to Kretlow et al. (1961), control of seed-borne diseases begins with the seed. Todd and Atkins (1959) considered that, since A. besseyi is seed-borne and infested seed serves as the inoculum, "white tip" disease could principal source of theoretically be controlled by eliminating the nematode in the seed. These hypotheses seem to have encouraged many people to try and find effective control of A. besseyi in rice seeds, hence the majority of workers concentrated on controlling A. besseyi in seed. Whether by design or as a result of the concerted effort in studying this method, hot water treatment of rice seed to control "white tip" disease was considered to be the best. However, Fortuner and Orton Williams (1975) conceded that perfecting this method had not been an

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easy task.

Several different treatment regimes with or without presoaking seed came out of studies by many workers (Cralley 1949, 1952; Yoshii and Yamamoto 1950d, 1951; Fukano and Yokoyama 1955; Kononova and Vinnichuk 1959; Todd and Atkins 1959; and Nandakumar et al. 1975).

Cralley (1952) treated dry seed at  $52 - 53^{\circ}$  C for 15 minutes and observed a reduction in infestation from 75 % to less than 1 %. Yoshii and Yamamoto (1950a) reported successful control of the nematode by treating dry seed at  $56 - 57^{\circ}$  C for 10 - 15 minutes. At the same treatment of  $57^{\circ}$  C, Nandakumar <u>et al</u>. (1975) reported successful control of <u>A. besseyi</u> but viability of seed was affected. Yoshii and Yamamoto (1950a) observed injury of seed only at temperatures as high as  $60^{\circ}$  C for more than 20 minutes.

Todd and Atkins (1959) succeeded in controlling <u>A. besseyi</u> by treating dry seeds at 55 - 61° C for 10 - 15 minutes and at 54° C for 15 minutes. Kononova and Vinnichuk (1959) treated seeds at 55° C for 10 minutes, followed by cooling at 18 - 20° C for 10 minutes and a second treatment at 50 - 52° C for 15 minutes, and a final cooling. This treatment controlled <u>A. besseyi</u> up to 98.8 % without damaging the seed and its viability.

Various periods of presoaking seed before treatment were also tried. Cralley (1952) suggested a cold presoak for 8 - 12hours followed by pre-heating for 15 seconds at 55° C, treatment at  $50 - 53^{\circ}$  C for 15 minutes and a final cooling in water for 15 minutes before drying. Nandakumar <u>et al.</u> (1975) succeeded in controlling <u>A. besseyi</u> without affecting viability of seed by presoaking at 28° C for 12 - 15 hours followed by treatment at 51° - 53°C for 15 minutes.

The latter workers confirmed a report by Cralley (1949) that presoaking was essential to activate the nematodes and predispose them to thermal treatment. Yoshii and Yamamoto (1950d) found that soaking in cold water for 16 - 20 hours at  $20^{\circ}$  C followed by treatment at  $50 - 52^{\circ}$  C for 10 - 15 minutes was effective in controlling <u>A. besseyi</u> but delayed seed germination by one day and that the seeds could be treated 3 months before sowing. Todd and Atkins (1959) reported a successful control of <u>A. besseyi</u> without affecting viability of seed by presoaking in cold water for 24 hours followed by treatment at  $51 - 53^{\circ}$  C for 15 minutes.

#### 2.2 The host plant : Rice

#### 2.2.1 General description

Rice is an important food crop which is produced in all continents. Its historical record as an important food dates back to much before written history (Adair 1972).

#### 2.2.2 Systematic position

According to Masefield <u>et al</u>. (1971), rice belongs to two genera <u>Zazania</u> and <u>Oryza</u>; where the species <u>Z. aquatica</u> belongs to the former genus while <u>O. glaberrima</u> Stued. and <u>O. sativa</u> L. to the latter. <u>Oryza sativa</u> and <u>O. glaberrima</u> differ slightly morphologically, the latter having shorter tunicate ligules of about 6 mm long, fertile lemma and palea and simple undivided panicle branches, while <u>O. sativa</u> has divided panicle branches and ligules of 15 - 45 mm long (PANS 1976). The genus <u>Oryza</u> has at least 20 species (Grist 1975; Chandler 1979) with <u>O. sativa</u> L. as the most important species in cultivation (Masefield <u>et al.</u> 1971). <u>Oryza sativa</u> species is believed to be polyphyletic having resulted from crossing of greatly diverse wild forms of rice with cultivated species. Both <u>O. sativa</u> and <u>O. glaberrima</u> are diploid with 24 chromosomes (2n = 24).

#### 2.2.3 The origin of rice or of rice culture

The question of the origin of rice and its culture has been a subject of discussion to a greater or lesser extent by many people including Copeland (1924), Efferson (1956), Grist (1975), Masefield <u>et al</u>. (1971), Graigmiles (1975) and Carpenter (1978), etc. The origin of rice seems to be a confused subject between origin of rice and the origin of the actual cultivation of rice.

Grist (1975) considers cultivated rices to have evolved from wild rice and not vice versa. Going by Vavilov's theory of the origin of plant species to be in the centre(s) of greatest diversity, Grist (1975) considered the origin of rice to be south west Himalayas, south-east Asia, India, China, Indo-China and that the origins of the cultivated forms of rice may be in the Himalayas, south-east Asia, India, the Philippines and Africa. According to Copeland (1924), linguistic evidence points to south-east Asia as the origin of rice. Adair (1972) considered the origin of cultivated rice to be southern Asia (Eastern India, Indo-China and southern China) and probably Africa.

0. sativa is considered to have originated from Asia where

it was a staple food for Chinese around 2 800 B.C. (Masefield et al. 1971) while O. glaberrima's origin is thought to be Africa, selected from the wild annual rice O. breviligulata Chev. and Roer or 0. brathii Chev. The other common African rice are the perennial 0. longistaminata Chev. and Roer. These two wild species grow side by side on the banks of streams in Zanzibar. Unfortunately, these wild species have been so little studied in East Africa that some in that region authors have not indicated their existence (Carpenter 1978). The latter author recognised the role played by the theory of plate-tectonics in enabling to explain the tropical origin of rice which species subsequently evolved in drifted apart continents. Different species are now found over each tropical region of the globe including South America and Australia.

As far back as 1000 BC, rice cultivation had already advanced to a level where varietal differences were recognised and recorded, based on growth habits, water requirements and nutritive values and paddy irrigation records date back to about 781 - 771 B.C. (Grist 1975).

Rice cultivation in Africa is thought to date back to much earlier before the landing on the Tanzania or Madagascar Coast by the first navigator from Java or Arabian Gulf (Carpenter 1978).

#### 2.2.4 Rice types

According to Chandler (1979) <u>O. sativa</u> varieties are divided into indica, japonica and bulu types whose origins seem to have been through selection domestication and through selection of the wild rices under pressures of different environments. The bulu type is

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considered to be the intermediate form between japonica and indica (PANS 1976). Contrary to the previous belief upheld by Grist (1975) that the indica and japonica rices are sub-species of <u>O. sativa</u>, they are now believed to be ecogeographic races which through hybridisation may, with time, even lose the existing race distinction. The indica types are widely grown in the tropics while the japonica types are grown in the temperate climates. These tropical and temperate rices are considered by some authors to be two different groups of varieties not directly interchangeable, though hybrids can be made to combine useful qualities of both.

#### 2.2.5 The rice plant

Rice is a typical graminaceous plant with fibrous roots and a grass-type-above ground system consisting of a main tiller which is the first to be formed and subsequently formed sub-tillers. The number of sub-tillers depends on the variety and environment. The stems bear monocotyledonous leaves and apical panicles bearing spikes on which flowers are borne. The rice plant is adapted for growing in standing water by having a hollow stem that allows oxygen to pass downwards to roots.

The root system of the rice plant consists of a primary root (radicle) and two additional roots which develop into short lateral roots and, adventitious roots. Except for the main root (seminal root) which emerges at germination the roots are nodal, arising from the nodal regions of the stem below the ground. The ultimate height of a rice plant is variable ranging from 1.22 to 1.83 m (Grist 1975) or from a few centimeters to 'floating varieties' exceeding 5 m.

The inflorescence is a panicle which bears spikelets that are laterally compressed, oval, oblong or lanceolate, with or without awn and are borne on a short pedicle. Each spikelet has three florets, the lower two are reduced to sterile lemmas. The fertile lemma may or may not be awned. The spikelet at maturity is called paddy. The rice kernel (grain) is enclosed in the husk.

#### 2.2.5.1 Rice kernel or grain (Caryopsis or brown rice)

The rice fruit is a caryopsis in which the single seed is fused with the wall of the ripened ovary (pericarp) forming a seed-like grain. The rice caryopsis varies widely among cultivars in shape and size, length and width. Grain shape is limited by amount of variation in the environment (Grist 1959). The husks (glumes) cover the grain and are formed from two specialised leaves, namely the lemma, which cover the dorsal side, and the palea which covers the ventral side. A space exists between the husk and the caryopsis. The outer tissue of the caryopsis is the pericarp which is impermeable to movement of oxygen, carbon dioxide and water vapour. Beneath the pericarp is the tegmen which is several layers thick and is rich in protein and oil with little starch. The aleurone layer is beneath the tegmen and is rich in protein, oils and vitamins with small amounts of starch. The innermost layer is the starchy endosperm with little protein.

The embryo (germ) is small and located on the ventral side of the caryopsis. It contains the embryonic leaves (plumule) and the embryonic primary root (radicle) which are joined by a short stem, the hypocotyl. The plumule is enclosed by a protective covering (the coleoptile), while the radicle is covered by a mass of soft tissue, (the coleorhiza). The scutellum (cotyledon) lies between the endosperm and the embryonic axis. The embryo is enclosed on the outer side by the aleurone layers.

Rice protein has one of the highest nutritive values (high protein efficient ratio) among cereal proteins (Houston 1972). High protein rice has better nutritional value because it has higher levels of all essential amino acids. Rice proteins have a high digestibility which improves with cooking. Rice was also found to have a high biological value, ranging from 67 - 89 (using white rats).

## 2.2.5.2 Changes in composition and properties during rice grain development

The morphology of the caryopsis changes rapidly during grain development. After fertilization, the caryopsis develops much faster longitudinally than transversally attaining its full length in 4 days from the day of flowering, maximum width and thickness in 14 and 21 days respectively. Optimum grain weight is attained after 28 days. Whereas tropical rices are usually harvested 30 days after flowering, in cooler climates, ripening can take up to 60 days. The consistency of the caryopsis goes through progressive changes from milky, dough, yellow to mature. Differentiation of the coleoptile, coleorhiza and acutellum begins by the third day after pollination. The plumule and the enclosing coleoptile appear after 5 days. Differentiation of the embryo is complete in at least 13 and not more than 20 days after flowering. Capacity for embryo to germinate is attained about 7 days after flowering.

When maturity is attained, optimum time for harvesting for maximum yields is when the field moisture content is between 25 and 32%.

### 2.2.6 Geographical distribution and levels of rice production

#### 2.2.6.1 World-wide production

Rice is produced in all continents, right from the equator to latitudes of 53° N (China) and 35 - 40° S and, upto elevations (in the tropics) of 2 400 m above sea-level. The People's Republic of China is reputed for producing more rice than any other country in the world. The world production of paddy in 1980 was 399.1 x  $10^6$ t and it has gone up to 465.9 x  $10^6$  t in 1985, pointing to its global importance (FAO, 1986). Asia produces the highest amount of paddy, approximating 427.5 x  $10^6$  t annually.

#### 2.2.6.2 Rice production in Africa

In Africa almost all countries produce rice (Table 2.2). However, among them Egypt, Madagascar and Nigeria are the major rice producers. The production, planted area under rice and average yield per hectare of paddy are given in Table 2.2.

In Tanzania, rice is grown under very variable ecological
## Table 2.2

Rice hectarage, yield and production in various countries of Africa 1979 - 1985

	1	L <b>97</b> 9–8:	1		1 <b>9</b> 83			1984			1985	
	Hact	Yield	Prod	Hact	Yield	Prod	Hact	Yield	Prod	Hact	Yield	Prod
COUNTRY	x10 <sup>3</sup>	Kg/ha	x10 <sup>3</sup>	x10 <sup>3</sup>	Kg/ha	x10 <sup>3</sup>	x10 <sup>3</sup>	Kg/ha	x10 <sup>3</sup>	x10 <sup>3</sup>	Kg/ha	x10 <sup>3</sup>
ALGERIA	NA	2743	1	NA	2667	1F	NA	3333	2F	NA	3456	2F
ANGOLA	20	1000	20	20F	1100	22F	20F	1100	22F	20F	1100	22F
BENIN	8	1172	10	7	717	5	6	1204	8	8	1304	10
BURKINA FASO	39	1141	44	23	1188	27	21	2048	43	22	2045	45
BURUNDI	4	2832	12	4F	2571	9	6F	3000	18	6F	3333	20F
CAMEROON	21	2100	45	30	3233	97	25	3200	80	271	3333	90F
CENT AFR REP	14	918	12	14F	881	13F	15F	880	13F	161	875	14F
CHAD	43	1220	53	38	471	18	31	50	2	161	1313	21F
COMOROS	12	1117	14	13F	1181	15F	13F	1169	15F	141	1121	16F
CONGO	4	631	2	4F	500	2F	4F	500	2F	41	500	2F
COTE DIVOIRE	383	1145	438	380	947	360	420	1167	490	470	1213	570*
EGYPT	416	5710	377	423	5773	2442	420	5324	2236	422F	5479	2312*
GABON	_	2305	1	-	2000	1F	-	1875	1F	-	1875	1F
GAMBIA	23	1604	37	16	1236	19	20	1350	27*	20	2200	44*
GHANA	107	837	89	40	1000	40	57	1158	66	87	738	64
GUINEA	434	821	356	550	720	396	556	725	403	561	837	470
GUIN BISSAU	70	771	54	90	944	85	145	724	105	140	786	10
KENYA	8	4654	39	9*	4082	36	9*	3489	31*	9F	3889	35F
LIBERIA	203	1237	251	210	1190	250	210*	1190	250*	210F	1200	252*
MADAGASCAR	1182	1738	205	1188	1807	2147	1170	1821	2131	1201*	1812	2178 <b>*</b>
MALAWI	37	1657	61	43F	1512	65F	44F	1545	68F	45F	1556	70F
MALI	165	1045	172	1 <b>12*</b>	1089	122	130*	962	125	160F	800	128
MAURITANIA	3	3262	10	4	3420	14	5	3712	17	6F	2727	15F
MAURITIUS	_	4696	-	-	6042	-	-	5612	-	_	4500	-
MOROCCO	6	4519	26	2	2341	4	2	2522	5	2F	5000	10F
MOZAMBIQUE	73	909	67	70F	786	55F	70F	786	55F	70F	786	55F
NIGER	21	1527	31	22	2097	45	18	2793	51	20	2800	56
NIGERIA	517	1988	1027	630	2032	1280	670 <b>*</b>	1940	1300*	700*	2043	1430*
RWANDA	1	3189	5	1	4712	6	1F	4519	6F	1F	4429	<b>6</b> F
SENEGAL	74	1300	96	52	2087	109	66	2055	136	78	1881	147
SIERRA LEONE	403	1250	504	400F	1522	609	350F	1314	460	400F	1250	500*
SOMALIA	5	2520	13	1F	2333	3	1F	2308	ЗF	<b>1</b> F	2308	ЗF
SOUTH AFRICA	1	2308	3	1F	2308	ЗF	1F	2308	ЗF	1F	2308	ЗF
SUDAN	4	2000	8	4F	2000	8F	ЗF	1200	ЗF	ЗF	2000	<b>6</b> F
SWAZILAND	2	2599	4	-	3471	2	-	8725	3	-	7500	ЗF
TANZANIA	267	1497	400	314	1303	409	389	1315	511	350	1220	427*
TOGO	18	883	15	13	790	10	11	1691	18	15F	1067	16
UGANDA	12	1314	15	17	1353	23	17	1176	20	25	1320	<b>3</b> 3
ZAIRE	289	872	252	339	800	271	325F	880	286	325F	892	290F
ZAMBIA	5	497	2	7	838	6	9	708	6	9F	778	<b>7</b> F
ZIMBABWE	1	588	-	. 1	1438	1	1F	1600	1F	1F	1200	1F

\* : Unofficial

F : FAO estimates

Source : FAO, Yearbook, 1985

situations, mostly by peasant farmers, in regions such as Mbeya, Tobora, Coast, Shinyanga, Mwanza, along river valleys in the Rufiji Basin, Kilombero Valley, the Great Ruaha and in the low-lying areas around Lake Victoria and Lake Malawi. Small amounts of upland rice are grown on the eastern slopes of Uluguru Mountains, the Usambara Mountains, and some parts of the Southern Highlands. Ebbels and Allen (1979) indirectly showed areas where rice is grown in Tanzania when they gave a list of localities where <u>A. besseyi</u> was identified on rice in 1970/71.

Peasants transplant their rice seedlings into swamps or flooded areas. Rain-fed and upland rice is broadcast and large scale irrigation schemes drill or broadcast their seed.

## 2.2.7 Economic importance of rice

The importance of rice in the world economy has been proven beyond doubt. At the anniversary of rice research at IRRI in 1979, special tribute was given to the first rice research leaders of IRRI "for having fully understood the significance of the rice plant in feeding the world" and for having appreciated that rice was a primary staple food for the world's low-income people who are known to feed primarily or at least partly on rice (Brady 1981). The historical record of rice as an important food dates back to prehistoric times (Adair 1972) and its importance continues to increase with the increase in the world population.

Although the production of rice in Africa and indeed in the African, Caribbean and Pacific (ACP) countries is small on a world-wide basis, rice is very important in a great number of these countries, being a staple food in many of them, including West Africa, Madagascar and Mauritius. In Tanzania, rice moved up from being the third most important food crop after maize and sorghum (Monyo, 1973), to being second to maize (FAO, 1978) where it has become highly competitive with maize as a staple food in urban areas.

## 3. GENERAL MATERIALS AND METHODS

Throughout these investigations, there are several methods and materials, which have been used in different experiments such as sampling for nematodes (live and dead), planting of rice seeds, germination procedures, soil sterilization, measurement of plant growth parameters, use of pesticides etc. In this chapter, these materials and methods have been described in general terms. Materials and methods specific to particular experiments are described in the appropriate sections.

## 3.1 Treatment of experimental plots

#### 3.1.1 Dazomet treatment

Rows 22.5 cm deep and 20 cm apart were made and the chemical was evenly applied to the rows and covered by soil. The plot area was worked by hand using hoes and rakes to mix the chemical and soil. The plot was levelled and watered with a light spray to seal the soil and to prevent premature escape of the active gas. After a week of allowing the chemical to act on pests and pathogens, the soil was worked again followed by watering to seal the gas. The working facilitated release of the toxic gas and the watering assisted with reactivating the remaining chemical to release the active gas. The working and watering of soil was repeated three times, at five day intervals. At the end of this period, it was considered that the toxic gases had been released through the working of soil and the watering. A germination test was conducted to determine if the soil was completely free of the phytotoxic gases. Soil from treated and untreated plots was taken and potted. The pots were sown with good quality lettuce seeds. Germination from the treated and untreated soil was equally good indicating that the soil was safe for planting.

## 3.1.2 Carbofuran treatment

Carbofuran was applied to planting holes and along the rows. In the hole application, the chemical was applied as evenly as possible over the seed. Row application involved spreading the chemical evenly along the rows. In both methods the chemical was immediately covered with soil.

## 3.2 Treatment of soil for pot experiment

## 3.2.1 Dazomet treatment

Soil was treated in 20 kg heaps by adding 4.96 g of chemical followed by mixing the soil thoroughly. After mixing each 20 kg lot, the treated soil was put on one heap. Control soil was subjected to the physical mixing and watering except chemical treatment. The two mounds of soil (treated and control) were spread to even mounds to facilitate moisture penetration during watering. The soils were watered evenly to provide moisture to activate the chemical in the treated soil. After watering, the soils were covered by plastic sheets to prevent moisture and active gases from escaping, then it was left for two weeks, followed by working the soil, watering and leaving it open. The working and watering was repeated each day for one week by which time the smell of gas could not be detected. Soil samples were taken for germination test to determine if the soil was free from phytotoxic gases. This basic method was followed with little variation for some experiments.

## 3.2.2 Carbofuran treatment

The required amount of chemical was calculated using the recommended rate of 17.5 kg of 3%G carbofuran/ha and extrapolating it to the amount of soil in each pot. The general formula of 1 ha furrow slice to be equivalent to  $2.24 \times 10^6$  kg of soil was used to calculate the chemical required in each pot. The chemical was placed on top of the seed and covered by soil. The pots were watered with tap water. Pots were placed onto receptacles to prevent irrigation water from dripping out of the pot.

## 3.2.3 Carbofuran seed treatments

This treatment was done in the laboratory. The amount of seed to be treated was weighed. It was found that the same number of seeds for different varieties gave different weights. Therefore, where equal numbers of seeds per plot were required, the equal number of seed for each variety were weighed separately and their respective amounts calculated according to their weights. One kilogram of rice seed requires 900 g of 5%G carbofuran.

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Carbofuran for equal seed numbers for each variety used was calculated to give the following weight equivalents:

Variety	Wt(g) for equal number of seed	Amount of carbofuran required(g)
Nyati	20.00	0.409
Kigunia	19.73	0.409
Lumoto	19.20	0.392
Sindano	19.94	0.407
Bagamoyo	19.33	0.395

Seeds were presoaked in water for 18 hours at room temperature followed by dipping them in carbofuran solution made by dissolving the calculated amount of chemical. Seeds were left to soak-in for 24 hours at room temperature in enough solution to cover seeds.

#### 3.3 Choice of pot size

Four-litre capacity pots were used except where the experiment was designed for termination during early stages of plant growth; in that case, smaller pots were used, such as three or two-litre sizes.

#### 3.4 Methods for calculating chemicals and fertilizer treatments

Calculations were based on general recommendations from the Soil Science Department of the Faculty of Agriculture, Forestry and Veterinary Science, Morogoro (Personal communication).

(a) 1 ha furrow slice (15 cm deep) was taken as being equivalent to 2.24 x  $10^6$  kg of soil. A furrow slice being the soil that is

normally disturbed during the ploughing and working of soil for cropping purposes.

(b) Recommended rate of carbofuran was 17.5 - 20 kg/ha of 3% active ingredient (FMC Technical Bulletin).

(c) Recommended rate for dazomet treatment was 10 - 20 kg/ha.

(d) To calculate chemicals and fertilizers for soil in pot experiments, one hectare had to be related to the equivalent one hectare furrow slice (2.24 x  $10^6$  kg soil) effectively 2.2 x  $10^6$  kg soil.

#### Example 1

#### 3.4.1 Adjustment for soil moisture:

To obtain percentage soil moisture, soil samples were taken, weighed, and dried to constant weight for obtaining dry weight. The difference between the dry and wet weight was the water content which was expressed in percentage water in the soil.

## 3.5 Assessment of nematodes in seeds

#### 3.5.1 Single seed

Nematode infestation was assessed for individual seeds and each seed represented a replication. Total number of seeds to be observed were determined by calculating the number of treatments, multiplied by the number of individual seeds that could practically be assessed individually. Seeds were dehusked singly by hand to separate the two glumes (lemma and palea) and the caryopsis. The separated glumes and caryopsis were all placed in a watch glass with a little water. The glass was placed in an incubator or on laboratory benches at temperatures between  $20 - 28^{\circ}$  C and the soaking period was 24 hours or 48 hours. After the soaking period, the watch glass was placed under the microscope and nematodes were counted, noting the dead or live nematodes and sometimes both. Nematodes were considered dead when they appeared stiff, motionless and straight. This was not a full-proof criterion, but the most practical under the circumstances.

#### 3.5.2 Multiple seed samples

Sample sizes were determined by either counting seeds to a given number per sample or by weight after thoroughly mixing the bulk sample. The seeds were dehusked by hand or by using a laboratory mortar and pestle. Nematodes were extracted using the modified Whitehead Tray method. After the chosen period of soaking, nematodes were put into dishes for counting. Nematodes per seed or nematodes per gram of seed were assessed.

#### 3.5.3 Flower samples

Individual flowers were obtained and were carefully opened and placed singly in a watch glass with a little water. The samples were left to extract and observed as for single seed samples.

#### 3.6 Land management and crop husbandry

#### 3.6.1 Land preparation

Experimental areas were tractor ploughed and harrowed. Levelling, working the soil to even tilth and demarcation of plots were done by hand using hoes and rakes. Field tapes, long rulers, ropes and wooden pegs were used to demarcate plots using a 90° angle criterion. Subsequently rows were marked within each plot by scratching a line to a depth of about 2-3 cm. A general recommended spacing for rice at the Faculty Farm was 20 cm between rows and 20 cm between plants (personal communication). However, slight modifications of the recommended spacing were employed for some experiments.

#### 3.6.2 Soil fertilization

A general fertilizer recommendation for the Faculty Farm was 90 - 100 kg N/ha and 50 kg P/ha. These fertilizers were available ammonium sulphate with 21% N and compounds of triple as superphosphate (TSP) with 46% P respectively. All the TSP and half the quantity of sulphate of ammonia were put as basal application at Quantities for the plot were calculated and subdivided planting. into grams per row. The fertilizers were applied to the scratched Holes for seed drilling were made at appropriate distances rows. using a sharpened stick to a depth of 1 - 2 cm. The remaining sulphate of ammonia was topdressed by either being further halved. with the one portion being applied at the height of tillering and the rest at booting stage or all at booting stage. Application was a side dressing 3 - 5 cm from the row of plants.

#### 3.6.3 Planting and irrigation

Treatments were allocated to plots following the experimental design and randomisation method. Seeds were placed into holes and covered lightly with soil. Supplementary irrigation was used whenever necessary. Watering was done from tap using rubber pipes and efforts were made to time the duration for watering each plot. During drier periods, irrigation was done twice a day, in the morning and evening.

## 3.6.4 Weeding

Plots were weeded 4 - 5 weeks after planting and plots were kept weed-free thereafter. Weeding was done by hand using small hoes and hand-forks as well as uprooting weeds by hand. Thinning to one plant per hole was done during the weeding. Where thinning was required, it was done before tillering to avoid confusion between tillers and separate plants.

## 3.7 Bird scaring

During seed filling stages up to harvest, birds were scared by workers who were employed from 0530 - 1830 h every day for about 6 - 8 weeks. These prevented birds from eating rice, right from the milk, yellow to dough stages.

#### 3.8 Data collection

#### 3.8.1 Determining sample plants

Plants to be assessed for growth parameters or yield were either randomly or systematically chosen. Alternatively, a central core area was marked and all plants inside the marked area were assessed. For growth, and yield assessments, plants, were tagged and numbered in such a way, that each plant had its complete parameters measured and its correct data attributed to it. Where a number of plants per plot were assessed, plants next to missing holes were avoided. A minimum of five plants and a maximum of ten plants were tagged for assessing growth and yield parameters per plot. The parameters measured included height, tiller number, fresh and dry weight of plants, leaf area, panicle size, number of spikes and spikelets, weight of ripened grain per main panicle, weight of 200 grains, and percent ripened spikelets.

## 3.8.2 Plant height assessment

The height of the main or mother tiller was assessed, in centimeters. The mother tiller was determined by being the first to boot. Height was measured using a field tape measure from the ground level to the tallest point of the tiller with its leaves and panicle lifted to their tips. This parameter was taken, when the plant had reached its full growth capacity.

#### 3.8.3 Tiller assessment

Tillers per plant were counted at harvesting.

## 3.8.4 Fresh and dry weight of plants

Plants were cut from the ground level, placed in individual labelled plastic bags, and weighed. Sample plants were bundled together into paper bags for each treatment and, dried to constant weight in a drying oven set at 80°C. The average dry weight per plant was calculated.

#### 3.8.5 Panicle Size

The mother tiller was used to assess the length of panicle. The length was taken from the node at the bottom of the panicle to the tallest spike.

## 3.8.6 Number of spikes and spikelets per panicle

Total number of spikes and spikelets per panicle from mother tiller were counted. Both filled and unfilled spikelets were recorded separately and their sum gave the total number of spikelets.

### 3.8.7 Percentage ripened grains

This was obtained by dividing the number of filled grains per panicle by the total number of spikelets multiplied by 100.

## 3.8.8 Harvesting and assessment of grain yield

Final yield per plot was obtained by marking plants or a central area and harvesting all panicles from the marked area for each plot. The panicles were sun dried, thrashed and winnowed. The paddy was weighed and grain moisture determined. The grain weight was adjusted to 12 - 15% moisture content.

#### 3.8.9 Weight of 200 grains

Two hundred grains were randomly taken from the total grain yield and weighed. In cases where the total yield was less than 200 grains, as for some pot experiments, and where the number was not far too little, the existing grain number was weighed and proportionally determined for 200 grains.

#### 3.9 Experimental location

The present study was conducted at the University farm of the Faculty of Agriculture, Forestry and Veterinary Science, Morogoro, Tanzania, now Sokoine University of Agriculture. The farm lies in the Morogoro region, at the foot of the Uluguru Mountains, between the latitudes 6.49' 27.14 S, and longitudes 37.36' 56.89at an altitude of 570 m above sea-level and receives an average annual rainfall of about 1000 mm.

## 3.10 Hot water treatment of rice seeds

#### 3.10.1 Treatment of presoaked seeds

Seeds for hot water treatment were placed in bags made of cotton material with a capacity of 800 g and were tied with a thick string around the neck. Enough and equal seed samples were treated at a time to allow free movement of water round the seeds in order to subject all seeds to even temperatures throughout the treatment period. A few weighting stones were placed into each bag to keep the seed samples submerged under treatment water but not to sink to the bottom. Samples were continually stirred during treatment using a stirring rod or a water bath with mechanical shaker.

The bags containing the seeds were labelled, tied and soaked in water for a set time at room temperature. Water was heated in a thermostatically controlled water bath to the required temperature. Seeds were removed from the soaking water for drying. Seeds destined to be presoaked only were removed from soaking water and dried for storage. A separate plastic bucket was filled with water heated to about 2° C above the treatment temperature. Samples were quickly dipped into the bucket to warm them, then transferred into the water bath for treatment. This was done to ensure that seeds placed for treatment did not lower the treatment temperature. Soon after placing them into the water bath, a stop clock was set for the required treatment time. After treatment, seeds were removed from the water bath and exposed to atmospheric temperature. The seeds were dried in the laboratory and samples taken for examination soon after treatment. Dried and labelled seeds were stored safely.

#### 3.10.2 Treatment of dry seeds

Samples were put in bags as described for treatment of presoaked seeds. A plastic bucket with water heated to about 2°C above treatment temperature was set. Seeds were placed into and quickly transferred from the plastic bucket to the water bath where the temperature was already set. The same procedure for treatment was followed as already described for the treatment of presoaked seeds.

## 3.11 Germination of rice seed

#### 3.11.1 Petri dish method

Samples, ranging from 20 to 200 seeds which had been randomly picked were placed in petri dishes. Petri dishes were lined with blotting paper, and moistened using distilled water. Seed samples were evenly placed over the wet blotters. The petri dishes were covered and seeds were kept moist but without free draining water. The samples were placed into incubators or laboratory benches at the required germination temperature. When the seeds started germinating, the samples were examined daily and germinated seeds were counted and removed, giving records of daily germination and date of commencement of germination. Alternatively seeds were left to reach maximum germination before counting and termination of the experiment.

#### 3.11.2 Germination chamber method

Germination chambers with temperature and relative humidity controls were used. Seed samples of up to 100 seeds per sample with replicates were germinated. Big sheets of two ply folded blotters were placed over germination trays. The bottom sheet was wetted and seeds were arranged evenly in rows for easy counting. The top sheet was placed over the seeds to cover them and more water was carefully applied to keep the seeds moist. The trays were placed into the germination chamber with a set temperature. The seeds were ready for counting when the plumule was opening to show the cotyledon and when the radicle was big enough, with the appearance of smaller roots.

Normal germinated seeds were those showing a healthy plumule and an emerging cotyledon, a healthy long radicle with smaller roots and root hairs. Abnormal germination was either roots only or shoot only with or without abnormal small roots. Weak seedlings were those producing weak radicles, plumules and feeble small roots. Dead seeds were found ungerminated, rotten, rotting or partly germinated and dead. Fresh looking ungerminated seeds were seeds that remained hard, without any sign of germinating or rotting.

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# 4. SURVEY OF THE INCIDENCE OF <u>APHELENCHOIDES</u> <u>BESSEYI</u> CHRISTIE 1942 IN RICE CULTIVARS GROWN IN TANZANIA

## 4.1 Introduction and Objectives

A search for the prevalence of <u>A. besseyi</u> in Africa was initiated in the 1960's. The first records of the occurrence of <u>A. besseyi</u> in Africa came from Sierra Leon and Senegal (Anon, 1965; Hooper and Merney, 1966). Peachey <u>et al</u> (1966) reported that <u>A. besseyi</u> did not occur in rice from Malawi, Swaziland, Tanzania, Uganda and Zambia. Annual report (EAAFRO, 1971) from East African Agricultural and Forestry Research Organisation confirmed the above observations of Peachy <u>et al</u> (1966), that neither <u>A. besseyi</u> nor <u>Ditylenchus angustus</u> was found in the samples of rice obtained from Tanzania (EAAFRO, 1971).

Although <u>A. besseyi</u> was known to be widely distributed in Africa, its presence in East Africa was not confirmed until 1970 - 1971 (EAAFRO, 1972; Taylor <u>et al</u>, 1972). By March 1973, the presence of <u>A. besseyi</u> was confirmed in various countries of Africa, including Ghana, Nigeria, Dahomey, Gabon, Madagascar, Cameroon, Zaire, Central African Republic, Chad, Togo, Ivory Coast, Mali, Upper Volta, Comoro Islands and Tanzania.

Taylor <u>et al</u> (1972) gave the distribution of white tip nematode of rice in difference cultivars of rice obtained from different locations of Tanzania (Table 4.1). Ebbels and Allen (1979) compiled a list of localities where <u>A. besseyi</u> was identified by EAAFRO workers for the period 1970 - 1971. EAAFRO workers considered their 1970 - 1971 survey of <u>A. besseyi</u> in Tanzania a preliminary study and had intended to make a follow up assessment of

Origin of sample Village/district	Rice cultivar	Seeds infes- ted	Average number of nematodes per infes- ted seed	Viable nema- todes
		Percentage	e F	ercentage
Mbarali	Salama	8	9.8	64
Mbarali	H4 Morogoro	14	15.3	-
Mkuyuni-Morogoro	Sindano	78	35.2	-
Mkuyuni-Morogoro	Cheza	32	57.1	71
Mkuyuni-Morogoro	Meli Kongo	14	13.9	-
Mkuyuni-Morogoro	Samanini	10	15.2	-
Mkuyuni-Morogoro	Junia	82	24.8	63
Mkuyuni-Morogoro	Kipakapaka	74	39.3	66
Mkuyuni-Morogoro	Ruzukwi	18	65.1	63
Kwalusonge Mazinde-				
Handeni	Kihogo	36	38.2	35
Magamba-Handeni	Kihogo	42	68.4	38
Kwachaga-Handeni	Mwacha Pande	18	23.1	17
Tamota-Handeni	Fire	2	4.0	0
Tamota-Handeni	Tule na Bwana	+2	-	-
Kwediuabu-Handeni	Zira	32	18.9	48
Bogorwa-Handeni	Pishori	24	19.8	-
Mwaya-Ulanga	India	+2	-	-
Stahabu-Pangani	Mwaropa	2	1.0	-
Mwera-Pangani	Chaka Jekundu	+2	-	-
Mazinde-Korogwe	Meli	+2	-	-
Mnazi-Lushoto	Afaa	10	20.5	-
Chato-Biharamulo	Afaa	+2	-	-
Chikuyu-Manyoni	Super	4	7.0	50
Chikuyu-Manyoni	Kihogo	6	20.0	71
Chikuyu-Manyoni	Kamoto	6	9.3	43
Chikuyu-Manyoni	"mixture"	6	35.0	80
Kwimba-Mwanza	Faya	2	40.0	53

Table 4.1Occurrence of Rice White Tip Nematode in Rice SeedsObtained from Different Regions in Tanzania

Source : Taylor et al (1972) FAO Plant Prot. Bull 20:41-42

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the incidence and effect of <u>A. besseyi</u> on the yield of rice grown in Tanzania. Unfortunately, the break-up of the East Africa Community prevented the second survey.

With the above stated observations in mind, it was decided to conduct a survey of <u>A. besseyi</u> in seeds of some rice cultivars grown in Tanzania. Objectives were to investigate the occurrence and intensity of <u>A. besseyi</u> in rice cultivars grown in Tanzania as a basis for further studies of this rice pest.

## 4.2 Materials and methods

A total of 196 samples of rice representing 196 cultivars were obtained from various parts of Tanzania but mainly from Kilombero Agricultural Training and Research Institute (KATRIN) near Ifakara in Morogoro Region and Mbarali Irrigation Scheme at Mbarali in Iringa region respectively.

Each rice sample was mixed thoroughly and sub-sampled for nematode assessment. Initially 300 seed samples were analysed for nematode infestation but later this number was found to be impractical. Seeds were singly dehusked, placed in a watch glass and covered with enough water to soak the seed and the husk. Samples were left on the laboratory bench for 24 hours. After the soaking period, nematodes were counted under the microscope and infested seeds were recorded. Percentage infestation and average number of nematodes per infested seeds were calculated.

Ideally, rice samples should have been obtained from farmers' fields during harvesting in order to ascertain the age of the seed. However, this was not possible because farmers were not willing to sell or give away any part of their crop during

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harvesting.

As such, the rice seed samples for nematode assessment were obtained from stocks of rice seed in farmers' stores. Therefore, there was no way of knowing the age of the seed or the precise storage conditions. However, the rice seed obtained through Mbarali Rice Scheme and Kilombero Agricultural Training and Research Institute (KATRIN) at Ifakara were from the previous cropping season.

## 4.3 Results and discussion

It was observed that 36 out of the 196 rice samples (18.4%) investigated were infested with <u>A. besseyi</u> in the seed. The number of nematodes observed varied from cultivar to cultivar (Table 4.2). The percentage infestation also varied between cultivars, ranging from 1 to 85%. The highest infestations were in cultivars like M'punga mwepesi, Kajibi, Africa and Faya with 85, 52, 24 and 21% respectively. Similarly, the total number of nematodes recovered were variable, ranging from 1 to 1056. The highest number of nematodes per seed were found to be 50 for cultivars Kihogo and Lunyuki, 59 for Kihogo 1/146, 69 for M'punga mwepesi, 70 for Africa and 81 for cultivar Nyuki. The average number of nematodes per seed ranged from 1 to 10.

However, it must be pointed out that the non-occurrence of <u>A. besseyi</u> in particular samples should not be interpreted in terms of resistance. For example, the same cultivars collected later on during the course of this study from other localities were found to be infested with <u>A. besseyi</u>. Therefore, the presence or absence of <u>A. besseyi</u> in rice samples may depend on the area from which the seed was collected and the degree of the incidence of

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Tanzania
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4.2
Table

Origin of Sample	Rice Cultivar	Total no of seeds observed	% seed infesta- tion	Total no of nematodes recovered	Highest no of nemato- des/seed	Mean no of nematodes/ infested seed
Mbeya	Afaa Kilombero	300	1.00	41	15	13.67
	Kihogo 1/146	300	1.00	86	59	28.67
Morogoro	Lunyuki	300	2.67	198	50	24.75
	Nyuki	100	18.00	377	81	20.94
" (Ifakara)	Faya India	100	1.00	N	N	2.00
	Basmati 217	100	12.00	47	6	3.92
	IR 20	100	1.00	1	1	1.00
	IR 579-48-1-2	100	2.00	ω	9	4.00
	IR 589-66-2-1	100	1.00	Ŋ	ß	5.00
	Eal no. 1365 SML	100	1.00	4	4	4.00
	Kajungu Long Straw	100	1.00	28	28	28.00
:	Kikarati	100	1.00	N	N	2.00
-	Saturn	100	1.00	1	1	1.00
-	IR 773 AG 36-2-1	100	3.00	57	42	19.00
	Eal no. 1513 Shimolata	100	2.00	N	1	1.00
	IR 154-61-1-1 X Sicostin					
	CP 231, 1062	100	2.00	8	7	4.00
=	IR 22	100	4.00	84	40	21.00
=	Eal no. 436 SML 242	100	1.00	N	N	S
	IR 532-527	100	1.00	80	Ø	ω
	1316 I R 665-1-1-6	100	1.00	ო	ო	က

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Table 4.2 (c	sontinued)		;			
Origin of Sample	Rice Cultivar	Total no of seeds observed	% seed infesta- tion	Total no of nematodes recovered	Highest no of nemato- des/seed	Mean no of nematodes/ infested
						seed
=	IR 579-163-2	100	1.00	4	4	4
=	Eal no. 1391 Accession	100	3.00	37	34	12.30
=	Mpunga Mwepesi	100	85.00	1056	69	12.40
=	Eal no. 1485 SML APWA	100	1.00	7	4	2.30
=	Faya Rangi 5	100	2.00	N	1	1.00
=	Dunduli Yamlimani	100	21.00	235	30	11.19
3	IR 520-1-26-3-3 Philipines	100	1.00	10	10	10.00
=	Nene Faya Rangi	100	3.00	14	œ	4.67
=	Chikarati	100	2.00	14	12	2
=	Manda Malawi	100	1.00	22	22	22
=	Kajibi	100	50.00	427	30	8.54
=	Kajibi	100	52.00	55	70	1.06
=	Afaa Mwanza 1/33	100	1.00	1	ч	ч
=	Afaa Mwanza 1/33	100	1.00	N	N	N
=	Sindano Ex Mwea	100	1.00	4	ч	4
=	Sindano Ex Mwea	100	1.00	1	4	1
=	Kihogo Red Line 7	100	12.00	149	50	12.40
=	Kihogo Red Line 7	100	6.00	69	69	11.50
-	Africa	100	21.00	312	48	14.86
81	Africa	100	24.00	605	70	25.20
=	IR 22	100	12.00	149	50	12.40
2	IR 22	100	6.00	69	30	11.50

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<u>A. besseyi</u> in such a geographical location. To establish cultivars that are resistant to <u>A. besseyi</u> in Tanzania, samples of the same cultivars would have to be collected from all areas where they are grown and examined for several years in succession. This would have to be augmented by screening for resistance experiments.

The results of this survey confirmed the findings of the EAAFRO workers (Taylor <u>et al</u>, 1972) regarding the presence of <u>A. besseyi</u> in seeds of many rice cultivars grown in Tanzania, the high percentage seed infestation in some of the cultivars and the very high nematode recovery for some cultivars.

Work in Japan (Fukano, 1962) suggested that for susceptible cultivars an infestation level of 30 or more live nematodes per 100 seeds is enough to inflict appreciable damage to the crop. Thorne, (1961) stated that, since there were 5 to 6 nematodes per grain, they must be capable of causing apparent damage to the young plant. Although the exact numbers of live and dead nematodes were not precisely disaggregated in the results, the majority of the nematodes observed were found to be alive.

It was concluded from the results of this survey and those of the previous survey (Taylor <u>et al</u>, 1972) that <u>A. besseyi</u> was prevalent in many rice cultivars grown in Tanzania. It was also concluded that the level of infestation in some cultivars was sufficiently high to warrant further investigations on the possible damage caused by this nematode and the possible ways of controlling it. Control of this nematode in the seed was considered the most practical approach, although other possible methods were also to be investigated. 5. HOT WATER TREATMENT OF RICE SEEDS TO CONTROL SEED-BORNE

#### A. BESSEYI

The present study having confirmed survey results obtained by EAAFRO workers (EAAFRO 1971) on the prevalence and intensity of <u>A. besseyi</u> infestation in seeds of rice cultivars grown in Tanzania, it was necessary to investigate methods of controlling the nematodes in the seeds and in the field. Hot water treatment of rice seed to control seed-borne <u>A. besseyi</u> was considered by the present author to be more socio-economically acceptable in Tanzania than chemical control.

Various temperatures and treatment times are suggested in the literature and have been dealt with in the literature review of this study. There were also variable cooling and heating regimes suggested in literature, including other variations in the treatment conditions. Dry seed treatement and treatment following presoaking came out consistently well in the suggested hot water treatment (Cralley 1949, 1952; Yoshii and Yamamoto 1950d; Todd and Atkins 1959; Muniappan and Sheshadri 1964; review by Fortuner and Orton Williams 1975; Nandakumar et al. 1975). However, it was concluded from the examination of literature that there were no universally recommended treatment regimes except suggestions by the above This was probably because of the variations in the authors. treatment conditions such as cooling time and temperature, soaking temperature, treatment temperature and time periods and the combinations and variations of these and others in the hot water treatment investigations as presented in the literature.

It was also apparent that dry seed treatment was done at considerably higher temperatures than hot water treatment following presoaking. Except for the recent publications by Garrity and Ventura (1986), and Ventura and Garrity (1986), very little work and/or less emphasis has been placed on the assessment of the effects of hot water treatment on the germination and emergence of different cultivars and genetic lines under different environments. As shown in the sections on introduction and objectives, there are large numbers of rice cultivars being grown in Tanzania.

With the above points in mind, it was considered necessary to investigate hot water seed treatment as a means of controlling seed-borne <u>A. besseyi</u> and its effects on the germination of treated rice seeds of different rice cultivars grown in Tanzania. To achieve these objectives, several experiments were carried out. The studies involved dry seed treatment and treatment of seed following presoaking.

Preliminary observational studies on the effect of dry rice seed treatment with hot water at 60°C for 10 minutes, on the seed-borne <u>A</u>. <u>besseyi</u> in rice cultivar M'punga mwepesi showed that dry seed treatment at the tested treatment regime controlled <u>A</u>. <u>besseyi</u> by 81.2%. It was also shown that nematode control assessment of treated seed, conducted 5 days after treatment gave better results than assessment done on the same day of treatment, when nematodes may appear all dead from treatment shock. These observations were useful in conducting subsequent experiments.

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5.1 Effects of hot water treatment of rice seeds without presoaking (dry seed treatment) at different temperatures and time periods on the control of seed-borne <u>A. besseyi</u> in eight cultivars of rice.

## 5.1.1 Introduction and Objectives:

The investigation was carried out at the initial stages of the study to provide baseline information. This study was an extension of the preliminary observations with cultivar M'punga mwepesi. The present study, examined a wider range of temperatures and time periods and more rice cultivars. It was decided to investigate the possibility of controlling <u>A. besseyi</u> using hot water treatment without prior soaking (dry seed treatment) at lower temperatures than those observed with cultivar M'punga mwepesi in order to find the optimal treatment regime and minimal adverse effects of hot water treatment on the germination of seeds.

#### 5.1.2 Materials and Methods

The treatment temperatures and time periods used were; 54, 55 and 56°C for 5, 10 and 15 minutes. Rice cultivars were chosen on the basis of the availability of seed and high nematode incidence in the seeds. The following cultivars and treatment combinations were used:

	54°C	55°C	56°C	
	5 10 15	5 10 15	5 10 15	
Cultivar	(mins)	(mins)	(mins)	Control
Bagamoyo	"			
Sindano	u			
Tulenabwana	U		01	
Meli	U		"	
Bluu	н		11	
Kalafulu	u		11	"
Faya Pemba		14	11	
Kihogo Morogoro	u	u	11	u

Treatment Regimes

The experiment was a complete randomised design with three Hot water treatment was done using a temperature replications. controlled water bath. After treatment, samples of 20 seeds were randomly picked and nematode assessment was done using single seed assessment method. Details of both methods of hot water treatment and nematode assessment are given in the General Materials and Methods Section of the thesis. Nematode assessment was done under laboratory temperature conditions and samples were soaked for 24 hours before nematodes were counted. Live nematodes were recorded and mobility was used as a criterion for judging live nematodes. Active and mobile nematodes were considered live while those lying straight, stiff and motionless were considered dead. Analysis of variance using transformed data (square root transformation) was conducted followed by an F-test for significance and a least significant different test. Comparisons of chosen means was done using F-tests. An analysis of variance for the factorial aspects of

the experiment was carried out and linear and quadratic regressions for time and temperature were fitted in.

#### 5.1.3 Results and Discussion

The results of the analysis of variance and the F-test showed that the treatment factors of time and temperature were highly significant (P = 0.01). The results of the LSD test on the square root of means showed that the control was significantly different from the rest of the treatments. Mean results from the same treatment time irrespective of different treatment temperatures were not significantly different.

The time factor was significant and so was the linear regression of square root of number of live nematodes on time (Figure 5.1). Temperature was not significant. The contrast results showed that the control was significantly different from all treatments combined and that there was no significant difference between each of the temperatures. Similarly, the results of the LSD test showed that the control was significantly different from each of the other treatments (Table 5.1).

While there was no significant difference between each of the temperatures, there were significant differences between the times: 5 and 10 minutes, 5 and 15 minutes and 10 and 15 minutes (P = 0.01) (Table 5.2). Table 5.3 shows the transformed means for time and their reconverted values.

From these results it was concluded that temperature independently did not matter very much but the time of exposure was the significant factor (P = 0.01). The numbers of live nematodes

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Fig 5.1: Rate of decrease in numbers of live nematodes (square root transformed values)

# Table 5.1 Effect of hot water treatment of dry seeds of rice at different temperatures and time periods on the control of seed-borne <u>A. besseyi</u> in eight cultivars or rice.

Control		2.78
55°C/5 mins		1.73
54°C/5 mins		1.68
56°C/5 mins		1.51
54°C/10 mins		1.37
56°C/10 mins		1.31
55°C/10 mins		1.30
54°C/15 mins		1.03
55°C/15 mins		1.03
56°C/15 mins		1.03
SED	=	0.1334
LSD (1%)	=	0.3441
CV (%)	=	31.3

F-value	
172.9	**
.0088	NS
.818	NS
.657	NS
13.35	**
50.19	**
11.77	**
209.9	**
	F-value 172.9 .0088 .818 .657 13.35 50.19 11.77 209.9

F 1.158 (1%) = 6.63

Key: \*\* = significant at P = 0.01

Table 5.3 Transformed mean values for time and their corresponding reconverted values (square root of x + 1)

Time (minutes)	5	10	15
Transformed values	1.638	1.327	1.035
Reconverted values	1.68	0.76	0.07

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decreased significantly with increase in treatment time (Figure 5.1). Therefore, nematode control in dry seed treatment, depends very much on the length of time of exposure to the thermal treatment where 5 minutes was comparatively less effective in controlling <u>A.</u> <u>besseyi</u> than 10 and the latter less effective than 15 minutes.

However, 15 minutes, especially, was able to kill a significant proportion of <u>A. besseyi</u> compared to 5 minutes. It should be noted that there still remained some live nematodes, after treatment for as long as 15 minutes for all temperatures.

There was no significant difference between the cultivars in the way the nematodes reacted to the thermal treatment. Therefore, this investigation demonstrated that nematode control by hot water treatment of dry seeds was independent of cultivar differences. Of the studied temperatures, any one of them could be adopted for hot water treatment as long as the time for exposure was effectively extended beyond 5 and even 10 minutes to 15 minutes. These results agree with Nandakumar <u>et</u> <u>al</u>. (1975) who stated that in seed treatment, it is essential to get the treatment temperature to the nematodes, that is, below the seed coat. Enough time of exposure is necessary to allow the treatment water to penetrate the different husk layers and for the heat to be duly transfered to the nematode. Treatment of dry seed may require comparatively more time for these processes to take place before the heat is transfered to the target nematode below the seed coat.

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5.2 Effect of hot water treatment of dry seeds on the germination of six cultivars of rice.

## 5.2.1 Introduction and Objectives:

This experiment was conducted during March - April to assess appropriate germination conditions for later experiments. It was also decided to study the effect of hot water treatment of dry seeds on the rate and germination of treated seeds. There were only a few temperature controlled germination chambers in the Faculty laboratories. It was therefore necessary to test the efficacy of the two types of environments available and the environment of the laboratory benches on the germination of hot water treated seed, germinated within closed petri-dishes.

## 5.2.2 Materials and Methods:

Four cultivars of rice namely, Africa, Kajibi, Mpunga mwepesi, Dunduli yamlimani were chosen for this study because of the availability of seed. Twenty seeds per sample were tested and two petri-dishes (replications) per environment were used. Hot water treatment of dry seed was done at  $62^{\circ}$ C for 15 minutes in a temperature controlled water bath. Details of the treatment method are given in the General Materials and Methods Section. Seeds were germinated within one week of being hot water treated. The incubator and the drying oven were both set at 30°C. The laboratory temperature was recorded daily at the same time during the day for the whole period of germination. The average laboratory temperature reading ranged between  $21^{\circ}$ C -  $24^{\circ}$ C during the course of germination.

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Seeds were placed over the blotting paper within petri-dishes and moistened with distilled water and the petri-dishes were covered with appropriate lids. The samples were placed into their respective environments and kept moist throughout germination test.

The experiment was a randomised block design with two replications. Germination observations were made to enable the recording of the first day of germination (onset of germination) and thereafter, germination was recorded daily until the termination of the experiment.

#### 5.2.3 Results and Discussion:

Germination started on the fifth day in all rice cultivars The onset of germination coincided with the for all treatments. peak germination. Most of the germination was complete by day 9. Table 5.4 shows the total germination and the percentage mean germination for each cultivar and germination environment. Cultivar factor was only significant in rice cultivar Dunduli yamlimani where it showed a significantly lower germination compared with the rest of the cultivars (Table 5.5). Hot water treatment of dry seeds at 62°C 15 minutes did not significantly affect germination of the four The lower germination obtained for cultivar tested cultivars. Dunduli yamlimani compared with the rest of the cultivars probably reflected the poor condition of this particular seed sample. The three environments were not statistically different from each other. It must be noted that this experiment was conducted during the warm months of March and April when there is not much difference between day and night temperatures. Therefore, at that time, it is possible

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		Inc	subator	Mean Percentage Germination	Drying	oven	Mean Percentag Germinatio	e Lab	• Bench	Percentage Germination
Cultivar	Treatment	1	N		ч	N		1	N	
Africa	Treated	17	17	85.0	15	17	80.0	17	None	85.0
	Control	19	15	85.0	20	19	97 . 5	17	20	92.5
Dunduli	Treated	14	13	67.5	16	17	82.5	17	19	0.08
Yamlimani	Control	14	13	67.5	17	10	67.5	13	15	70.0
Kajibi	Treated	18	20	95.0	20	16	0-06	20	17	92.5
	Control	17	20	92.5	16	19	87.5	19	20	97.5
Mpunga	Treated	20	17	92.5	19	14	82.5	19	20	97.5
Mwepsi	Control	20	17	92.5	18	19	92.5	19	19	95.0

Total and mean percentage germination after 14 Days (Mean of 2 replications) Table 5.4

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Table 5.5 Cultivar means and their levels of significance

Africa	Dunduli yamlimani	Kajibi	Mpunga mwepesi
17.46	14.83	18.50	18.42
LSD(5%) =	1.691		
SE =	2.010		
CV (%) =	11.6		

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to successfully germinate the seeds in the open laboratory.

The non significant results for the treatment were confirmed by the rate of germination results where all treatments for all cultivars started germination on the 5th day of planting. The experiment, therefore, demonstrated that hot water treatment without presoaking at the tested regimes, did not affect the rate of germination of seeds of the studied rice cultivars.

It was concluded that hot water treatment without presoaking at 62°C for 15 minutes did not delay onset of germination, rate of germination nor significantly reduced the ultimate germination. The three environments were equally efficient for that period of the year. The results provided data for onset and rate of germination of hot water treated seeds without presoaking.

5.3 A study of the effects of hot water treatment without presoaking (dry seed treatment) on the control of the seed-borne <u>A. besseyi</u> and on the germination of seeds of five rice cultivars 5.3.1 Introduction and Objectives:

This experiment was conducted in the months of June to July as a follow up of previous dry seed treatment studies. In this study, it was decided to include soaking in water as a treatment. Previous dry seed treatment investigations had shown that <u>A. besseyi</u> could survive temperatures as high as 62°C for 15 minutes. It was therefore decided to examine this aspect further and include a higher temperature. Possible damage to the seed was considered and a germination test was included in the investigation. It was also decided to follow up previous observations on the assessment of decided to follow up previous observations on the assessment of nematodes on the day of treatment and one week after the treatment. Soaking period for maximum revival of <u>A. besseyi</u> was also included in the investigations.

This study was conducted to assess the effects of dry seed treatment (with hot water) on the control of seed-borne <u>A. besseyi</u> and to establish the best time (days) to assess control of <u>A.</u> <u>besseyi</u> in treated seeds. It was also intended to examine the period of soaking for maximum revival from quiescence of <u>A. besseyi</u>.

The latter two objectives were also required to provide baseline information needed for streamlining methodologies for subsequent experiments with this nematode. The effect of hot water treatment on the germination of treated seeds was also studied.

Five cultivars were chosen for this study based on the survey for <u>A. besseyi</u> results, which showed seeds of these cultivars to harbour this nematode in considerably high levels.

#### 5.3.2 Material and Methods:

Rice cultivars Dunduli yamlimani, Mpunga mwepesi, Kajibi, Kihogo Red Line-7 and Africa were used. Treatment temperature chosen were 60°C and 62°C for 10 and 15 minutes respectively. Sampling for treatments was done as shown on the diagram below:



P1 - P4 were each hot water treated at one of the selected temperature regimes. P5 and P6 were soaked in water at room temperature (22°C) for 10 and 15 minutes respectively. The following treatments, P1 - P6 and sample III were each further subdivided into 1, 2 and 3 subsamples respectively. Subsequently, subsamples 1 were dehusked for assessment of nematode kill while subsamples 2 and 3 were left to dry on laboratory benches and stored for one week after which subsamples 2 were further sampled for nematode assessment. Individual seeds were assessed for nematodes. Nematodes lying straight, motionless and stiff were considered dead. Nematode counts were taken 24 hours after soaking the seed and repeated again for samples that had been soaked for a total of 48 hours.

A seed germination test was conducted within one week of treatment using seeds from subsamples 3. The experiment was conducted in randomised block design with two replications. Hundred seeds per treatment were germinated in petri-dishes under laboratory conditions. Two sets of petri dishes per treatment were lined with blotting paper and seed samples were placed onto the blotting paper.

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The blotting paper was kept moistened with distilled water. Germination counts were made and analysis of variance was conducted followed by contrasts to compare chosen means.

## 5.3.3 Results and Discussion

Cultivar, treatment and cultivar x treatment were all significant at P = 0.01. There were significant differences between hot water treatment at 60°C and 62°C for the 10 minutes exposure time and the same comparisons were even more significantly different for the 15 minutes exposure time (P = 0.01), suggesting that the increase in time from 10 to 15 minutes effected more serious damage to the seed. Soaking treatment did not significantly affect germination compared to the control but both control and the soaking treatment were significantly different from hot water treatment P = 0.01 (Table 5.6).

There were inherent cultivar differences in germination as shown by cultivars Kajibi and Africa which had lower germination in the controls compared with the other cultivars (Table 5.7a). The effects of treatments were significantly related to the cultivar factor (Table 5.7c) suggesting that cultivars were differently affected by the hot water treatment.

Generally, hot water treated samples gave significantly lower percentage germination than the control and soaked seeds, showing that hot water treatment at 60°C and 62°C for 10 and 15 minutes did significantly lower germination of the tested cultivars (Table 5.7b). Among the studied cultivars, there were two with poor germination even without hot water treatment and two with what

Table 5.6 Effect of hot water treatment at 60°C and 62°C for 10 and 15 minutes on the germination of seeds of five rice cultivars

Contrasts	F-value	Significance (F(1.3.4))
Control vs all other treatments	19.905	**
Control vs soaked	0.111	NS
Control vs hot water treatment	39.476	**
Seed soaked vs all hot water treatment	58.387	**
Hot water treatment at 60°C vs 62°C	2.281	NS
Seed soaked vs hot water treatment at 62°C	63.223	**
10 min vs 15 min for hot water treatment at 60	0°C 3.732	NS
10 min vs 15 min for hot water treatment at 62	2°C 1.524	NS
Hot water treatment at 60°C vs 62°C for 10 min	n 7.117	*
Hot water treatment at 60°C vs 62°C for 15 min	n 78.181	**

- Table 5.7 Percentage germination means (2 replications) for seeds of five rice cultivars, hot water treated (without presoaking) at 60°C and 62°C or 10 and 15 minutes.
- (a) Cultivars Dunduli M'punga Kajibi Kihogo Africa Yamlimani Mwepesi Red Line 7 Means 84.65 76.76 24.98 84.39 37.60 LSD(5%) = 3.98SE = 1.3796
- (b) Treatment Control S22T10 S22T15 H60T10 H60T15 H62T10 H62T15 Means 68.42 66.09 69.42 61.36 56.90 56.20 53.35

LSD (5%) = 4.71 SE = 1.6327

## Treatment

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(c) Cultivar S22T10 Control S22T15 H60T10 H60T15 H62T10 H62T15 Dunduli 90.85 87.45 89.50 91.80 84.15 74.80 74.00 Mpunga 84.30 82.10 84.00 69.90 70.00 78.15 68.90 23.10 40.30 14.55 12.95 17.30 34.40 Kajibi 32.25 Kihogo RL7 90.95 88.15 91.65 88.80 80.70 78.05 72.45 36.70 32.70 Africa 43.75 49.65 41.65 41.75 17.00

LSD (5%) = 10.53 SE = 3.65 CV (%) for the whole experiment = 84

# <u>Key:</u>

S22T10 = soaking at 22°C for 10 min S22T15 = soaking at 22°C for 15 min H60T10 = Hot water treatment at 60°C for 10 min H60T15 = Hot water treatment at 60°C for 15 min H62T10 = Hot water treatment at 62°C for 10 min H62T15 = Hot water treatment at 62°C for 15 min Garrity and Ventura (1986) considered as good germination (more than 85%). It must be noted that germination was conducted under laboratory conditions where temperatures for that time of the year (June and July) were low and could get much lower than 20°C at night. Temperatures during the period of this germination experiment can be considered suboptimal and could have exacerbated the adverse effect of hot water treatment on the germination, resulting in some treated and sensifised seeds losing their viability.

Although all the cultivars were significantly lower in their percentage germination by hot water treatment (Table 5.7), cultivars; Kajibi, Africa and Mpunga mwepesi with the lowest germination percentages for the control, were more significantly affected by hot water treatment than the other cultivars which had better germination in the control (more than 85%). The latter point agrees with Garrity and Ventura (1986) who stated that seeds with initial low germination before treatment (below 85%) tend to have their germination more affected by hot water treatment than those with high germination. The latter point might mean that seeds with low germination before treatment are of poorer quality.

The cultivar differences observed, seem to have been due to the conditions of the seed samples rather than from genetic differences. The effect of the seed condition was probably compounded by hot water treatment and germination environment under suboptimal temperatures.

It can be concluded that hot water treatment of rice seed of the studied cultivars at 60°C for 10 and 15 minutes significantly lowered germination of all cultivars, especially at 62°C for 10 and

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15 minutes respectively over that of the control or soaked seeds. The cold conditions during germination is believed to have exacerbated the effects of hot water treatment on germination. Cultivars with low germination before treatment were more affected by hot water treatment compared with those with inherent high germination (at P = 0.01 compared with P = 0.1). The latter aspect of the result agrees with findings of Garrity and Ventura (1986).

The age of the seed, the previous storage conditions and other aspects of seed quality, all of which affect viability of the seed, are important factors in the determination of the level of tolerance by the seed to the adverse effects of hot water treatment. Some of these factors plus the effect of cold during germination are believed to have had an effect on the germination especially of more sensitive (treated) seeds.

Analysis of variance and test of significance conducted with the results of an assessment on the same day of treatment gave significant differences for the factors; cultivar, treatment, revival period and the interaction between cultivar and treatment. The assessment conducted one week after treatment, as well as confirming the significant results obtained for the latter analysis, also gave significant results for the cultivar x period and treatment x revival period interactions. The analysis of variance conducted with combined results (same day and one week after) to examine the comparison of the two assessment days confirmed the significant differences between the factors; cultivar, treatment, revival period and the interactions between cultivar x treatment and treatment x revival period. The comparison of the mean percentage dead nematodes for the control compared against the mean percentage dead nematodes for the treated samples as a whole for both assessment days were highly significant (P = 0.01). The control showed significantly less dead nematodes compared to the hot water treatments (Table 5.8).

The contrast results demonstrated that the two temperatures and the two treatment times were not significantly different (Table 5.9) and that they all significantly controlled <u>A. besseyi</u> leaving 0.05 and 0.06% live nematodes for each assessment day respectively. Therefore, at those treatment regimes (60°C and 62°C for 10 and 15 minutes respectively), control of <u>A. besseyi</u> was almost complete, leaving very few live nematodes whose survival could not make much difference between the treatment regimes. The control and water soaked treatment did not significantly differ and similarly the two hot water treatments for each time period were not significantly different.

The effect of different treatments (excluding the control) on the percentage dead <u>A. besseyi</u> for each of the two assessment days is presented in Table 5.10. Water soaked treatments had significantly lower percentage dead nematodes compared with the two hot water treatment temperatures, which themselves did not significantly differ. However, there were slight decreases in the percentage dead nematodes for each of the hot water treatment in the assessment done one week after treatment compared to that of the same day of treatment. The latter results suggest that some presumed dead nematodes were actually alive. These results confirm those obtained in the dry seed experiment with the cultivar Mpunga

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Table 5.8 Contrasts of the means for the percentage dead nematodes for the control against treated samples (hot water treatment), as a whole

<sup>1</sup> On the day of treatment					
Overall hot water treatment mean	versus	Control mean	F-value		
99.95		60.00	128.3 **		
<sup>2</sup> One week	after tratme	nt			
Overall hot weater treatment mean	versus	Control mean	F-value		
99.40		51.70	828.1 **		
<u>Key</u> :					
** = significant at P = -0.01					
$^{1}MS = 21.98$ with 30df					
<sup>2</sup> MS = 100.5 with 30df					

Table 5.9 Contrasts of the means for the percentage dead nematode for the two temperatures and two treatment periods for the assessment done one week after treatment.

Hot Water Treatment					
Temp (°C)	60°C	6	0°C	F - val	ue
Time (min)	10 15	10	15	30 df	
Means	99.6 98.3	99.7	100	21.98	NS
Contrast of 62°C vers	sus 60°c			0.369	NS
Contrast of 10 and 15	5 minutes at	62°C		0.384	NS
Contrast of 10 and 15	5 minutes at	60°C		0.020	NS
Contrast of temperatu	ires versus t	ime		0.291	NS
Contrast of 62°c vers	sus 60°C for	10 minutes		0.002	NS
Contrast of 62°C vers	sus 60°C for :	15 minutes		0.657	NS

# Table 5.10Effect of day of assessment on the percentage dead nematodes<br/>for each treatment (excluding control)

Treatment	On day of treatment: mean % dead nematodes	One week after treatment: mean % dead nematodes
Hot water 60°C	100.00	99.85
Hot water 62°C	99.9	99.95
Water soaked	51.5	53.25
LSD (5%)	6.473	3.027
LSD (1%)	8.718	4.077
SED	3.171	1.483
SE	2.242	1.049

mwepesi described at the beginning of this chapter.

Summary of significance levels for the effect of hot water treatment on the percentage dead nematodes is shown on Table 5.10. There were more significant differences in the percentage dead nematodes between cultivars when the assessment was done one week after treatment than on the same day of treatment (Table 5.11a).

Examination of the percentage dead nematodes on each of the assessment days between the two revival periods gave similar results, with the control and water soaked treatments giving significantly lower percentage dead nematodes than the two hot water treatments (Table 5.11b).

On the same day of assessment of treatments, only rice cultivar Africa gave significantly lower percentage dead nematodes when revival period extended to 48 hours from 24 hours (Table 5.12). However, one week later cultivars Dunduli yamlimani and Kihogo Red Line-7 had significantly lower percentage dead nematodes compared with the results obtained on the same day of treatment.

The comparative effects of the two revival periods on the percentage dead nematodes was significant at both, same day and one week after treatment at P=0.1 and P=0,01 respectively (Table 5.13) with 48 hours resulting in significantly lower percentage dead nematodes. The treatment effects compared with each revival period (24 and 48 hours) gave significant results for the control and for the water soaked seeds with significantly lower numbers of dead nematodes for revival period of 48 hours compared to that for 24 hours (Table 5.14a). The comparisons of the percentage dead

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Table 5.11	Summary of significance of the effect of hot water
	treatment on the percentage dead nematodes

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a. Effect of cultivar differences on the percentage dead nematodes for each assessment day

Cultivar	Same day of	treatment	Week after treatment
Mpunga		89.3	91.71
Africa		86.5	85.64
Kajibi		78.7	76.71
Kihogo		74.7	73.29
Dunduli yamlimani		73.3	69.64
LSD (5%)		7.739	3.618
LSD (1%)		10.423	4.873
SED		3.790	1.773
SE		2.680	1.253

b. Effect of day of assessment and soaking period on the percentage revival of <u>A.</u> <u>besseyi</u> following hot water treatment

Treatment	Same day of 24 hours	treatment 48 hours	Week of 24 hours	treatment 48 hours
Hot water 60°C	100.0	100.0	99.8	99.9
Hot water 62°C	100.0	99.8	99.1	98.8
Water soaked	58.4	44.6	60.7	45.8
Control	64.2	55.4	58.9	44.8
LSD (5%)	11.211	L	6.0	55
LSD (1%)	15.098	3	8.1	54
SED	5.490	)	2.9	65
SE	3.882	2	2.0	97

Variety	<u>On the</u>	On the same day		after
	24 hrs	48 hrs	24 hrs	48 hrs
Afrika	92.4	80.6	88.86	82.43
Dunduli	75.4	71.1	79.14	67.43
Kajibi	80.7	75.4	78.29	75.14
Kihogo	76.3	73.1	74.00	65.29
Mpunga mwpesi	90.1	88.4	92.43	91.00
SED SE LSD (5%) LSD (1%) CV%	5.36 3.79 10.94 14.74 12.5	0 5 0	2.5 1.7 5.1 6.8 5.9	06 72 17 92

Table 5.12Percentage dead nematodes counts at each of the revival<br/>period (24 and 48 hours) for the different cultivars on the<br/>two assessment days

# Table 5.13The comparative effect of the two revival periods (24 to 48<br/>hours) on the control of the seed borne A. besseyi on the<br/>two assessment days

		On the same day			One week after		
		24 hrs	48 hrs		24 hrs	48 hrs	
		83.0	77.7		82.54	76.26	
SED SE		2.40 1.697			1.121 0.793		
LSD	(5%)	4.901			2.289		
LSD	(1%)	6.600		•	3.083		
CV%		12.5			5.9		

Table 5.14Effect of different treatments revival period (24 and 48<br/>hours) and day of assessment on the control of seed borne A.<br/>besseyi assessed on the same day and one week after<br/>treatment.

a.	Treatment	<u>On the sa</u>	me day	One wee	k after
	Control	24 hrs	48 hrs 55 4 ns	24 hrs	48 hrs
	SED SE LSD (5%) LSD (1%)	6.3 4.4 12.9 17.4	94 983 946 936	2.9 2.0 6.0 8.1	65 97 55 54
	Treatment	<u>On the sa</u>	me day	One wee	k after
		24 hrs	48 hrs	24 hrs	48 hrs
	Hot water 60°C	100.0	100.0	99.1	98.8
	Hot water 62°C	100.0	99.8	99.8	99.9
	Water soaked	58.4	44.6	60.7	45.8
	SED	4.4	83	2.0	97 ·
	SE	3.1	.70	1.4	83
	LSD (5%)	9.1	.55	4.2	81
	LSD (1%)	12.3	29	5.7	66
b.	Revival period	<u>On the sa</u>	ume day	One wee	k after
	24 h	83.00	)	82.	54
	48 h	77.74	L	76.3	26
	SED = 1.774				
	SE = 1.25				
	LSD (5%) = 3.35				
	LSD(1%) = 4.72				

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revival periods respectively gave no significant results (Table 5.14b). It must be noted that there were more live nematodes (although not in significant numbers) for the 48 hours revival period and for the assessment done one week after treatment compared to that done on the same day of treatment, after only 24 hours soaking.

Results presented above show that the control (untreated) had about 48% dead nematodes. This is too high a percentage of dead nematodes to be expected from control samples, unless the seeds were very old and degenerated, whose viability is reduced and would therefore be expected to be degenerating with the pest they harbour. This was not the case, because control seeds were checked for germination and germination results for some cultivars were quite high. The possible explanation seems to lie on the temperature during the revival.

Tamura and Kegasawa (1957) found 20°C to be the optimum temperature for <u>A. besseyi</u> to swim away from soaked seeds. During the cooler months in the University Campus area in Morogoro, night temperatures can fluctuate to below 15°C when the mean minimum temperatures lie between 15°C and 17°C. Low night temperatures may have caused more nematodes to fail to revive and thereby yielding more dead nematodes than normal.

It was observed during the course of this study, that extractions of <u>A. besseyi</u> by soaking seed samples in water over laboratory benches during periods of low temperatures yielded more dead than live nematodes, sometimes all dead nematodes. When nematode extraction was done from seeds of the same batches under warmer conditions, such as in incubators, more live nematodes were revived. Hence, based on the results of the present study and that of the previous experiments, it was decided to use temperature controlled incubators to extract nematodes during cooler months.

The results showed that the control and the soaked seed treatment did not significantly differ, and that these two treatments yielded significantly fewer dead nematodes compared to hot water treatments. The results also demonstrated that hot water treatment at 60°C and 62°C for 10 and 15 min can significantly control <u>A. besseyi</u>. Results also demonstrated that treatment temperatures and time regimes were not themselves significantly different (Table 5.9). The percentage number of dead and live nematodes assessed on the same day as opposed to those from the assessment conducted one week after did not yield significant differences for the cultivars or the treatments.

Except for cultivar Mpunga mwepesi and the water soaked treatment, the rest of the cultivars and treatments had less dead nematodes (though not in significant numbers) for the assessment done one week after treatment compared to that done on the same day of treatment. That means some of the nematodes presumed dead when the assessment was done on the same day of treatment were found alive one week after treatment. These results confirm the previous observations made with dry seed treatment of seed of cultivar Mpunga mwepesi. It is likely that on the same day of treatment (revivals of  $\underline{A}$ . <u>besseyi</u> having been done soon after treatment) some nematodes may have been in a state of shock, precipitated by thermal treatment

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from which they were able to recover later.

Comparisons of revival period showed that 48 hours of reviving nematodes, resulted in more live nematodes than after only 24 hours. This result was significant in both control and soaked treatment showing that the comparative optimum period for revival of <u>A. besseyi</u> in dry seeds by soaking dehusked grain and husk in water is 48 hours.

It was decided from the results of the present and previous studies that, warm environment with temperatures not fluctuating to below 20°C should be used for both germination of rice seeds for all experimental work and for nematode revival for study of live and/or dead nematodes in rice seeds. Laboratory open bench environment was considered unsuitable for these purposes during the cooler months of the year when incubators should be used for extraction of <u>A. besseyi</u> and, germinators for seed germination.

Therefore, regardless of treatment, 24 hours is not the maximum period for revival of nematodes from quiescence in dry seed of same or different cultivars of rice. The actual day of assessment independently, was not important. The importance of the day of assessment was associated with the treatment and the cultivars with which the days of assessment interacted. This may be related to the physiological condition of the different cultivars and that of the nematodes they harbour, which in turn may be related to the moisture content in the seeds and the level of dehydration of nematodes contained therein.

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# 5.4 Hot water treatment following presoaking

#### 5.4.1 Introduction:

It is well known that cultivated rices constitute thousands of cultivars. For example, Webb et al (1968) were able to assemble as many as 4 381 rice cultivars from 49 countries to study their characteristics. Wilson and Tidbury (1944) observed 148 varieties/cultivars in Zanzibar Island. Despite the fact that the latter authors were of the opinion that some of those varietal names were synonyms, they were nonetheless convinced that there were, all the same, a great many different varieties under cultivation on Zanzibar Island. In Tanzania Mainland, there is probably also a synonymous situation in the nearly 200 varieties/cultivars being grown (Table 1.1). That taken into account, there must still be well over 100 different cultivars constituted from the local, introduced and improved rices that are being grown in Tanzania. Leonard and Martin (1963) estimate that there are about 8 000 botanically different rice cultivars in the world and stated that differences in water supply, altitude and season of cultivation as well as differences in consumer demands necessitate the growing of many cultivars.

Most parasites are generally believed to be in physiological balanced state with their hosts, that factors bringing distress to the pest also bring about an equally distressing effect to the host. Nandakumar <u>et al</u>. (1975) stated that, in seed treatment, it is essential to get the treatment temperature to the nematodes (below the seed coat) and, at that point, temperatures become critical to the life of the seed.

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Cralley (1949) reported that presoaking was essential to activate the nematodes and predispose them to hot water treatment. Fortuner and Orton Williams (1975) stated that it is essential to presoak rice seed before hot water treatment for control of A. besseyi in order to bring the nematode out of quiescenence and predispose it to thermal treatment. Meanwhile, rice seeds thus presoaked become physiologically active to start germination processes. In that physiological state, rice seeds also become predisposed to hot water treatment. Yoshii and Yamamoto (1950d) from the results of their studies, observed that, hot water treatment following presoaking was effective in controlling A. besseyi but delayed germination of seed by 1 day. It would seem from examining literature that more work on the effect of hot water treatment following presoaking on different rice cultivars grown in Tanzania was necessary.

In view of the great importance placed on the hot water treatment for control of <u>A. besseyi</u> and the seeming gaps in the present knowledge of the effect of such treatment on different rice cultivars, coupled with the knowledge of the very many cultivars being actively grown in Tanzania to date, it was considered important to study hot water treatment following presoaking. It was essential, to study various aspects of hot water treatment on rice seed following presoaking with a view to achieving the following objectives:

1. To assess temperatures and treatment periods that will significantly control <u>A. besseyi</u> with minimum damage to the rice seeds.

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2. To assess the effect of hot water treatment following presoaking on the germination and emergence of seeds of selected rice cultivars.

5.5 Effect of hot water treatment (following presoaking in water for 18 hours) at 54°C for 15 minutes on the control of seed-borne A. besseyi

#### 5.5.1 Introduction and Objectives:

From the preliminary studies with hot water tratment at 54°C for 15 minutes, it was observed that complete control of <u>A</u>. <u>besseyi</u> was achieved when infected rice seeds were treated following presoaking. Therefore, a follow up study to assess the efficacy of this treatment regime in the control of <u>A</u>. <u>besseyi</u> in the seeds of three rice cultivars was conducted. The three cultivars were chosen based on the availability of seeds with live nematodes.

#### 5.5.2 Materials and Methods:

Seeds were hot water treated following presoaking in water for 18 hours and were subsequently (after 1 week) assessed for nematodes using single seed assessment method. Treatment of seeds and nematode assessment were done according to methods given in the Materials and Methods Section of the thesis. Fifty, single seed samples were assessed per cultivar, treatment and replication. Samples were assessed 48 hours after soaking to revivie <u>A. besseyi</u>.

# 5.5.3 Results and Discussion:

In the control (untreated), there were 29.95, 21.43 and 24.85% dead nematodes for cultivars Sindano, Nyati and Bagamoyo respectively. After hot water treatment, all nematodes recovered were dead showing that hot water treatment of presoaked seeds at 54°C for 15 minutes, completely controlled seed borne <u>A. besseyi</u>. This treatment regime had previously been used on many occasions to control <u>A. besseyi</u> in seeds used for other experiments. For that reason, this regime was adopted for <u>A. besseyi</u> control in the seeds used in field experimental studies. The results therefore confirmed previous observations on the efficacy of this treatment regime to control seed borne <u>A. besseyi</u>. Therefore, it was decided to concentrate on the assessment of the effects of the latter treatment regime and several others on the germination aspects of seeds of several rice cultivars.

5.6 Effect of hot water treatment on the germination of presoaked seeds of four rice cultivars

#### 5.6.1 Introduction and Objectives

The experiment was a follow-up of the previously conducted observation on germination of treated seeds following hot water treatment of presoaked seeds. Limited facilities were available at the National Seed Testing Laboratory, and a germination test with four rice cultivars and one treatment regime could be facilitated. The lowest previously tested treatment regime that had been found to completely control <u>A. besseyi</u> was chosen (54°C for 15 minutes). The choice of cultivars was based on the availability of seeds and on the fact that most of the chosen cultivars had been included in previous dry seed treatment studies.

Previous observations showed that soaking seeds in water for up to 72 hours, especially during warmer months, resulted in sprouting and it became very difficult to find nematodes at that stage. It was therefore, decided to reduce soaking time to what could be considered adequate period for the purpose of seed imbibing water and long enough for the nematode to hydrate and be activated. Soaking period of 18 hours was therefore considered satisfactory.

Therefore, the objective of this study was to further examine the effects of hot water seed treatment, following presoaking, on the germination of seeds of four rice cultivars. In this experiment normal germinated seeds, abnormal seedlings and any other abnormalities that may have been due to seed damage were also recorded.

#### 5.6.2 Materials and Methods:

Experiment was conducted as a completely randomised design with three replications. Hot water treatment was conducted in the Faculty laboratory and seed germination tests were done at the Seed Testing Laboratory situated in the Faculty Campus. Rice cultivars Kihogo, Kigunia, Lumoto and Nyati were treated at 54°C for 15 minutes following presoaking for 18 hours at laboratory ambient Treatment was carried out in temperature controlled temperature. water bath as described in the General Materials and Methods Section of the thesis. Germination test was done a week after treatment, in germination chambers set at 28°C. Seeds were left to germinate for 10 days. Two hundred seeds from each treatment were germinated on moist blotters. Normal and abnormal germinations, dead and rotting seeds, and seeds that looked fresh and felt hard but were ungerminated were recorded. Analyses of variance using transformed data (square root transformation) for the various parameters was conducted followed by an F-test for significance.

#### 5.6.3 Results and Discussion:

Table 5.15 shows the percentage means for the various

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Table 5.15Mean percent germination and Summary of analysis of variance<br/>for the effect of hot water treatment (54°C for 15 min)<br/>following presoaking on the germination of form rice<br/>cultivars (transformed means and square root transformation

Cultivar	% germ seeds	inated	% frea ungerm seeds	sh inated	% abnorn seedling	nal zs	% dead a rotting	and seeds
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Percent G	erminatio	on						
Lumoto	92.83	99.50	2.17	0.00	3.00	0.17	2.00	0.33
Nyati	80.33	94.00	15.67	2.67	4.00	3.00	0.00	0.33
Kigunia	91.83	91.83	5.30	3.50	2.83	4.33	0.00	0.33
Kihogo	69.17	92.83	22.67	4.67	8.17	2.50	0.00	0.00
Summary o	f signif	icance f	or the ar	nalysis	of varia	nce		
Lumoto	9.634	9.975	1.35	0.71	1.814	0.805	1.470	0.902
Nyati	8.959	9.695	3.97	1.76	2.119	1.858	0.707	0.902
Kigunia	9.583	9.583	2.39	1.98	1.748	2.196	0.707	0.880
Kihogo	8.301	9.635	4.69	2.26	2.881	1.732	0.707	0.707
SED	0.2	L63	0.588	3	0.34	139	0.2	338
SE	0.19	530	0.415	59	0.24	132	0.10	65
LSD (5%)	0.4	586	1.246	56	0.73	333	0.49	96
LSD (1%)	0.63	318	1.717	75	1.00	05	0.68	33
CV %	2.8		30.2		22.2		32.80	כ

parameters and the corresponding transformed values (square root transformation). Hot water treatment following presoaking significantly affected germination as shown by almost all studied variables. The cultivar differences were significant to a greater or lesser extent for all the studied variables. The effects of treatment were significantly related to rice cultivars for the percentage germination of normal seeds and the percentage abnormal seedlings. The percentage number of dead seeds were not significantly affected by the treatment.

Cultivars Nyati Kihogo had significantly and lower germination percentage of normal seedlings in the treated as compared to the control. The other two cultivars did not differ significantly in their normal seedling germination between the treated and the control. The same cultivars (Nyati and Kihogo) had a significantly higher number of seeds that failed to germinate (ungerminated seeds) which looked fresh, felt hard and were not rotten or rotting, compared to the control. The other cultivars did not differ significantly between the treatments for these variables.

For the percentage abnormal seedlings, there was a significantly higher percentage in the treated seeds compared to the control for cultivars Lumoto and Kihogo and, for the percentage dead and rotting seeds, cultivar Lumoto had a significantly higher percentage in the treated than in the control.

The results presented above demonstrate that hot water treatment of rice seeds at 54°C for 15 minutes following presoaking in water for 18 hours significantly decreased the percentage germination for normal seedlings of two of the studied cultivars (Nyati and Kihogo) and not for the others. The cultivars whose percentage normal seed germination was significantly decreased had a corresponding higher percentage of ungerminated seeds in the treated compared to the control showing that the thermal treatment had prevented their germination. The results of these two variables also demonstrate cultivar differences in the ability to tolerate hot water treatment.

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Two cultivars (Lumoto and Kihogo) had significantly higher percentages of seedlings that were abnormal in the treated compared to the control. Cultivar Lumoto had a zero percentage seeds that could be considered ungerminated but a significantly higher percentage of abnormal seedlings and also a higher percentage of dead and rotting seeds. These results suggest that in the latter cultivar, the effects of hot water treatment did not prevent actual germination but affected the development of the seedlings. These results agree with Levitt (1956) who stated that some seed type may remain viable after treatment with high temperatures but the subsequent development of the seedlings may be adversely affected. It is noteworthy that the same cultivar had the highest percentage normal germination (99.50 and 92.83% respectively).

Therefore, cultivar Lumoto could be considered tolerant to the tested treatment regime such that the effects of the treatment were only able to produce abnormal seedlings rather than inflict total loss of viability thus preventing germination altogether. The reverse can be said for cultivar Nyati. For cultivar Kihogo, there were significant increases in both ungerminated and abnormal seedlings, suggesting that this cultivar was the most sensitive, resulting in serious loss of vigour and equally serious loss of viability.

The temperature range, within which seeds germinate is said to be determined by the source of seeds, genetic differences within the species (for example, varietal differences) and the age of the seed (Mayer and Poljakoff - Mayber, 1978). It can be concluded that while hot water treatment adversely affected normal germination, some cultivars were more tolerent than others. The adverse effect of treatment temperature was manifested by failure of seed to germinate (destroyed viability) and by seeds which produced abnormal seedlings. Abnormal seedlings had developed either root or shoot only or some weak seedlings with or without malformed root system (Plate 5.1 and 5.2). The results also demonstrated that hot water treatment was not instrumental in the death to the seeds categorised as dead and rotting.

These results lead into further investigations to elucidate the effects of hot water treatment following presoaking on the germination of seeds of 22 rice cultivars and their reaction to the thermal treatment.

5.7 Effect of hot water treatment following presoaking on the germination of seeds of twenty two rice cultivars

#### 5.7.1 Introduction and Objectives:

In view of multitude of rice cultivars being grown in Tanzania, it was considered important to investigate further the effect of hot water treatment following presoaking on the germination of cultivars than those hitherto tested. It was also



Plate 5.1 Abnormally germinated rice seeds showing "Shoots only"



Plate 5.2 Abnormally germinated rice seeds showing "roots only"

decided to test and/or further assess more treatment temperatures and time periods as treatment combinations.

Some of the rice cultivars investigated in this study, came from the National Rice Development Programme, as well as those considered popular within localities.

#### 5.7.2 Materials and Methods:

The experiment was a factorial design in a randomised block layout with three replications. The following factors were used:

a) Treatment

- (i) Temperatures 54, 55 and 56°C
- (ii) Treatment time 5, 10 and 15 minutes
- (iii) Control

b) Cultivars - 22

All seeds for treatment were presoaked in water for 18 hours; treatment combinations were as follows:

(i)	54°C/5 minutes	(vi)	55°C/15 minutes
(ii)	54°C/10 minutes	(vii)	56°C/5 minutes
(iii)	54°C/15 minutes	(viii)	56°C/10 minutes
(iv)	55°C/5 minutes	(ix)	56°C/15 minutes
(v)	55°C/10 minutes	(x)	Control

#### The following rice cultivars were assessed:

(1)	Kihogo Red	(12)	Sindano
(2)	Nyati	(13)	Mchuzi wakuku
(3)	Afaa Mwanza 0/906	(14)	Faya Pemba

(4) Afaa Kilombero 0/906 (15) Bluu (5) Kihogo Selection No 7 (16) Kihogo Selection No 22 (6) Kalafulu (17) Kigunia (7) Taiwan T - 14 (18) Meli (8) Lumoto (19) Afaa Mwanza 1/159 (9) Bagamoyo (20) Tulenabwana (10) Lunyuki (21) Faya Theresa (11) Kihogo (22) Faya

Parameters to be measured included germination and observations on any abnormalities.

Each rice cultivar sample was mixed thoroughly and subsampled into treatment lots consisting of 500 g. Each sample was tied inside a treatment bag with weighting stones and soaked in water for 18 hours at laboratory temperature. Hot water treatment was carried out using a temperature controlled water bath as described in the Materials and Methods Section of the thesis. After treatment, seeds were dried for 24 hours on laboratory benches and germination was carried out a week later in seed germinators at the National Seed Testing Laboratory

Germinators were set at 28°C and seeds were placed in special germination papers and placed on trays. A total of 100 seeds per treatment per replication were used. Assessment was started as soon as the first germinated seeds had developed the first leaf and counting was done every two days by removing germinated seeds. The experiment was continued for 18 days, and on the last assessment day, ungerminated seeds (those that looked fresh

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and not rotting), dead seeds (seeds in the process of rotting) and weakly germinated seeds (seeds that germinated into comparatively weak seedlings) were recorded.

Data was transformed using angular transformations. Analysis of variance for factorial aspects of the experiment were conducted and linear and quadratic regressions were fitted for each of the quantitative factors (temperature and time). A Student-Newman-Keuls Multiple Range Test was conducted to compare means. In addition, analyses of variance, including all treatments, was conducted followed by F-tests for significance and Student-Newman-Keuls Multiple Range Tests to compare treatment means.

#### 5.7.3 Results and Discussion:

For all the variables, time x cultivar and time x cultivar x temperature interactions, were not fitted in the model because they were not significant. This resulted in increased residual degrees of freedom, as effects that were not fitted in the model were automatically accounted for in the residual degrees of freedom.

The results of the analysis of variance for normally that time, temperature, cultivar. germinated seeds showed temperature x cultivar were highly time x temperature and significant (P = 0.01) and so were the linear regressions on time and temperature for these variables (Figures 5.2, 5.3 and 5.4). The temperature and on time were quadratic regressions on not significant. As time and temperature increased, so did the rate of decrease in the percentage normal germination. Cultivars were significantly different, with each cultivar having a different



linear regression on temperature (Figure 5.5). The interaction between time and temperature was significant, with each temperature having a different linear regression (Figure 5.4). At 56°C the percentage germination decreased more rapidly with increase in time.

The results of the analysis of variance for the fresh-looking ungerminated seeds showed that time, temperature, temperature x cultivar cultivar, time x temperature and were significant factors, and so were their corresponding linear regressions on time and temperature (P = 0.01). The quadratic regressions on time and temperature were not significant. With increase in temperature or time, the percentage number of seeds that failed to germinate (ungerminated seeds) increased (Figures 5.6 and There was a significant interaction between time and 5.7). temperature and for each temperature there was a linear relationship Therefore, there was a significant increase in the with time. percentage of ungerminated seeds with increase in time for each of Cultivars were significantly the temperatures (Figure 5.8). different and they significantly interacted with temperature. It shown that, for almost all cultivars, with changes was in temperature, there was an increase in the rate of percentage seeds that failed to germinate (positive slope). However, for one cultivar, Faya, there was a decrease in the rate of percentage seeds that failed to germinate (negative slope) (Figure 5.9).

The results of the analysis of variance for the factorial aspects for the percentage weakly germinated seeds showed that time, temperature, and temperature x cultivar were significant and so were their linear regressions on time and temperature except that of

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temperature x cultivar, which was not significant. The quadratic regressions were not significant. There were significant differences between the times and the temperatures as independent factors and their corresponding linear regressions were significant (Figures 5.10 and 5.11). Therefore, as temperature or time increased, there were significant changes in the number of seeds that germinated into weak seedlings. However, temperature and time did not affect each other to influence percentage weakly germinated seeds. Cultivars reacted significantly differently and interacted significantly with temperature. The rate of change in the percentage numbers of seeds that germinated into weak seedlings increased with change in temperature for most of the cultivars (positive slopes) while for five cultivars the rate actually decreased (negative slopes - Figure The latter cultivars were Meli, Afaa Mwanza 0/906, Afaa 5.12). Mwanza 1/159, Bagamoyo and Tulenabwana.

The results of the analysis of variance for the percentage number of dead and rotting seeds was significant for the cultivar factor only. Cultivars reacted differently. Although there were no significant interactions between cultivar and temperature or between cultivar and time, there were nevertheless linear relationships between cultivar and temperature (Figure 5.13). For some cultivars, there was a positive increase in the rate of percentage number of dead seeds with increase in temperature (positive slope) while for others the rate of increase actually reduced with increase in temperature (negative slopes - Figure 5.13).

Tables 5.16 to 5.19 show the results of the Student-Newman-Keuls Multiple Range Test (SNK Multiple Range Test)









Table 5.16Effect of hot water treatment (following presoaking) on the<br/>germination of seeds of 22 rice cultivars Ranked means for<br/>percentage normal germination (angular transformed data)

Cultivar Mean Afaa Mwanza 1/159 51.500 Afaa Mwanza 0/906 53.800 Afaa Kilombero 0/906 54.700 Lunyuki 56.100 Meli 56.400 57.100 Sindano Tulena bwana 58.900 Taiwani T-14 61.500 62.000 Kigunia Bluu 64.800 Lumoto 67.000 67.500 Kalafulu 68.500 Kihogo Red 68.800 Kihogo 69.200 Faya Theresa 71.200 Nyati 71.500 Faya 73.900 Kihogo Selection 7 74.900 Kihogo Selection 22 75.300 Bagamoyo 75.500 Mchuzi wakuku 78.600 Faya Pemba Significant P =0.05

"Means joined by the same lines were not statistically different"

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Table 5.17Effect of hot water treatment (following presoaking) on the<br/>germination of seeds of twenty-two rice cultivars Ranked means<br/>for percentage fresh looking ungerminated seeds (angular<br/>transformed data)

Cultivar	Mean	
Faya Pemba	2.900	
Kihogo	6.000	
Bagamoyo	6.300	
Nyati	7.000	
Mchuzi wakuku	7.700	
Kihogo Selection 7	8.000	
Kihogo Selection 22	9.400	
Sindano	10.300	
Kihogo Red	11.500	
Lumoto	11.900	
Lunyuki	12.000	
Kalafulu	12.100	
Bluu	12.100	
Faya	13.100	
Afaa Kilombero 0/906	13.700	
Faya Teresa	14.100	
Afaa Mwanza 0/906	14.200	
Kigunia	15.500	
Taiwani T.14	15.800	
Afaa Mwanza 1/159	18.900	
Tulena bwana	19.200	
Meli Signigicant at $P = 0.05$	19.600	

"Means joined by the same lines were not statistically different"

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Table 5.18 Effect of hot water treatment (following presoaking) on the germination of seeds of twenty-two rice cultivars Ranked means for percentage weakly germinated seeds (angular transformed data)

Cultivar	Mean
Nyati	5.500
Faya Pemba	6.700
Mchuzi wakuku	9.300
Kihogo Selection 22	9.300
Kihogo Selection 7	9.800
Kihogo Red	10.000
Faya Teresa	10.400
Faya	10.600
Bagamoyo	10.700
Lumoto	11.800
Kihogo	14.200
Kalafulu	15.300
Kigunia	16.400
Bluu	17.200
Tulena bwana	18.600
Meli	20.500
Taiwani T.14	22.000
Afaa Mwanza 1/159	25.600
Lunyuki	26.700
Sindano	27.300
Afaa Mwanza 0/906	28.700
Afaa Kilombero 9/906 Significant at P = 0.05	30.700

"Means joined by the same lines were not statistically different"

Table 5.19Effect of hot water treatment (following presoaking) on the<br/>germination of seeds of twenty-two rice cultivars Ranked means<br/>for percentage dead and rotting seeds (angular transformed<br/>data)

Cultivar	Mean
Afaa Kilombero 0/906	1.100
Taiwani T.14	1.700
Faya	3.100
Kalafulu	4.800
Kihogo Selection 22	4.900
Bagamoyo	5.300
Mchuzi wakuku	6.000
Faya Pemba	6.700
Sindano	6.900
Kihogo Selection 7	7.300
Faya Teresa	7.700
Afaa Mwanza 9/906	8.300
Bluu	8.400
Lunyuki	9.300
Afaa Mwanza 1/159	9.400
Kihogo Red	11.300
Kihogo	12.100
Tulena bwana	12.100
Kigunia	12.400
Meli	12.700
Lumoto	13.900
Nyati Significant at $P = 0.05$	14.800

"Means joined by the same lines were not statistically different"

of significance for cultivar means for the analyses involving all treatments. Cultivars Faya Pemba up to Lumoto had the highest germination percentages compared with the rest and were not significantly different from each other. The opposite extreme included cultivars such as Afaa Mwanza 1/159, Afaa Mwanza 0/906 and Afaa Kilombero 0/906 which had the lowest germination percentages. The intermediate group of cultivars included Faya Theresa on the higher germination percentage scale to Sindano on the lower scale. Cultivars which had highest percentage germination (Faya Pemba, Mchuzi wakuku, Bagamoyo, Kihogo Selection No 22 and Kihogo Selection No 7, Faya and Nyati) had the lowest percentages of ungerminated and weakly germinated seeds (Tables 5.16, 5.17 and 5.18).

Treatments and cultivars interactions were significant (P = 0.01) for percentage normal germinated seeds, percentage weakly germinated and ungerminated seeds. For the percentage dead and rotting seeds, only the cultivar factor was significant (P = 0.01).

Percentage dead and rotting seeds were not significantly influenced by treatment except by the cultivar factor. Cultivars Afaa Kilombero 0/906, Taiwan T - 14 and Faya had the least percentage dead seeds while Kihogo Red, Kihogo, Tulenabwana, Kigunia, Meli, Lumoto and Nyati were a group of cultivars with the highest percentage dead seeds (Table 5.19).

The results of the comparisons of all treatment means, for all variables, are shown on (Table 5.20) Hot water treatment at 56°C for 15 minutes was significantly different from all other treatments. Treatments at 55°C for 5, 10 and 15 minutes and 56°C for 10 minutes were not significantly different. The control, Table 5.20Summary results of the effect of hot water treatment<br/>(following presoaking) on the germination of seeds of 22<br/>rice cultivars (Angular transformed data)

Treatment	% normal	% fresh	% weakly	<u>% dead</u>
Control	77.05 b	5.86 b	3.32 a	8.13 a
54°C/5 mins.	75.04 bc	8.48 ab	8.80 ab	6.83 a
54°C/10 mins.	72.43 bc	11.12 ab	9.26 ac	7.00 a
54°C/15 mins.	69.28 bc	12.63 ab	11.10 bc	8.41 a
55°C/5 mins.	68.24 bc	12.69 ab	11.18 bc	7.89 a
55°C/10 mins.	62.77 a	17.89 ac	14.43 bc	8.46 a
55°C/15 mins.	64.89 ac	16.73 ac	13.52 bc	8.99 a
56°C/5 mins.	63.45 a	17.9 ac	13.20 bc	9.14 a
56°C/10 mins.	55.15 a	25.66 c	16.30 bc	7.63 a
56°C/15 mins.	45.69	33.53	17.69 b	9.50 a

Means having the same letter are not statistically different

Student Newman - Keuls multiple range test

treatments 55°C for 5 minutes, 54°C for 5, 10 and 15 min were not statistically different. Hot water treatment at 56°C for 15 min resulted in significantly more percentage ungerminated seeds compared to all other treatments. The percentage ungerminated seeds in the control, in treatments at 54°C for 5, 10 and 15 min and in 55°C for 5 min were not significantly different.

The ranked treatment means for the percentage weakly germinated seedlings showed that the control, 54°C for 5, 10 and 15 min constituted a group of non-significant treatments.

The results of the analyses of variance for the factorial aspects of the experiment showed that time, temperature and cultivar factors were very important in the normal germination of rice seeds treated following presoaking and in the determination of seeds that fail to germinate or those that germinate into weak seedlings. Both time and temperature seriously reduced normal germination (Figures 5.2 and 5.3) by either destroying seed viability (Figures 5.6 and 5.7) or by reducing seedling vigour (Figures 5.10 and 5.11) and, in some cases, affected both. The effect of time and temperature on the germination of rice seed was interrelated in a manner that, for each temperature increase, there was a significant reduction in percentage normal germination (Figure 5.4) and significant increases in weakly germinated or ungerminated seeds (Figures 5.10, 5.11 and 5.8).

These results show that the reduction in the percentage germination was related to the increases in either ungerminated or weakly germinated seeds or both. Hence hot water treatment of rice seeds could be considered as causing loss of viability and reduction in seedling vigour.

Mayer and Poljakoff - Mayber (1978) stated that different seeds have minimum, maximum and optimum temperatures for germination and that these conditions are determined by the source of seeds and the genetic differences within the species.

Present study concluded that there were some cultivars whose seed germination was seriously affected by hot water treatment especially at 56°C for 15 min. while other cultivars were actually positively affected.

For percentage dead seeds, it was demonstrated that hot water treatment was not instrumental in the death of the seeds but the cultivar differences or environmental condition of the seeds prior to the treatment were more likely instrumental to the death of seeds.

It was demonstrated that the effect of temperature on the studied aspects of germination of treated rice seeds following presoaking varied with cultivars, with some groups of cultivars exhibiting great sensitivity, while the other groups could be considered as being of intermediate sensitivity ranging from tolerant to indifferent (Figure 5.5). It is noteworthy, that genetically related cultivars such as Afaa Mwanza 1/159, Afaa Mwanza 0/906 and Afaa Kilombero 0/906 reacted in a related manner within each others range and so were such cultivars as Kihogo Selection No 22, Kihogo Red, Kihogo Selection No 7 and Kihogo. This observation seems to agree with Mayer and Poljakoff - Mayber (1978) who stated that temperatures at which different seeds germinate and the range within which they germinate is partly determined by genetic

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differences within a species.

It was observed that cultivar Faya had a very small negative regression slope (almost zero) for percentage normal germination and а corresponding negative regression slope for percentage ungerminated seeds but for the percentage weakly germinated seeds the regression slope was positive (Figures 5.5, 5.9 and 5.12). This result suggests that this cultivar was minimally affected by temperature in so far as viability was concerned. However, seed vigour was definitely reduced (Figure 5.12). These results agree with Levitt (1956) that seeds may still be viable after treatment at high temperatures, but that their seedling development may nevertheless have been adversely affected. Loss of vigour can result in the seedling's failure to cope with suboptimal growth conditions.

Ballard (1969) stated that less vigorous seeds are more adversely affected by harsh environmental conditions such as low temperatures and low oxygen in the soil. The proportions of ungerminated to weakly germinated seeds by cultivars could be interpreted as having been influenced by the way hot water treatment affecting their physiology. These different reactions may reflect cultivar differences in the ability to tolerate heat treatment with some cultivars loosing viability where others, merely lost vigour.

It must be emphasised that the age of the seed samples used were not known, and also their previous storage history, and hence, the overall seed quality. Consequently, the poor performance in the germination of some of the cultivar may have been exacerbated by their overall poor quality. For example, cultivar Sindano, from previous experiments exhibited high tolerance to hot water treatment at 54°C and 56°C for up to 15 minutes. In this experiment, it germinated poorly but however, the effect of hot water treatment to this cultivar was reduced vigour (giving weak seedlings) rather than loss of viability altogether. Therefore, the poor germination performance by Sindano in this experiment seems to suggest seed quality deterioration. There may be more cultivars among those that germinated poorly whose quality had deteriorated. Hence the tentative grouping of cultivars into categories according to their reaction should be viewed in that context.

Treatment as a factor did not significantly influence the percentage of seeds that were designated dead and rotting. However, since there was a linear regression with time (Figure 5.13) there could be some small proportion in that category that were killed by hot water treatment. The overall results confirm the observations made for the factorial analyses where temperature and time were found to interact.

Temperature was critical in the germination of seeds. For example, the control and hot water treatment at 54°C for 5, 10 and 15 minutes and at 55°C for 5 minutes were not significantly different in the percentage normal germination and ungerminated seeds. However, for higher temperatures, as short as 5 minutes treatment was found to belong to such group of treatments as 56°C for 5 and 10 minutes and 55°C for 10 and 15 minutes, meaning that an increase in temperature could effect more changes in the germination of seed exposed for the same period of time.

However, the intensity of hot water treatment was more apparent in the weakly germinated seeds than in the ungerminated seed variables. For example, a higher and wider range of treatment regimes such as: 54°C for 5, 10 and 15 minutes and 55°C for 5 minutes were not significantly different from the control for loss of seed viability. However, reduction in vigour (weakly germinated seeds) was effected at a much lower treatment regime much closer to the control (54°C for 5 and 10 minutes). Therefore, while there were seemingly larger treatment ranges and higher temperatures and longer period of treatment for effecting loss in viability (ungerminated seeds), the physiological damage to seeds was effected at lower treatment regimes much closer to the control resulting in reduced seedling vigour.

5.8 An examination of the abnormal germination of seeds of six rice cultivars treated with hot water following presoaking

### 5.8.1 Introduction and Objectives

Previous germination experiments with hot water treated rice seeds following presoaking had shown some abnormally germinated seedlings, having root or shoot only, or malformed root system. This experiment was designed to further investigate this phenomenon.

### 5.8.2 Material and Methods:

A random selection of six rice cultivars was made from the 22 cultivars studied in the previous season. Rice cultivars used were:

1 - Tulenabwana

- 2 Afaa Kilombero 0/906
- 3 Afaa Mwanza 0/906
- 4 Nyati

### 5 – Runyuki

### 6 - Kalafulu

Hot water treatments consisted of: 54°C, 55°C and 56°C for 5, 10 and 15 minutes respectively. Treatments were done as described in the General Materials and Methods Section of the thesis. Seeds were germinated a week after treatment in the germination chambers of the National Seed Testing Laboratory. Germination chambers were set at 28°C. Two hundred seeds were germinated per cultivar per treatment and per replication. The experimental design was a randomised block with two replications. Abnormal seedlings were recorded.

Analysis of variance was conducted followed by an F-test for significance for the percentage seedlings which had developed shoot only. Subsequently, a Student Newman Kauls (SNK) Multiple Range Test was conducted to compare the means of significant variables.

### 5.8.3 Results and Discussion:

Cultivars Runyuki, Kalafulu and Afaa Mwanza 0/906 had 3, 1 and 1 seedlings with root only without shoot respectively. Consequently no analysis of variance was conducted for the latter variable. Seedlings with shoot only for each treatment and cultivar are shown in (Table 5.21) and ranked treatment and varietal means and their respective LSD values are shown on (Table 5.22).

Treatments and cultivars were both significant (P = 0.01)suggesting that there were significant differences in the numbers of abnormally germinated seedlings (with shoot only and no roots) between treatments and between cultivars. The control had the least

		1			
° <sub>1</sub>	C <sub>2</sub>	с <sub>з</sub>	C <sub>4</sub>	с <sub>5</sub>	с <sub>6</sub>
0.0	7.2	2.0	0.0	13.0	2.0
3.0	17.0	34.0	1.0	14.0	5.0
6.0	10.0	6.0	0.0	0.0	3.0
5.0	10.0	0.0	2.0	7.0	0.0
11.0	19.0	0.0	4.0	6.0	9.0
2.0	7.0	0.0	3.0	4.0	0.0
8.0	21.0	0.0	4.0	0.0	7.0
4.0	7.0	23.0	0.0	0.0	5.0
0.0	8.0	0.0	0.0	1.0	10.0
8.0	8.0	7.0	27.0	10.0	16.0
	C <sub>1</sub> 0.0 3.0 6.0 5.0 11.0 2.0 8.0 4.0 0.0 8.0	C1         C2           0.0         7.2           3.0         17.0           6.0         10.0           5.0         10.0           11.0         19.0           2.0         7.0           8.0         21.0           4.0         7.0           8.0         8.0	$\begin{array}{cccc} C_1 & C_2 & C_3 \\ \hline 0.0 & 7.2 & 2.0 \\ \hline 3.0 & 17.0 & 34.0 \\ \hline 6.0 & 10.0 & 6.0 \\ \hline 5.0 & 10.0 & 0.0 \\ \hline 11.0 & 19.0 & 0.0 \\ \hline 11.0 & 19.0 & 0.0 \\ \hline 2.0 & 7.0 & 0.0 \\ \hline 8.0 & 21.0 & 0.0 \\ \hline 4.0 & 7.0 & 23.0 \\ \hline 0.0 & 8.0 & 0.0 \\ \hline 8.0 & 8.0 & 7.0 \end{array}$	$C_1$ $C_2$ $C_3$ $C_4$ $0.0$ $7.2$ $2.0$ $0.0$ $3.0$ $17.0$ $34.0$ $1.0$ $6.0$ $10.0$ $6.0$ $0.0$ $5.0$ $10.0$ $6.0$ $0.0$ $11.0$ $19.0$ $0.0$ $4.0$ $2.0$ $7.0$ $0.0$ $3.0$ $8.0$ $21.0$ $0.0$ $4.0$ $4.0$ $7.0$ $23.0$ $0.0$ $0.0$ $8.0$ $0.0$ $0.0$ $8.0$ $8.0$ $7.0$ $27.0$	$C_1$ $C_2$ $C_3$ $C_4$ $C_5$ $0.0$ $7.2$ $2.0$ $0.0$ $13.0$ $3.0$ $17.0$ $34.0$ $1.0$ $14.0$ $6.0$ $10.0$ $6.0$ $0.0$ $0.0$ $5.0$ $10.0$ $0.0$ $2.0$ $7.0$ $11.0$ $19.0$ $0.0$ $4.0$ $6.0$ $2.0$ $7.0$ $0.0$ $3.0$ $4.0$ $8.0$ $21.0$ $0.0$ $4.0$ $0.0$ $4.0$ $7.0$ $23.0$ $0.0$ $0.0$ $0.0$ $8.0$ $0.0$ $0.0$ $1.0$ $8.0$ $8.0$ $7.0$ $27.0$ $10.0$

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## Table 5.21 Percentage seedlings with shoot only

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Table 5.22An examination of the abnormal germination of seeds of sixrice cultivars treated with hot water following presoaking

Ranked varietal percentage mean for seedlings with shoot only

Cultivars	Transformed <u>Means</u>	Reconverted <u>Means</u>
Nyati	5.33	0.93
Tulenabwana	6.35	1.22
Runyuki	6.99	1.43
Kalafuru	8.53	2.20
Afaa Mwanza 0/906	9.45	2.70
Afaa Kilombero 0/906	12.20	4.47
SED = 1.545		

- LSD(5%) = 3.09
- Ranked treatment means

Treatments	Transformed <u>Means</u>	Reconverted <u>Means</u>
Control	5.55a	0.94
54°C/15 minutes	5.76a	1.01
55°C/10 minutes	6.07a	1.12
55°C/15 minutes	6.45a	1.26
56°C/10 minutes	7.87a	1.88
54°C/10 minutes	8.39a	2.13
55°C/5 minutes	8.54a	2.21
56°C/5 minutes	8.63a	2.26
54°C/5 minutes	11 <b>.</b> 52b	3.99
56°C/15 minutes	12 <b>.</b> 97b	5.05
SED = 1.995		
LSD(5%) = 3.99		

CV(%) = 59.8 (for this variable as a whole)

mean numbers of seedlings with shoot only abnormality and was significantly different from hot water treatment at 56°C for 15 minutes, the latter treatment having the highest numbers of seedlings with this type of abnormality (Table 5.22).

Cultivars Afaa Kilombero 0/906 and Afaa Mwanza 0/906 showed the highest mean numbers of shoot-only abnormal seedlings (4.47 and 2.70 respectively) compared to the control with 0.93 abnormal seedlings and, it was noted that the latter are genetically related.

The results demonstrated that hot water treatment of rice seeds at 56°C for 15 minutes following presoaking in water for 18 hours can significantly increase the proportions of seeds that can germinate into abnormal seedlings (with shoot only without a root system) in some cultivars that are sensitive to this type of thermotherapy. The findings confirmed the abnormal germination results of a previous experiment (presented in the same chapter of the thesis) with four rice cultivars treated at 54°C for 15 minutes following presoaking in water for 18 hours. In the previous experiment with 22 genetically related cultivars such as the Afaa series, were shown to germinate within the same range with equal proportions of ungerminated and weak seedlings, suggesting that they were affected in a similar manner.

These findings agree with Ventura and Garrity (1986) who stated that different genotypes may differ substantially in their sensitivity to hot water treatment, suggesting that genetically related cultivars would react in similar manner to this factor of

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treatment. The results also agree with Levitt (1956) who stated that seeds may still be viable after treatment with high temperatures but subsequent development of their seedlings may nevertheless be adversely affected, which points to some physiological damage that may result from the treatment.

The incidence of seedlings with shoot-only abnormality was much higher than that of seedlings with only the roots. These results seem to suggest that the root system is more sensitive to hot water treatment compared to the shoot, hence comparatively more seedlings failed to develop roots.

It was concluded from the results of this study and from those of four and twenty-two cultivars presented in this chapter, that hot water treatment can adversely affect normal germination of seeds of susceptible cultivars, significantly reducing percentage normally germination by destroying viability and/or producing abnormally germinated or weak seedlings. The abnormalities can range from failure to form root or shoot systems or, formation of abnormal root systems or weak shoot systems.

The results pointed to a relationship between hot water treatment and abnormal germination of a specific nature, such as seedlings with shoot only. Plates 5.1 and 5.2 show examples of the abnormalities referred in this study, shoot and root-only seedlings, as observed earlier on in the course of the investigation.

Further studies are proposed to investigate incidence and nature of the root and shoot only abnormality in hot water treated seeds following presoaking. Such a study should include a wider range of treatment temperatures and rice cultivars known to be sensitive and tolerant to hot water treatment. The rice seeds to be used in such a study should be of the same age (less than 1 year) and of uniform maturity and equal moisture content and from optimum storage conditions, that is, good quality seed.

5.9 The relationship between seeding depth with the germination of rice seeds treated with hot water (following presoaking) and between germination of rice seeds planted in soil treated with carbofuran.

### 5.9.1 Introduction:

Previous observations had shown that germination tests conducted in the laboratory under warm temperature conditions were giving high germination rates compared to those in the field using the same seed samples. Hot water treated samples were giving worse results in the field compared to the laboratory and field controls. It was observed that seeds that were potentially viable from the laboratory tests actually did germinate but failed to emerge. It was further observed that these seeds were rather more deeply seated compared to those that had successfully germinated in the same treatment. These observations prompted investigation of these phenomena since they have strong bearing on the performance of treated seeds in the field.

A number of experiments were conducted, each trying to elucidate the observations made. One, involved investigations on the effect of seeding depth on germination, of hot water treated seed and on the germination of seed planted in carbofuran treated soil. The latter experiment was repeated under environmentally controlled conditions in germination chambers with more hot water treatment regimes, more cultivars and increased levels of sowing depths.

5.10 Effect of two sowing depths on the germination of presoaked rice seeds, treated with hot water, and on the germination of seeds sown in soil treated in different ways with carbofuran

5.10.1 Objectives:

This experiment was conducted in June. The objective of the experiment was to investigate the effect of hot water treatment of rice seeds at 54°C for 15 minutes, following presoaking for 18 hours on the germination of seeds at two sowing depths. This treatment regime was chosen because it was found earlier, to give consistantly 100% nematode control. The other objective of the experiment was to investigate whether carbofuran treatment, applied in two different ways was of any advantage in the germination of seeds planted at different depths.

### 5.10.2 Materials and Methods:

Carbofuran at 17.5 kg/ha of 3G and half that rate, was used and applied along planting furrows and in planting holes (stations). The two planting depths were 1.5cm and 6.0cm respectively. Only two depths were used because the germination was done in rectangular metal trays which facilitate a depth determination. The trays were of the following dimensions: 44cm long, 14cm wide and 7cm deep. Seeds previously hot water treated and dried were planted at spacings of 10cm within rows to give 5 seeds per row and three rows giving a total of 15 seeds per tray. Sandy loam soil was incorporated with fertiliser before being placed into the trays. Fertiliser was applied at the rate of 100kg N/ha and 50 kg P/ha. Carbofuran was applied over the seeds along the rows and on top of the seeds (for hole application). The seeds were covered with preweighed soil to give the required depths. Germination test was conducted in the open, outside the laboratory to simulate field conditions. Germination records were taken at 2 - 3 day intervals for a period of 2 weeks.

The experiment was factorial design in a randomised block layout, with two replications. The treatments and their combinations were as follows:

1. Carb/rec rate (17.5kg/ha of 3G) to holes sown deep (6cm) ... ... 2. shallow (1.5cm) .. з. deep furrow ... ... shallow 4. 5. Carb ½ rate (½ of 17.5kg) to holes sown deep .. u 6. shallow 7. furrows deep 8. •• ... shallow 9. Hot water treated seeds sown deep 10. Hot water treated seeds sown shallow 11. Control seeds sown deep 12. Control seeds sown shallow Key: Carb/rec rate is carbofuran recommended rate, and ½ rate is half of the recommended rate.

Analyses of variance and F-tests for significance were conducted to compare chosen means. Contrasts were made for two of the main days of germination namely; the last day of the counts and the 11th day when most treatments had attained their peak germination. The following contrasts were made:

- 1. Hot water versus control.
- 2. Hot water versus control deep.
- 3. Hot water shallow versus control shallow.
- 4. Hot water versus carbofuran.
- 5. Hot water deep versus carbofuran rec. rate deep applied to holes.
- 6. Hot water shallow versus carbofuran rec. rate shallow applied to holes.
- 7. Hot water deep versus carbofuran ½ rate deep applied to furrow.
- 8. Hot water shallow versus carbofuran ½ rate shallow applied to furrow.
- 9. Hot water deep versus water shallow.
- 10. Control deep versus control shallow.
- 11. Carbofuran rec. rate deep versus carb/rec. rate shallow.
- 12. Carbofuran rec. rate deep versus carb/rec. ½ rate shallow.
- 13. Carbofuran deep versus carbofuran shallow.

### 5.10.3 Results and Discussion:

Table 5.23 shows the means for all the variables for all the assessment days and their respective least significant values.

	Mean	number o	f seeds	that ger	minated	out of 1	5 seeds	(mean of	2 repli	cations)			
					Day	s of ass	essment	after so	wing				
Treatments	9	Ø	0	10	11	12	13	14	15	16	19	20	21
C/Rec/holes/deep	000.0	0.330	0.670	3.670	4.670	7.000	7.000	7.670	8.330	8.330	8.330	8.330	8.330
C/Rec/holes/shallow	3.000	6.000	7.670	7.670	9.330	9.330	10.670	10.670	10.670	10.670	10.670	10.670	10.670
C/Rec/furrow/deep	000 0	0.670	1.670	3.330	6.330	7.670	10.330	10.670	11.330	11.330	11.670	11.670	11.670
C/50%/holes/deep	000 0	0.000	0.670	3.000	7.670	000 6	11.000	11.000	11.670	11.670	11.670	11.670	11.670
C/50%/holes/shallow	5.000	7.670	9.330	9.330	9.330	9.670	9.670	9.670	10.000	10.000	10.330	10.330	10.330
C/50%/furrow/deep	000 0	0.330	0.670	4.330	6.330	7.670	9.330	9.330	9.330	9.670	9.670	9.670	9.670
C/50%/furrow/shallow	0.670	7.330	10.330	10.670	10.670	10.670	10.670	10.670	10.670	10.607	11.000	11 000	11.000
Hot water deep	000 0	000.0	0.000	0.000	0.330	1.670	2.000	3.000	3.330	4.330	4.330	4.670	4.670
Hot water shallow	1.000	3.000	4.000	5.330	5.330	7.330	8.000	8.000	8.000	8.000	8.330	8.330	8.330
Control deep	000 0	000 0	0.670	3.670	5.330	8.670	11.670	12.000	12.670	12.670	13.330	13.330	13.330
Control shallow	4 330	7.330	8.660	000.6	9.330	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
L.S.D. 05	3.027	3.259	3.627	5.148	3.633	3.691	3.512	3.922	3.915	3.722	3.687	3.658	3.658
L S D 0 01	6.003	4.430	4.930	6.997	4.939	5.016	4.773	5.331	5.322	5.059	5.011	4.972	4.972
S.E.	1.031	1.111	1.237	1.755	1.239	1.258	1.197	1.337	1.334	1.269	1.256	1.247	1.247
C.V. %	1238.600	59.240	49.130	53.120	28.630	26.690	22.700	24.820	23.990	22.410	21.830	21.600	21.600

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# Table 5.23 (continued)

# Key:

C/Rec/holes/deep	II	Carbofuran recommended rate to holes sown deep
V/Rec/holes/shallow	Ш	Carbofuran recomended rate to holes sown shallow
C/Rec/furrow/deep	11	Carbofuran recommended rate to furrow sown deep
C/Rec/furrow/shallow	11	Carbofuran recommended rate to furrow sown shallow
C/%/holes/deep	11	Carbofuran half rate to holes sown deep
C/%/holes/shallow	11	Carbofuran half rate to holes sown shallow
C/%/furrow/deep	II	Carbofuran half rate to furrow sown deep
C/%/furrow/shallow	IJ	Carbofuran half rate to furrow sown shallow
Hot water deep	II	Hot water treated seeds sown deep
Hot water shallow	11	Hot water treated seeds sown shallow
Control deep	11	Control seeds sown deep
Control shallow	II	Control seeds sown shallow

	Assessed 11 and 21 days af	ter Planting	
	Contrasts	at 11 days after planting	Contrasts at 21 days after planting
<u>S</u>	itrasts	F-value	<b>F-value</b>
ъ.	Hot water versus control	13.200 **	17.180 **
م	Hot water deep versus		
	control deep	8.150 **	24.110 ***
m	Hot water shallow versus		
	control shadow	5.210 *	0.896 NS
4.	Hot water versus carb.	216,740 ***	1.010 NS
ີ ເ	Hot water deep versus		
	carb.rec. holes deep	6.140 *	4.310 *
<b>.</b>	Hot water shallow versus		
	carb. shallow rec. holes	5.210 *	11.570 **
2	Hot water deep versus carb.		
	deep (50% rec. rate) furrow	11.730 **	8.040 *
8.	Hot water shallow versus carb.		
	shallow furrow	9.290 **	0.577 NS
<b>.</b>	Hot water deep versus hot		
	water shallow	8.146 **	4.306 *
10.	Control deep versus control		
	shallow	5.213 *	3.560 NS
11	Carb. rec. rate deep versus		
	carb rec. rate shallow	8.753 *	0.1607 NS
12.	Carb. 50% rate deep versus		
	carb. 50% rate shallow	5.865 *	0,000 NS
13.	Carb. deep versus carb.		
	shallow	14.474 **	0.080 NS
-04			
NG.			
*	- Significant at the 5% level	NS - not Significant	
*	- Significant at the 1% level	carb – carbofuran	
Ť	* - Significant at the 0.1% level	rec – recommended	

Contrasts of Carbofuran, Hot Water Treatment and Sowing Depth on the Germination of Rice Seeds Table 5.24

Table 5.24 show the results of F-tests conducted to compare selected treatments at two of the assessment days (days 11 and 21).

There were significant differences (P = 0.01) at all assessment days after day 6. On day 11 all contrasts made were significantly different, to a lesser or greater level as shown. However, by day 21 (last day of assessment), 7 out of 13 of these contrasts were no longer significantly different.

On day 11 carbofuran with shallow seeds and control shallow seeds germinated better and had achieved two-thirds of germination, while the other treatments (minus hot water deep) with seeds sown 'deep' had only achieved about one third germination. By day 11 hot water shallow treatments had only achieved one third germination similar to treatments sown deep. Meanwhile all the other treatments sown 'shallow' had by then achieved two-thirds germination.

All the contrasts for day 11 were significantly different from each other. On day 21 all the treatments had attained their maximum germination resulting in non significant differences between many contrasts. The differences between the significant contrasts on days 11 and 21 demonstrate the slower rate of germination in some treatments which nonetheless managed to germinate as well as the fast germinators by the end of the experiment.

Days to maximum germination were at least 12 to 13 for the control shallow and carbofuran recommended rate/holes/shallow. Except for the carbofuran/½/furrow/shallow which took 19 days to achieve their maximum germination, the rest of the carbofuran treatments regardless of method of application and depth took longest to achieve their maximum germination (19 to 20 days).

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Maximum germination for the control 'shallow' was attained on the 12th day while for deeply sown seeds it was attained on the 15th day.

The results presented above show that hot water treatment delayed germination and significantly reduced the number of germinated seeds compared to all other treatments. Hot water treated seeds sown deep were the least successful in germination, attaining less than one-third germination by the end of the experiment. While, other comparable treatments in terms of depth, had achieved greater than two thirds germination by the end of the experiment. Therefore, hot water treatment delayed the rate of germination, but under shallow germination depth (1.5 cm), this treatment achieved comparable germination to the rest of the succesfully germinated treatments by the end of the experiment.

These results show that, increased depth seriously affected germination of hot water treated seeds, and the effect of depth was in the emergence and not the actual viability of the seeds. However, there was a proportion of hot water treated seeds that was affected in its viability as assessed by the germination period day 21.

All the shallow treatments (except hot water treatments) had achieved their initial germination by day 6. However, their counterpart seeds sown deep started emerging between day 8 and 9. The control sown deep was one day later compared to some of the carbofuran treatments. However, for all treatments it was less than 6 days and probably 6 days for hot water treatment. Increase in depth significantly affected some treatments, for example carbofuran

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deep compared to carbofuran shallow; but for the control, the opposite was the case where the control seeds planted shallow had lower germination than control planted deep at day 21. This could not be easily explained, hence efforts were made in the subsequent experiment to examine this further. The co-efficient of variation values were very high on the early days of germination which reflect the variability in the rate of germination and emergence which stabilised as the maximum germination and emergence was achieved (days 16 to 21).

These results show that rice seeds cannot emerge well from higher depths (6cm) when their emergence rate is delayed. However, hot water treated seeds were the most affected by deep seeding (6cm) and their rate of emergence was delayed beyond day 8 and 9. The results further show that there was more than the function of depth for hot water treated seeds to emerge. Hot water treatment had probably affected the seeds in such a way that reduced their seedling vigour compared to the other treatments sown at the same depth. The latter debilitation of the seedlings probably resulted in significantly less of them finally emerging above the surface. This aspect of depth and emergence was a factor for further investigations in the subsequent experiment.

5.11 Effect of sowing depth on the germination of hot water treated seeds of three rice cultivars (treated at 48°C and 60°C for 10 and 20 minutes following presoaking in water for 18 hours)

5.11.1 Introduction and Objectives:

This investigation was a follow up of a previous experiment where two germination depths were studied. It was aimed at further

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studying the effect of seeding depth using three depth levels and three rice cultivars. These were tested against two treatment temperature regimes of 48°C and 60°C at 10 and 20 minutes respectively. The experiment was conducted under controlled environment, in germination chambers set at 20°C. It was decided also to record numbers of germinated seeds that fail to emerge in order to assess the effect of these treatments on the emergence and hence vigour of the seedlings.

### 5.11.2 Materials and Methods:

The experimental factors involved are shown below:

(1) Seeding depth:

1cm below the soil
3cm below the soil
7,5cm below the soil

(2) Cultivars:

Bluu

Bagamoyo

Sindano

(3) The seeds were hot water treated following pre-soaking at room temperature for 18 hours and subsequently dried prior to planting. In the case of the control, seeds were not subjected to any treatment.

Treatment combinations were as follows:

- (1) Control (unsoaked and untreated seeds)
- (2) Seed presoaked (in water for 18 hours) and not treated
- (3) Seeds presoaked and hot water treated at 48°C for 10 minutes

(4) Seed presoaked and treated at 48°C for 20 minutes
(5) Seeds presoaked and treated at 60°C for 10 minutes
(6) Seed presoaked and treated at 60°C for 20 minutes
(7) Unsoaked seeds treated at 48°C for 10 minutes
(8) Unsoaked seeds treated at 48°C for 20 minutes
(9) Unsoaked seeds treated at 60°C for 10 minutes
(10) Unsoaked seeds treated at 60°C for 20 minutes.

The experiment was a factorial in a complete randomised layout with 3 replications. Rice seeds used were from a previous One hundred and five seeds were crop which had no nematodes. planted per treatment per replication. Parameters to be studied included; normal germination of seeds, ungerminated seeds (seed that felt hard, looked fresh and not rotten), dead seeds (that were germinated seedlings that had rotting) and not emerged (germinated-unemerged). After assessment of the germinated and emerged seedlings, soil samples from individual germination trays were examined and the numbers of ungerminated seeds were assessed. Those seeds that had germinated but had not emerged were recorded as germinated and unemerged. Seeds that had failed to germinate and were showing signs of rotting were recorded as dead seeds. There were some, seeds which turned out to be empty husks were also recorded.

### 5.11.2.1 Soil Preparation:

Soil used in this experiment was clayey and was seived and mixed with river sand. The soil mixture was 1:2 sand and clay. The resultant soil mixture was steam sterilised. Trays assigned for the 7.5cm depth were filled first with pre-weighed soil to give the required depth level. Seeds for the appropriate treatment and depth were planted in rows. The trays were filled with pre-weighed soil, to make up the top depth level. When all the treatments were completed, the germination trays were placed in the germination chambers set at 20°C. The experiment was left for 18 days before germination assessment was commenced. The final assessment of unemerged seedlings and dead seeds was completed 26 days after the experiment was set up.

Analysis of variance for the factorial aspects of the experiment (using angular transformed data) were conducted and linear and quadratic regressions were fitted in the temperature and time aspects of the treatments. Tests of significance were carried out to further examine means of significant variables. A further analysis of variance involving all treatments was conducted followed by Student-Newmen-Kuels multiple range tests.

### 5.11.3 Results and Discussion:

Summary of significance levels from the analysis of variance tables for the effects of three sowing depths on the germination of hot water treated seeds of three rice cultivars is shown on (Table 5.25). Means for all studied variables and their LSD values are given on (Table 5.26) and the summary for significance levels for the cultivar and depth factors are presented on (Table 5.27).

All treatment factors; cultivar, depth, hot water treatment and their interactions were significant (P = 0.01) for all studied variables and their corresponding linear and quadratic regressions

		% Normal germination	% ungerminated seeds	% unemerged seedling	% Dead seed
Source of Variation	đf	F - value	F - value	F - value	F - value
Variety	2	17.380 **	20.229 **	10.843 **	10.578 **
Depth	N	790.227 **	7.280 **	1090.984 **	* 505 *
Linear		1475.885 **	13.680 **	2048.502 **	4.558 *
Quadratic	-1	104.570 **	0.880 NS	133.405 **	2.060 NS
Hot water (H WT)	б	1218.630 **	4690.105 **	39,758 **	7.926 *
Vart. depth	4	21.479 **	2.239 NS	12.814 **	4.065 *
Dev. Lin	0	40.366 **	3.711 *	23.449 **	4.313 *
Dev. Quad	ດ.	2.592 NS	0.768 NS	2.179 NS	3.816 *
Vart. HWT	18	5 342 **	14.331 **	9.320 **	3.132 **
Depth HWT	18	31.032 **	1.813 NS	38.656 **	2.350 **
Lin. Dev	თ	55 119 **	2 706 NS	69.801 **	3.850 **
Quad. Dev	თ	6 945 **	* 616 * 0	7.510 **	0.849 NS
Vart. Dept HWT	36	8 838 **	1.380 *	7.352 **	1.813 NS
Dev. Lin. Dev.	18	13.819 **	1.506 *	13.087 **	1.729 NS
Dev. Quad. Dev.	18	2.838 **	1.254 NS	1.617 NS	1.898 NS
Residual (MS)	178	23.920 NS	7.379	19.830	4.135
NS - Not signific * - Significant ** - Significant	ant at the 0.05 at the 0.01	level level			

Summary of significance levels from analysis of variance tables for the effects of three sowing depths on the germination of hot water treated seeds, of three rice cultivars. Table 5.25

	   	of 3 repli	cations)				
4	eatment	ŝ	% Germinated	% Dead seeds	% Ungerminated	% Empty husks	% Germinated
VARS	HLdQ	HWT	86608		S C C C C C C C C C C C C C C C C C C C		unemergeo seeds
ч	٦	1	91.43	1.90	3.81	2.86	00-0
1	-	N	95.87	00 0	1 90	1.90	0.32
ч		ო	95.87	0.63	1.27	0.63	1.59
1	1	4	95.87	0.32	1.27	0.00	2.54
٦	-1	ъ С	4.76	2.54	88.57	4.13	00.0
ч	Ч	9	0.32	1.90	96.51	1.27	00.0
ч	Ч	7	93.65	1.27	0.63	2.86	1.59
٦	1	8	91.74	1.50	1.27	2.54	2.86
1	Ч	6	92.38	1.90	1.59	2.86	1.27
٦	H	10	94.60	2.86	0.95	1.27	0.32
1	N	Ч	92.04	1.27	1.92	2.23	2.54
٦	2	0	92.70	1.59	2.54	0.32	2.86
-	<ul> <li>N</li> </ul>	ო	94.91	0.95	0.63	0.32	3.18
	2	4	90.48	0.95	2.22	2.86	3.49
-	2	ى س	5.40	2.54	89.21	1.90	0.95
	. 0	9	1.90	0.95	93.65	2.22	1.27
	2	2	95.56	0.95	0.63	0.31	2.54
		80	97.78	0.00	0.63	0.63	0.95
-	0	თ	88.25	1 90	2.22	3.81	3.81
		10	86.03	2.54	3.17	4.44	3.81
	<u>מ</u> ו	-	55.24	2.86	2.86	0.00	39.05
	ო	N	81.34	2.53	2.53	3.16	10.44
	ი ო	ι m	34.81	0.94	2.53	1.27	60.44
-	ი ი	4	55.24	00 0	0.95	0.95	42.86
-	ო	ы	2.54	5.08	88.89	0.95	2.54
٦	ო	9	0.95	68.6	86.03	0.95	3.17
ч	ო	7	44.30	4.11	0.63	4.75	46.20

Effect of seeding depth, cultivars and hot water treatment on germination of rice seeds (Mean percentages Table 5.26

011174098416160468666666666666666666666666666666	seeds	% Dead £
0 1 1 1 7 4 6 9 8 4 1 8 1 8 6 8 8 8 8 1 8 1 8 1 8 1 8 1 8 1		
	2	1.2
	2	2.2
ч ч ч ч ч ч ч ч ч ч ч ч ч ч ч ч ч ч ч	0	0.0
4 4 4 6 6 6 6 7 8	0	0
4 9 8 9 4 4 6 4 6 7 8 9 8 9 4 4 8 4 8 7 8 9 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7	6	4.76
8 8 8 9 1 8 1 8 2 8 9 9 8 9 9 1 8 1 8 1 8 9 8 9 8 9 8 9 8		4.42
988 989 989 989 989 989 989 989 989 989		3.81
8 8 9 7 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9		3.17
4 1 6 1 6 7 4 9 8 6 7 7 1 7 1 8 9 7 9 8 9 7 7 8 9 7 7 8 9 8 1 8 1 8 9 7 7 8 9 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1		3.17
ч с ч с ч с ч с ч с ч с ч с ч с ч с ч с		0.32
9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9		1.59
- 0 8 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		1.90
8 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	_	1.59
2 4 0 8 8 6 7 2 8 8 0 4 2 2 8 0 4 2 2 8 0 4 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		0.95
4 9 8 8 9 9 4 9 8 9 4 9 8 9 4 9 8 9 4 9 9 8 9 9 4 9 9 9 9	_	3.49
85 6 87 6	<i>'</i> 0	4.76
85.40 87.62 87.62 8.25 8.25 7.22 8.25 7.22 8.25 7.22 8.25 7.22 8.25 7.22 8.25 7.22 7.22 8.25 7.22 8.25 7.22 7.22 8.25 7.22 8.25 7.22 7.22 8.25 7.22 7.22 7.22 7.22 7.22 7.22 7.22 7	~	6.0
87.62 3.49 8.25 8.25 0.00 8.25 2.22 1.22 2.23 2.60 2.60 2.60 2.60 2.60 2.60 2.60 2.60	~	5.08
3.49 9.25 9.25 9.25 9.25 9.25 9.25 9.25 9.2		11.75
2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5		1.27
1.27 8.25 0.00 5.00 0.00		36 0
2,22 8,25 0,00 6,00	_	2.54
1.25 0.00 1.25		0.32
8.25 0.00	~	0 0
0.00	~	0.63
1 50		0.95
		1 - 50
86.67		5-40
B7_94		50 9

Table 5.26 (continued)

•
н	reatmen.	ts	% Germinated	% Dead seeds	% Ungerminated	% Empty husks	% Germinated
VARS	HTAO	HWT	Beeda		seeds		unemerged seeds
N	в	7	65 . 19	0.63	00.0	3 80	30.38
N	e	Ø	68.99	6.33	0.00	4.43	20.25
N	e	თ	79.43	0.63	0.00	0.63	19.31
2	e	10	38.56	0.63	1.58	1.27	57.95
ო	ч	ч	88.57	2.86	2.86	4.76	0.95
ო	٦	N	96.51	1.27	0.00	1.27	0.95
ო	ч	ო	94.91	0.00	3.50	1.59	00.0
ო	ч	4	99.05	0.63	0.00	0.32	00.0
ო	ч	Ŋ	27.62	0.63	67.62	0.00	4.13
ო	ч	9	3.49	1.27	93.02	1.59	0.63
ო	٦	7	91 75	0.63	5.08	1.90	0.63
ო	г	Ø	97.78	0.00	1.27	0.63	0.32
ო	٦	თ	94 60	1.27	2.54	1.59	00.00
ო	Ч	10	94 60	0.32	3.49	0.95	0.63
ო	N	-1	90.16	2.54	2.54	1.90	2.86
ო	0	2	94 29	0.63	1.90	1.27	1.90
e	2	ო	98.10	0.32	0.32	0.00	1.27
ო	2	4	97.14	0.32	0.32	0.00	2.22
ო	2	51 Cl	13.65	1.27	69 84	2.22	13.01
ო	2	9	1 90	4 44	90°79	1.27	1.59
ო	2	7	92 06	2 54	1.27	0.95	3.17
ო	N	Ø	91 11	2.54	0.32	1.59	4.44
e	2	თ	63°09	00.00	2.83	1.56	2.52
e	2	10	88 57	1.59	4.76	3.81	1.27
ო	e	-	41 90	2.86	2.86	2.86	49.52
ო	ო	0	73 33	3.17	1.59	2.22	19.68
ო	e	ო	50.32	0.63	0.95	0.63	47.47
ო	e	4	67.62	0.63	0 00	0.32	31.43

Table 5.26 (continued)

							1
Ĥ	reatment	ts	% Germinated	% Dead seeds	% Ungerminated	% Empty husks	% Germinated
VARS	DPTH	TWH	ReedS		86608		unemerged seeds
е С	ო	ى د	3.17	1.27	74.60	2.22	18.73
ო	ო	9	0.63	6.03	88.57	0.63	4.13
e	ო	7	52.38	0.95	2.86	1.90	41.90
ო	ო	80	56.64	2.85	2.85	4.43	33.23
ო	ო	თ	86.03	1.27	1.27	0.63	10.79
ო	n	10	79.05	0.63	0°*0	0.32	20.00
LSD LSD	.05		8.070	3.282	4.475	2.982	7.212
LSD	5		10.797	4.391	5.987	3.990	9.649
SE			2.824	1.148	1.566	1.043	2.523
CV %			7.86	95 . 81	14.21	92.92	43.70
Key -	vars =	cultivars; ]	Dpth = depth; HWTR =	Hot water treatment	ţţ		

Table 5.26 (continued)

cult	errect or three sowing deputivars	n to the germination of n	ot water treated seed	s of three rice
(a)	Cultivar means for combi treated at 48°C and 60°C	ned seedling depths (1.0, for 10 and 20 minutes fol	3.0 and 7,5 cm) for s lowing presoaking.	eeds hot water
Cultivar	Mean % germinated seeds	Mean % ungerminated seeds	Mean % unemerged seeds	Mean % dead seeds
Sindano	68.7	20.2	10.6	1.51
Bagamoyo	65.4	19.4	9.1	2.83
Bluu	64.6	17.7	12.2	1.88
SED	0.73	0.40	0.65	0.30
TSD (2%)	1.44	0.80	1.29	0.59
(b) Cultivar m	eans for seeds hot water tr	eated and sown at 1 cm dep	th	
Cultivar	Mean % germinated seeds	Mean % ungerminated seeds	Mean % unemerged seeds	Mean % dead seeds
Sindano	78.9	17.9	0.83	0.89
Bagamoyo	71.2	21.8	1.58	2.51
Bluu	75.7	19.8	1.05	1.49
SED	1.26	0.70	1.13	0.51
LSD (1%)	2.55	1.39	ı	1.04

Summary of significance levels for the cultivar and depth factors from the experiment to study the effect of three source of three sizes Table 5.27

(c) Cultivar m	eans for seeds hot water to	reated and sown at 3 cm dep	th	
Cultivar	Mean % germinated seeds	Mean % ungerminated seeds	Mean % unemerged seeds	Mean % dead seeds
Sindano	76.0	17.5	3.43	1.62
Bagamoyo	71.3	19.9	2.41	3.71
Bluu	74.5	19.7	2.53	1.37
SED	1.26	0.70	1.13	0.51
TSD (1%)	2.55	1.39		1.04
(d) Means for	seeds hot water treated and	i sown at 7.5 cm depth		
Cultivar	Mean % germinated seeds	Mean % ungerminated seeds	Mean % unemerged seeds	Mean % dead seeds
Sindano	51.1	17.5	27.7	2.03
Bagamoyo	53.8	18.7	23.4	2,28
Bluu	43.7	18.8	33.1	2.79
SED	1.26	0.70	1.13	0.51
LSD (5%)	2.55	1.39	2.28	1.04
LSD (1%)	3.47	8	3.10	ł

Table 5.27 (Continued)

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were also significant (P = 0.01). After analysis of variance, the empty husk variable was not examined further because treatment factors for the latter variables had not been significant. As a result of the latter variable and some miscounts of the seeds, a few total germination percentages were slightly below or above hundred.

A11 rice cultivars had a significant reduction in germination from 3cm to the 7.5cm seeding depths (Figure 5.14). Cultivar, Bluu had a significantly lower percentage of fresh looking ungerminated seeds (P = 0.01) compared with the other two cultivars which themselves did not significantly differ. Effect of different sowing depths on the germination of seeds by the different cultivars differed with cultivar Bagamoyo and depth. In comparative terms, Bagamoyo had the least reduction in percentage normal germination of the three cultivars at 7.5cm seeding depth. However, all rice cultivars were not significantly different in percentage ungerminated seeds at 7,5cm depth.

For the percentage germinated but unemerged seeds, cultivars did not differ significantly for the 1cm and 3cm sowing depths. However, for the 7.5cm sowing depth, all the three cultivars were significantly different with cultivar Bagamoyo having the least number of seeds that germinated and failed to emerge (Table 5.27). The rate of change in the percentage germinated and unemerged seedlings increased with depth for all cultivars; showing that, all cultivars were more affected in their ability to emerge from 7.5 cm seeding depth (estimated slopes were; 5.362 for Bluu; 3.692 for Bagamoyo and, 4.472 for Sindano). The increase in the percentage unemerged seedlings was significantly greater for 7.5cm seedling

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Fig 5.14 : Effect of Depth × cultivar on percentage normal germination of rice seed



Fig 5,15: Effect of Depth × cultivaron the percentage dead and rotting seed.

depth for all cultivars (Figure 5.17).

Percentage numbers of dead seeds varied with cultivar and depth (Table 5.27 and Figure 5.15) with cultivars Bagamoyo having the highest dead seeds and Sindano the least at 1cm depth. Rate of change in percentage dead seeds also differed with cultivar (Blue = 0.218; Sindano = 0.162 and Bagamoyo = -0.0089) indicating that there were significant increases in the percentage dead seeds with increase in seeding depths for cultivars Sindano and Bluu, while for cultivar Bagamoyo the reverse was true.

The effects of depth, irrespective of cultivar or treatment, on some studied aspects of germination is shown in Figures 5.16 – 5.19. Means covered by the same LSD lines were not significantly different. There was a significantly lower germination (P = 0.01) from a 7.5cm seeding depth compared with that of 3cm and 1cm depths (Figure 5.16). The latter two seeding depth were not significantly different.

There was a significantly higher percentage of germinated but unemerged seedlings from a 7.5cm seeding depth (P = 0.01) compared with the two other depths (Figure 5.17) which themselves did not significantly differ. Percentage ungerminated seeds were significantly higher at 1cm depth compared to 3cm and 7.5cm sowing depths and the latter two depths were not significantly different (Figure 5.18). There was a significantly lower percentage dead seeds from a 1cm seeding depth compared to that of the other two depths which themselves were not significantly different (Figure 5.19).



Effect of seeding depth on the germination of hot water treated seeds

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Cultivar Sindano had a significantly lower percentage of fresh looking ungerminated seeds (P = 0.01) compared with the other two cultivars which themselves did not significantly differ (Figure 5.20). Effect of sowing depth on the germination of seeds by the different cultivars showed cultivar Bagamoyo to have the highest percentage ungerminated seeds at 1cm sowing depth. At 3cm seeding depth, cultivars Bagamoyo and Bluu were significantly different (P = 0.01) from Sindano. At 7.5cm depth, all the three cultivars were not significantly different in terms of percentage ungerminated seeds. Cultivars Bagamoyo and Bluu had comparatively higher percentage ungerminated seeds at all seeding depth compared to variety Sindano. However, all cultivars had negative slopes (Sindano = -0.25; Bluu = -0.156 and Bagamoyo = -0.065) indicating that the percentage ungerminated seeds were decreasing with increase in the seedling depth.

For the percentage germinated but unemerged seeds, all cultivars were not significantly different for the 1cm and 3cm sowing depths. However, for the 7.5cm sowing depth, all the three cultivars were significantly different with cultivar Bagamoyo having the least number of seeds that germinated and failed to emerge (Figures 5.21). The rate of change with depth in the percentage germinated and unemerged seedlings increased for all cultivars showing that, all cultivars were more effected in their ability to emerge from 7.5cm seeding depth (estimated slopes were; 5.362 for Bluu; 3.692 for Bagamoyo and, 4.472 for Sindano). The increase in the percentage unemerged seedlings was significantly greater at 7.5cm seedling depth for all cultivars (Figure 5.21).



Fig 5.20: Effect of depth x cultivar on the percentage fresh looking ungerminated seeds



Fig 5.21: Effect of depth × cultivar on percentage germinated unemerged seeds

The results presented above showed that the three rice cultivars studied exhibited some differences in their ability to germinate into normal seedlings, with cultivar Sindano germinating significantly better than the other two cultivars which themselves did not significantly differ. Germination performance by the three cultivars by depth, showed that cultivar Sindano germinated best at 1cm and 3cm depth at which levels Bagamoyo cultivar was the least in germination. At 7.5cm depth, cultivar Bagamoyo germinated best and cultivar Bluu was the least in percentage normal germination.

The comparative ability to germinate better at 7.5cm seeding depth by cultivar Bagamoyo whose seeds are the smallest of the three cultivars where cultivar Bluu with the biggest seeds had the poorest normal germination is noteworthy.

Cultivars Sindano and Bluu had positive regression slopes for percentage dead seeds with depth (0.162 and 0.218 respectively) suggesting that the number of dead seeds increased with depth for the two cultivars while the reverse was the case for cultivar Bagamoyo with a - 0.089 regression slope (Figure 5.15). These results indicate that these rice cultivars have different optimal seeding depths. Cultivar Sindano maintained the best viability when shallow seeded (1cm). On the other hand cultivar Bagamoyo, had the best percentage normal germination when seeded at 7.5cm depth. The differential reaction by the cultivars to seeding depth can be attributed to their inherent seed condition which could be genetic or environmentally conferred.

In general and irrespective of treatment, normal germination was significantly decreased at the deeper seeding depth of 7.5cm

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(Figure 5.16) demonstrating that, the latter seeding depth by itself is not conducive for germination of rice seeds. The results demonstrated that, rice seeds have optimal depths from which they can successfully germinate and emerge. The results agree with a statement made by Purseglove (1972) that in a dry seed bed the optimum sowing depths for rice is 5 - 6cm in light loams, but not more than 2 - 3cm in heavy clays.

This can be explained by the fact that the ability of a seedling to emerge successfully from any given depth is a function of its amount of vigour which in turn is determined by many factors including amount of stored food, and the overall quality of the seed (Heydecker and Coolbear 1977). The latter is governed by the environment on which it was formed, which means nutritional and climatic conditions at the site of production. The position of the seeds on the mother plant and the stage at which the physiological ripening process was terminated were also considered important as these determine the rate, degree and uniform nature of maturation of the seeds and hence the ability of individual seeds to germinate and successfully emerge from below the soil (Heydecker and Coolbear, 1977).

Seeds of Rice cultivars, irrespective of treatment, differed in their percentage failure in germination and emergence. This result showed that failure to emerge by some proportion of seeds that had germinated, was due to cultivar differences rather than due to treatment. It is noteworthy that cultivar Bagamoyo has a comparatively smaller size seed than the other two. Heydecker and Coolbear (1977) discussing seed quality stated that larger size seed

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or greater seed weight is not necessarily an advantage except in food storage; and the smaller seeds are probably only disadvantaged in the timing of their seedling emergence. The results obtained in this study seem, to differ from Heydecker and Coolbear (1977) statements concerning the advantages in the timing of seedling emergence by bigger seeds compared with smaller seeds.

Summary of the results of the Students - Newman - Kuel multiple range test to separate significant treatment treatment means for all the variables is shown on (Table 5.28). Unsoaked seeds treated at 60°C for 10 minutes and seeds soaked but not exposed to hot water treatment gave a significantly higher normal germination compared with the rest of the treatments. Presoaked seeds treated at 60°C for 10 or 20 minutes gave significantly lower percentage normal germination compared with the rest of the treatments (7.443 and 1.129 respectively).

Seeds presoaked and treated at 60° for 10 and 20 minutes had a significantly higher percentage ungerminated seeds (91.08 and 82.99% respectively) compared to the rest of the treatments, which did not differ significantly. The percentage ungerminated seeds for the latter treatments ranged from 1.36 to 3.31.

Seeds presoaked and treated at 60°C for 20 minutes had the lowest seeds that germinated and failed to emerge. The treatments: presoaked and not treated, presoaked and treated at 60°C for 10 minutes and, seeds unsoaked and treated 60°C for 10 minutes were not significantly different, having 5.2, 5.5 and 7.2% unemerged seedlings respectively. On the other hand, the control, seeds unsoaked and treated at 48°C for 10 minutes and 20 minutes,

Table 5.28 Effect of three sowing depths on the germinations of hot water tree	vater tre	ated seed	of	three r	rice
CULTIVATE IOLIOWING presoaking (Angular Transformed Data)					

Treatments	Mean % germinated <u>seeds</u>	Mean % ungerminated <u>seeds</u>	Mean % unemerged <u>seeds</u>	Mean % dead seeds
Presoaked/60°C/10 min	7.443	82.990	5.200 a	3.00
Presoaked/60°C/20 min	1.129	91.080	2.060	4.94
Presoaked/48°C/10 min	79.129 a	2.120 a	16.320 b	2.09
Presoaked/48°C/20 min	79.215 a	2.200 a	12.680 c	1.650
Presoaked only	87.308 b	3.310 a	5.500 a	
Unsoaked/60°C/10 min	87.538 b	1.990 a	7.190 a	1.580
Unsoaked/60°C/20 min	80.546 ac	2.200 a	14.760 bc	1.164
Unsoaked/48°C/10 min	79.467 a	2.170 a	14.980 bc	1.460
Unsoaked/48°C/20 min	82 <b>.</b> 963 c	1.360 a	15.250 bc	1.570
Control	77.567 a	2.710 a	16.040 b	1.790

<sup>&</sup>lt;u>Key</u> : Means having the same letter were not significantly different Students - Newman - Kuel multiple range test.

presoaked seeds treated at 48°C for 10 and 20 minutes, dry seeds treated at 60°C for 20 minutes had the highest unemerged seedlings and, were not significantly different.

Presoaked seeds treated at 60°C for 10 minutes gave significantly higher percentage of dead seeds. The rest of the treatments were not significantly different.

Results above demonstrated that presoaking seeds in water for 18 hours followed by hot water treatment at 60°C for 10 or 20 minutes was too drastic, resulting in a significant reduction in viability to 7.443 and 1.129% respectively with a corresponding 82.99 and 91.08% seeds whose germination potential was destroyed. On the other hand, seeds that were treated at 60°C for 10 or 20 minutes without prior soaking resulted in 87.54 and 80.55% normal germination respectively, with treatment at 60°C for 10 minutes actually gave the highest percentage normal germination compared with all other treatments including control.

These results show that rice seeds hot water treated without presoaking can tolerate significantly higher temperatures compared to those treated in the same way following 18 hours presoaking. The results agree with Mayer and Poljakoff - Mayber (1978), who stated that many types of seeds are able to resist extreme temperatures if treated without presoaking. Results of treatments at 60°C for 10 or 20 minutes of presoaked seeds demonstrated that, the latter treatments were drastic resulting in the destruction of seed viability.

Treatment at 60°C for 10 or 20 min (following presoaking) resulted in significantly higher percentages of seeds that were dead

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and rotting. The percentage unemerged seedlings for most of the other treatments including the control (Table 5.28) were statistically not significantly different, suggesting that for those treatments a greater proportion of the unemerged seedlings did not fail due to the effects of hot water treatment but were probably lacking in vigour due to other factors.

This study demonstrates that hot water treatment at 60°C for 10 or 20 minutes following presoaking effected total destruction of viability for almost all the seeds. On the other hand, hot water treatment at 48°C for 10 or 20 minutes of unsoaked or presoaked seeds and hot water treatment of unsoaked seeds at 60°C for 10 minutes did not impail germination, but rather improved it. (Table Soaking seeds without hot water treatment for 18 hours 5.28). followed by germination resulted in the least percentage failures to emerge (excluding the two extreme treatments at 60°C for 10 or 20 minutes following presoaking) compared with the rest of the treatments including the control. This could have been due to the added advantage given by the period of imbibition to initiate germination process, giving a faster rate of emergence before conditions such as oxygen concentration in the soil were depleted. Ballard (1969) stated that conditions during the first minutes or hours of imbibition can make the difference between good and bad germination and between vigorous and poor growth. Heydecker and Coolbear (1977) observed that there are germination advantages in hydrating and subsequent drying back of seeds, such as advancing germination. Unsoaked seeds treated at 60°C for 10 minutes, seeds that were only presoaked without hot water treatment and, seeds

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presoaked and treated at 48°C for 20 minutes had significantly greater germination compared with the control. Therefore, these treatment regimes did not adversely affect germination potential but rather, they positively influenced germination. Chappendale (1933) soaked seeds of Dactylis glomerata L. at 20°C for 17 hours before drying back and attributed the subsequent germination advantage to an increased rate of water uptake by breaking the permeability barriers. The outer tissue of the caryopsis (the pericarp) is impermeable to movement of oxygen, carbon dioxide and water vapour. Therefore, presoaking will facilitate breaking the latter permeability barrier, placing presoaked seeds at an advantage over unsoaked seeds. The results also demonstrate to what extend dry seeds can tolerate high temperatures, while at the same time taking advantage of hydration giving them a germination advantage.

Results of this study also demonstrated that the three rice cultivars studied have different tolerance to hot water treatment; with Sindano being significantly more tolerant compared with the other two and, that the effect of hot water treatment resulted in more ungerminated seeds than unemerged seedlings for all cultivars. The observations confirmed the above statement that the tested treatments following presoaking were two extremes, the one almost totally destroying viability, while the other did not result in significant adverse effects on germination. Thus, for those treatments whose effects on germination were comparative to the control, viability and vigour were not significantly affected. However, three treatments (soaking only, dry seed treatment at 60°C for 10 minutes and presoaked and hot water treatment at 48°C for 20 minutes) resulted in better germination and emergence demonstrating an added advantage on germination over the control conferred by these treatments.

Figure 5.22 presents the trend in the percentage normally germinated seeds with depth for each of the ten treatments and the corresponding estimated linear regression slopes. There was generally a decrease in the percentage normal germinated seeds as shown by the negative estimated slopes. The graphs in (Figure 5.22) constituted two main groups at significantly different levels of germination percentage axis showing the extent of the levels of extremity of these treatment groups. The one group lying between 50 and 90 % germination for the three seeding depths while the other group of treatments (following presoaking) at 60°C for 10 and 20 minutes respectively lay between 12 and 1% germination. For the latter group there was a minimal decrease with depth in percentage germination especially for the treatment 60°C/20 minutes. This is because the latter two treatments killed almost all the seeds.

There was a decrease in the percentage ungerminated seeds with depth and a corresponding increase in the percentage seeds that were classified as dead and rotting. This reflects a flow in the subjective criterion used to separate seeds that were assumed dead by other causes from seeds failing to germinate because their viability was destroyed by hot water treatment. Due to prolonged period of the experiment, and due to germination delays caused by temperature and seeding depth, the seeds that otherwise would have been classified as fresh looking ungerminated were more likely getting rotten by the time these seeds were assessed. Consequently,

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Fig 5.22: Rate of change with depth of Percentage normal germination for each hot water treatment.

they were put in the category of dead and rotting seeds.

The result of the effect of different treatments on germination demonstrated that unsoaked seeds treated at 60°C for 10 minutes and seeds that were soaked and treated were not only better in germination than the other treatments but had their germination improved compared to the control.

Figure 5.23 shows the graphical representation of the percentage germinated and unemerged seedlings. These results go further to elucidate the fact that most of the treatments were as good as the control with respect to seed emergence. The treatments such as soaking without hot water treatment and treatment at 60°C for 10mm without soaking or at 48°C for 20 minutes following presoaking, resulted in an improvement in the vigour to enable seeds to emerge comparatively better than the control from lower depths.

Figure 5.24 is a graphical representation of the percentage dead seeds with depth for each treatment. Seeds presoaked and hot water treated at 60°C for 20 minutes had a significantly greater rate of change with depth compared with the rest of the treatments showing that with increase in depth, the number of seeds that died increased. This means that some of the seeds left viable after these treatments were so weak, that the environments in the deeper seeding levels resulted in total loss of viability.

Percentage dead and percentage empty husks had very high co-efficient of variation showing that the chances for latter variables to appear are low giving a patchy frequency distribution. The latter variable was not significant for all treatment factors and their interaction confirming the fact that they were non-viable



Fig. 5.23: Percentage germinated and unimerged seeds at various depths after different treatments.



Fig. 5.24: Rate of change with depth of Percentage Dead and Rotting Seeds for each hotwater treatment

and therefore, were not affected by treatments.

It can be concluded that hot water treatment at 60°C for 10 or 20 minutes following presoaking significantly lowered seed germination in all the rice cultivars. Cultivar Sindano resulted in a significantly higher percentage seeds that germinated and failed to emerge. Most of the seeds that did not emerge from the 60°C for 10 or 20 minutes treatment following presoaking had not germinated, indicating that they had totally lost viability as a result of this treatment.

The 7.5cm depth can be considered suboptimal for successful emergence of seedlings. The results also confirm the statement by Heydecker and Coolbear (1977) that seed from the same plant may differ in their quality depending on the growth and harvest conditions. The difference in quality may be reflected in their ability to perform in germination and subsequent growth.

However, some treatments like soaked and untreated seeds, unsoaked seeds treated at 60°C for 10 minutes and presoaked seeds treated at 48°C for 20 minutes had their rate of germination increased beyond that of the control and, their rates of change in the percentage unemerged seedlings were correspondingly lower than that of control with significantly lower percentage unemerged seeds and, a significantly higher percentage germination (Figures 5.22, 5.23 and 5.24). Therefore, seeds from these treatments could be considered as having had greater viability potential and increased vigour beyond that of the control. Hence, these latter treatments could be considered as possible treatments for improvement of these aspects of seed germination.

#### 6. CONTROL OF A. BESSEYI

#### 6.1 Introduction

It was observed that most of the recommended pesticides, including nematicides, were often not available in Tanzania, mainly due to shortage of foreign currency. Tanzanian farmers resorted to pesticides, only when other forms of pest management were not available. The reasons for low priority of chemical control methods in Tanzania was financial constraints, scarcity of pesticides and inadequate knowledge of their use.

Carbofuran, a nematicide-insecticide with systemic activity was chosen for use in the present study because it was recommended for nematode control in rice (FMC Tech. Bull.) and, it was available in the country for banana insect control. Dazomet was used in these investigations to fumigate and sterilise the soil.

Carbofuran was tested at the recommended (17.5 kg of 3G/ha) and at half the recommended rate for the control of white tip nematode in rice (FMC Tech. Bull.). Various methods of application and time of treatment were tested. A number of experiments were conducted to study various methods of using carbofuran in the control of A. besseyi.

6.2 A preliminary field study of the effect of <u>A.</u> <u>besseyi</u> on some aspects of growth and yield of a rice cultivar "Mpunga mwepesi"

In the screening tests, 36 out of 195 rice cultivars assessed were found to be infested with <u>A. besseyi</u>. Highest seed infestations were in cultivars such as Mpunga mwepesi and Kajibi, with 85 and 52% seed infestations respectively. From 100 seeds of each of the cultivars: Mpunga mwepesi, Kajibi and Nyuki, 1 056, 605 and 55 nematodes respectively were recovered. Consequently, rice cultivar Mpunga mwepesi was chosen for use in the preliminary field study conducted to assess the effect of controlling <u>A. besseyi</u> on some aspects of growth and yield.

#### 6.2.1 Materials and methods

The experiment was a randomised block design with four replications of six treatments. Seeds of cultivar "Mpunga mwepesi" infested with <u>A. besseyi</u> and hot water treated to control <u>A. besseyi</u> were planted in untreated plots, in plots treated with carbofuran at planting and in plots pretreated with dazomet (to control soil nematodes and other pests/pathogens).

## 6.2.1.1 Chemical treatment

Dazomet was used to pretreat the soil in order to minimise the effects of soil nematodes and other soil pathogens/pests in an effort to isolate the effects of <u>A. besseyi</u> and those of controlling <u>A. besseyi</u> on some aspects of growth and yield of a rice cultivar "Mpunga mwepesi". A granular formulation of dazomet was used in this experiment and was applied to a depth of 22.5 cm along the furrows that were 20 cm apart, at the rate of 595 g of dazomet per plot of 15 m<sup>2</sup>. Details of soil treatment in the plots is given in the Materials and Methods Section of the thesis.

Carbofuran 3G was applied at planting at the rate of 17.5



kg/ha to the plots. The chemical was applied to furrows 10 cm apart to the depth of 7.5 cm.

#### 6.2.1.2 Hot water treatment of seeds

It being the first attempt with hot water treatment, choice of hot water treatment of seeds without presoaking at 60°C for 10 minutes was made as a compromise from many suggested treatment regimes in the review by Fortuner and Orton Williams (1975). A prewarmed thermos flask was filled with water heated to 60°C, about 300 g lots of dry seeds were treated at constant temperature of 60°C for 10 minutes. After treatment, seeds were removed from the flask and water was quickly drained. subsamples from the treated and untreated seeds were taken for assessment for live and dead nematodes and the remainder was taken to the field for planting on the same day of treatment. Seeds that remained after planting were returned to the laboratory and dried on laboratory benches. The dried and stored seeds were subsampled and assessed once more for live and dead nematodes, 5 days after treatment.

## 6.2.1.3 Planting, fertilizer application and weeding

Experimental area was tractor ploughed, disc harrowed and plots were prepared using hoes and rakes. Planting was done by hand. Treatments were allocated to the plots randomly and seeds were sown in rows 20 cm apart and two seeds drilled 10 cm apart to be thinned to one, 5 weeks later. Seeds left after planting from treated and untreated samples were returned to the laboratory to be stored and were subsequently used for nematode assessment, 5 days after treatment. Phosphate fertilizer was applied at planting at the rate of 50 kg P/ha and nitrogen was applied at the rate of 90 kg N/ha. Nitrogen was applied in split application, half at planting and the rest at 50% booting stage. Fertilizer was applied along planting rows and top dressing with nitrogen was applied between rows. Weeding was done by hoes, three times before maximum tillering. No weeds were found in the plots treated with dazomet. 6.2.1.4 Nematode assessment in seeds used in the experiment

Assessment for nematode kill was done from hot water treated and untreated seeds on the day of treatment and 51 days after treatment. Seeds were singly dehusked and both husk and seed were placed in individual watch glasses where water was added to cover the samples. Details of nematode assessment is given in the Materials and Methods Section of the thesis.

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#### 6.2.1.5 Growth parameters

Ten plants taken at random from each plot were used to determine the following parameters; fresh weight, dry weight and panicle length. Fresh weight was taken by weighing the plants cut at the ground level. For practicability, after taking all the other parameters, the plant material belonging to the same treatment were bundled together per replication and put into a brown sampling bag. The samples were placed in the drying oven for drying to constant weight. Dry weight was then assessed.

#### 6.2.1.6 Yield parameter

Ten plants from a marked central plot area were used for panicle assessment. Mother tiller (first to boot) was used in measuring this parameter. Each panicle was taken separately and the number of filled and unfilled spikelets were determined. Percentage ripened grains was considered as the ratio between the total number of spikelets formed (filled and unfilled) and the number of filled spikelets (regardless of whether or not filled) on the same panicle. 6.2.1.7 Nematode population determination in flowers and harvested seeds

This was done starting from the stage of flowering at weekly intervals up to the stage of grain maturity. Flowers were randomly sampled from panicles of randomly chosen plants. A total of 50 flowers were sampled from each plot. The flowers were opened and placed in a watch glass and covered with little water and left for about 24 hours. After this period, each sample was observed under the microscope and nematodes were counted. This was repeated several times during grain filling stage until the grains were ripe.

## 6.2.2 Results and discussion

There were 0 and 18.8% live nematodes in the treated seeds on the date of treatment and 5 days after treatment respectively. The control had between 98 and 100% live nematodes when assessed on the same day of treatment and 5 days after treatment respectively (Table 6.1). Nematodes were found to a greater or lesser extent in all treatments and in most of the investigated flowers and spikelet development stages (Table 6.2). Control seed (untreated) whether sown into carbofuran or dazomet treated soil or where sown in untreated soil yielded much higher nematode numbers at seed maturity.

Mean fresh and dry weight of rice plants and mean panicles length are presented in Table 6.3. Plants from hot water treated seeds gave the lowest fresh and dry weights and the greatest mean panicle lengths of all treatments. Mean panicle lengths were much smaller for plants grown from untreated seed and in untreated soil compared with the rest of the treatments.

The control (untreated seed and untreated soil) gave the least mean number of spikelets per plant and the least percentage filled grains compared with the rest of the treatments (Table 6.4)

Control seeds (infested with nematodes) sown in untreated soil gave the least panicle sizes, the least mean number of spikelets per plant, least percentage seed filling and the least grain yield compared with rest of the treatments, while hot water treated seeds sown in carbofuran treated soil or untreated soil gave the highest grain yield (Tables 6.3 and 6.4). Carbofuran and hot water treatments gave high percentage seed filling and corresponding

Treatment	Total No. of Seeds Examined	No. of Seeds with Nematodes	% Seed Infesta- tion	Total No. of Nema- todes Recovered	Total No. of live Nematodes	Total No. of dead Nematodes	% Live Nematodes
An the Dov of Treetowt							
TI ALL TAY OF TICA CHIELD							
Treated	300	114	38.0	849	ο	849	0
Control	300	122	40.7	819	803	16	98.4
Five Days After Treatment							
Treated	300	133	44.3	824	155	669	18.8
Control	300	119	39.7	643	643	ο	100.0

Nematode assessment of hot water treated rice seeds used in the experiment to assess the effects of controlling <u>A</u>. <u>besseyi</u> on some aspects of growth and yield of a rice cultivar "Mpunga mwepesi" Table 6.1

Prevalence of  $\underline{A}$ . <u>besseyi</u> in sample flowers and spikelets at different stages of rice flower and seed development Table 6.2

(50 flower or spikelet samples)

Treatment	Nematode infestation	At Flower Opening	2nd Week after Flowering	3rd Week after Flowering	4th Week after Flowering	At Seed Maturity
Untreated soil and treated seed	No of infested flower Total No of Nematodes	seeds 3	ຎຓ	6 210		00
Carbofuran treated and untreated seed	No of infested flower Total No of Nematodes	seeds 6 51	5 24	5 197	9 299	8 220
Dazomet treated soil and untreated seed	No of infested flower total No of Nematodes	seeds B	6 350	10 60	4	7 152
Carbofuran treated soil and treated seeds	No of infested flower Total No of Nematodes	seeds 4 5	8 11		00	00
Dazomet treated soil and treated seeds	No of infested flower Total No of Nematodes	seeds 1 1	6 1	С	00	00
Untreated soil and untreated seeds	No of infested flower Total No of Nematodes	seeds 18 75	15 189	22 474	13 218	25 1189

•

Treatment	X Fresh wt. (g) /plant	X Dry wt. (g) /plant	X panicle length cm /plant
Seed treated with hot water	84.73	15.75	18.21
Carbofuran treated soil planted with untreated seeds	134.40	25.85	17.56
Dazomet treated soil planted with untreated seeds	185.45	34.28	17.34
Carbofuran treated soil planted with treated seeds	96.98	19.40	17.63
Dazomet treated soil planted with treated seeds	102.88	17.68	16.76
Untreated seed planted into untreated soil	117.03	21.90	13.86

# Table 6.3Effect of A. besseyi on some aspects of growth<br/>of a rice cultivar "Mpunga mwepesi"

## Table 6.4Effect of A. besseyi on some aspects of yieldof a rice cultivar "Mpunga mwepesi"

Treatment	X No of spikelets/ plant	X %age filled grains	X grain yield g/ 0.675m <sup>2</sup>	Total No. of Nematodes/ 50 sampled spikelets
Seed treated with hot water	2277.75	20.068	165.53	0
Carbofuran and untreated seed	2096.25	25.666	156.30	220
Dazomet and untreated seed	2601.00	15.486	42.75	152
Carbofuran and treated seed	2083.50	18.502	183.03	0
Dazomet and treated seed	2319.00	10.551	37.65	0
Untreated seed planted in untreated soil (control)	to 1968.25	6.854	32.55	1189

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high grain yields. Dazomet treatments gave higher vegetative material (Table 6.3) and high numbers of spikelets but fewer of them were filled compared with the rest of the treatments. The corresponding nematode counts at maturity are shown on Table 6.4.

These results show that hot water treatment controlled <u>A. besseyi</u> (Tables 6.1) resulting in improved grain yield components compared with the control (Table 6.4). Soil treated with carbofuran at planting sown with untreated seeds also improved yield but sowing treated seeds into carbofuran treated soil gave the highest yield. Dazomet treated soil, sown with treated or untreated seed gave high vegetative material and lower yield.

Vegetative growth from dazomet treatments were high, being highest of all where the treated soil was planted with untreated seed. The latter trend was also true for carbofuran treated soil sown with untreated seed. Similar trend was observed for the number of spikelets but the percentage filled seeds and grain weight was lowest in all dazomet treatments (comparable to control) and highest in the hot water and carbofuran treatments.

It had been observed, in the field, that dazomet treated plots had plants that were vegetatively overgrown compared with all other treatments, as if they had far too much nitrogen. The latter plots also delayed in flowering and grain ripening. This observation prompted an investigation on the possible cause.

Dazomet treated and untreated soil (that was to be used for another experiment), and the chemical dazomet were analysed for available nitrogen (Appendix 6.1). The results showed that the chemical dazomet itself when applied at the rate of 356.4 g/800 kg soil added 22.7 mg N to each kg soil. The actual physical treatment itself resulted in additional 68.3 mg N/kg soil. Since all plots

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soil added 22.7 mg N to each kg soil. The actual physical treatment itself resulted in additional 68.3 mg N/kg soil. Since all plots had a blanket nitrogen treatment at the recommended rate of 90 kg N/hå, plots treated with dazomet (even though the rate was not the same as in the analysed soil), must have ended up with far more nitrogen than the recommended rate. It was concluded that the vegetative overgrowth observed in the experiment under discussion was caused by over fertilisation with nitrogen and that, the latter growth was at the expense of the grain yield. Nematode populations from dazomet treatments were comparable to carbofuran treatments and both of them, were lower than those in the control. Dazomet plots probably provided an environment that was not conducive to <u>A. besseyi</u> multiplication.

Grain filling was considerably low for all treatments (Table 6.4). This was due to the cool weather, low rainfall and low relative humidity experienced during the experiment. The experiment was conducted after the optimum period for rice growing in Morogoro, April to August.

Although the results were not statistically analysed, there were clear improvements in all the studied yield variables in hot water treatments, compared with the controls (Tables 6.3 and 6.4) which corresponded to decreases in nematode populations in the hot water and carbofuran treatments as compared with the controls. These results were construed as demonstrating that hot water treatment was able to control <u>A. besseyi</u> in the seed by 81.2% resulting in considerably low <u>A. besseyi</u> populations throughout the plant growth until maturity (Table 6.2) and a corresponding improvement in the growth and yield of rice cultivar Mpunga mwepesi (Table 6.3 and 6.4).

Similarly carbofuran treatments also controlled <u>A. besseyi</u> populations during growth and maturity (Table 6.2) with improvement both in growth and yield of this rice cultivar, and even better yield results were obtained when carbofuran soil treatment was combined with hot water seed treatment (Table 6.3 and 6.4). Since hot water treatment had controlled <u>A. besseyi</u> by 81.2%, it can be concluded that carbofuran soil treatment combined with treated seeds gave better yields by probably controlling the remaining <u>A. besseyi</u> in the seed and other root nematodes and insect pests. However, carbofuran soil treatments combined with untreated seeds resulted in much less control of <u>A. besseyi</u> during the growth and reproduction phases of rice compared to hot water treatment (Table 6.2) but compared to the control, carbofuran gave considerable nematode control (81.5%) at final nematode assessment.

The effect of carbofuran treatment on the grain yield from untreated seeds (Table 6.4) was comparable to that of hot water treatment. Since carbofuran control of <u>A. besseyi</u> was considerably less than that of hot water treatment, the comparable yields of these two treatments were probably due to the fact that carbofuran is an insecticide – nematicide and therefore could have also controlled insect pests of rice as well as other nematodes apart from <u>A. besseyi</u>. The other possibility is that the numbers of live <u>A. besseyi</u> nematodes after control with carbofuran were probably below threshold levels for serious damage to the growth or yield of this particular rice cultivar. The latter two factors could also have operated together resulting in comparable yields obtained for the hot water and carbofuran treatments respectively.

It can therefore be concluded that both hot water and carbofuran treatments were able to control <u>A. besseyi</u> and increased grain yield of the studied rice cultivar, by 4.1 and 3.8 times more, respectively, than the control.

6.3 Effect of hot water treatment of seeds and soil treatment with carbofuran at planting on the grain yield of a nematode infested rice cultivar "Meli"

This study ws conducted to assess the effect of <u>A. besseyi</u> control by hot water treatment and carbofuran soil treatment at planting on the grain yield of a nematode infested rice cultivar "Meli". This experiment was also designed to compare the possible yield benefits that could accrue from the use of these treatments to control <u>A. besseyi</u> on the same rice cultivar. Rice cultivar "Meli" was chosen because of its high level of nematode infestation in seeds (Table 6.5) and its seed phenotypic homogeneity.

## 6.3.1 Materials and methods

A randomised block design with four replications was adopted and the trial was conducted between the months of February and June. Treatments involved were:

a) Control - (seed untreated and unsoaked)
Sample	Control seeds Live Nematodes	Treated seeds (54°C for 15 min) live nematodes
1	22	0
2	5	0
3	5	0
4	22	0
5	7	0
6	3	0
7	5	0
8	11	0
9	6	0
10	3	0
11	6	0
12	1	0
13	54	0
14	29	0
15	6	0
16	5	0
17	1	0
18	9	0
19	1	0
Total	201	0
Mean number of nematodes	infested seed	10.6
% seed infestation		38%
Highest seed infestation		54 nematodes

#### Table 6.5 Seed infested with <u>Aphelenchoides</u> <u>besseyi</u> out of a total of 50 sampled seeds from the lot used in the experiment to study the effect of controlling <u>A. besseyi</u> using carbofuran and hot water treatments on the grain yield of a rice cultivar "Meli"

Samples 1 to 19 = samples containing nematodes out of a total of 50 observed (not in order of incidence)

- b) Hot water treatment (seed presoaked in water for 18 hours under room temperature followed by hot water treatment at 54°C for 15 min and dried)
- c) Carbofuran treatment planting holes were treated with carbofuran at the rate of 17.5 kg/ha of 3G carbofuran during seed sowing.

Seeds to be planted were assessed for A. besseyi using single seed nematode assessment method described in the Materials and Methods Section of the thesis and live nematodes were recorded using motility as criterion for nematodes. Nitrogen was applied in two splits at a rate of 100 kg N/ha. All the phosphate was applied during planting at the rate of 50 kg P/ha. Half the nitrogen fertiliser was applied at planting and the rest 50% booting stage. The experimental area was tractor ploughed and disced followed by preparation of the plots using hoes and racks. The experiment was under rainfed conditions supplemented by irrigation. Plots were kept weed free by hoe weeding. Seeds were planted 20 cm apart within rows and, 20 cm apart between rows over a 15 m<sup>2</sup> plot area. Seeds were drilled in at the rate of 4 seeds per hole and thinned to one, after 4 weeks. Grain was harvested from an effective area of 9.89 m<sup>2</sup> at maturity using knives to cut the panicles. Harvested paddy was sun dried in brown paper bags. Paddy was manually thrashed from the panicle and winnowed. Grain weight was assessed at 13% moisture content and the weight of 200 seeds randomly sampled was assessed per treatment.

Analyses of variance for the grain yield and the weight of 200 seeds were carried out followed by F-tests of significance. A least significance difference (LSD) test for the significant factor (weight of 200 seeds) was carried out to separate significant means.

#### 6.3.2 Results and discussion

Summary of analysis of variance for the weight of grain and of 200 seeds is given in Appendix 6.2. The results of the LSD test to separate significant means for the weight of 200 grains is given on Table 6.6.

Treatments did not significantly differ for the grain yield. (Appendix 6.2). However, there was a increasing trend in the mean grain yield from the control through carbofuran to the hot water treatment suggesting that hot water treatment had resulted in a comparatively greater yield than the control (27%) and, carbofuran (16%) treatments. The latter treatment gave a 10% increase in yield above the control. Therefore, it could be concluded that both hot water and carbofuran treatments were beneficial . Since hot water treatment had resulted in 100% control of A. besseyi in the planted seeds, it was concluded that this control method had resulted in the observed increase in grain yield as compared to the yield obtained from nematode infested rice (control). The yield increase in carbofuran treatments over the control could have resulted from the control (of the parasitic activities) of soil nematodes and insects as well as the control of A. besseyi.

The weight of 200 seeds was significant at 5% probability

Table 6.6	Effect of hot water treatment of seeds (at 54°C for 15 minutes
	following presoaking) and of soil treatment with
	carbofuran at planting to control A. besseyi on the
	grain yield and the weight of 200 grains
	of a rice cultivar "Meli"

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Treatment	Grain Yield kg/9.89 m <sup>2</sup>	Weight of 200 seeds (g)
Hot water	3.88	5.13
Carbofuran	4.50	5.03
Control	3.53	4.83
LSD 5%	NS	0.23
SE	0.3649	0.2173
CV (%)	18.4	2.5
Means joined by the same lines were	not significantly	y different (P=0.05)

(Table 6.6) with the weight of 200 seeds from carbofuran treatments being significantly greater than those from the control. The weight of 200 seeds from hot water treatment was not significantly different from that of carbofuran and control. The result showed that the increase in yield of rice observed from hot water treatment compared to control was mainly due to more grain than from the increase in the unit weights of the grain while that observed from carbofuran treatment was due to significantly increased unit weight of the grain above that of the control (Table 6.6).

It can, therefore, be concluded that hot water treatment increased the numbers of grain produced (though not significantly) compared to carbofuran and the control. However, carbofuran significantly improved the weight of individual grains compared with the control.

The 27% increase in yield over the control resulting from hot water treatment above that of the control, when considered in terms of large scale production, can bring considerable profit. Therefore, results of this nature should be viewed not only from the statistical point of view but also be related to the farmers practical and economic situation. For example, during 1986-87, 1987-88 and 1988/89 the producer prices for paddy per kilogram was 9.60, 14.40 and 17.30 Tanzanian shillings respectively; by far the highest of all cereals (Tanzanian Economic Trends, July 1988). The benefits accruable from a 27% increase in yield for a ton of paddy at 1988-89 producer price could not be considered non-economical; especially when achieved from a low cost technology such as hot water treatment. 6.4 Effect of different methods of carbofuran application in the control of <u>A. besseyi</u> and on the yield of a nematode infested rice cultivar "Bluu"

This experiment was designed to study different methods of carbofuran application in soil to control <u>A. besseyi</u>. The objective was to try to find the most appropriate method of application that is both effective and practical. Rice cultivar Bluu was chosen because of its high nematode infestation in seed (77.75 nematodes/100 g seed) and its phenotypic homogeneity.

6.4.1 Materials and methods

The experiment was a randomised block design with three replications. Treatments involved carbofuran 3G soil treatment at the recommended rate of 17.5 kg/ha, and half the recommended rate. The chemical was applied at planting, along the planting furrows or into the planting holes. Treatment combinations were as follows:

1.	Carbofuran recommended rate to holes	(C/Rec/Holes)
2.	Carbofuran recommended rate to furrows	(C/Rec/Furrows)
з.	Carbofuran half rate to holes	(C/½/Holes)
4.	Carbofuran half rate to furrows	(C/½/Furrows)
5.	Control (untreated plots)	(Control)

Phosphate fertilizer at the rate of 50 kg P/ha was applied along the planting furrows during planting. Nitrogen was applied at the rate of 100 kg N/ha along the furrows in split application at planting and when 50% of the plants had reached booting stage.

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The experiment was conducted between February and June. Land was tractor ploughed, hoe levelled and hand planted. Spacing was 20  $\times$  20 cm between rows and 20 cm between plants. Yield was assessed from a central area of 1.8 m<sup>2</sup>. Grain weight and husk weight were assessed. Analyses of variance for the grain weight and husk weight were conducted followed by contrasts of chosen means.

#### 6.4.2 Results and discussion

Although there were no significant differences from the analysis of variance and the F test, (Appendix 6.3), the results of contrasts made for different rates and ways of application of carbofuran showed that carbofuran applied to holes was significantly better than the furrow application for the grain yield (Table 6.7). Similar contrasts made for the husk weight gave no significant results (Table 6.8).

Grain yield increased from the carbofuran half rate to the recommended rate when applied to holes and decreased from half to recommended rate for the furrow application (Figure 6.1). Husk weight stayed more or less the same for the recommended to the half rate for hole application but significantly increased from half to recommended rate for furrow application (Figure 6.2).

The results suggest that hole application is better than furrow application and that with increase in the rate of chemical applied to holes, there was an increase in grain yield. The chemical was more thinly spread when applied to furrow and therefore the results of the furrow application for the yield and husk weights may not have been related to the effect of chemical.

grain yield of a rice cultivar, "Bluu"				
Contrast	7 (1,8,5%) = 5,32	F	Significance	
Carbofuran treatments versus Contro	51	0.22	NS	
Carbofuran recommended rate versus	half rec. rate	0.48	NS	
Holes versus furrows applications		7.26		

Table 6.7 Effect of different methods of carbofuran application on the

#### Key:

\* = significant at P = 0.05

### Table 6.8Effect of different methods of carbofuran application on the<br/>husk weight of a rice cultivar "Bluu"

Contrast	F	Significance
Carbofuran treatments versus control	0.50	NS
Carbofuran recommended rate versus half rec. rate	1.84	NS
Holes versus furrow applications	0.002	NS



Fig.6;1 Effect of different methods of applying carbofuran on the grain yield (g/1.8m<sup>2</sup>) of a nematode infested rice cultivar "Bluu".



Fig..6.2 Effect of different methods of applying carbofuran on the husk weight (g/1.8m<sup>2</sup>) of a nematode infested rice cultivar "Bluu."

It is felt that an increased rate of the chemical beyond the recommended rates might have given a clearer trend in the results. On the other hand, carbofuran is a very expensive chemical which, even at these recommended lower rates, is beyond the means of many peasant farmers. Hence, efforts were made to test the efficacy of lower than the recommended rate. Furrow application method was of interest because of its easy applicability like the furrow fertilizer application method commonly used. However, the results showed that the latter method was less efficient compared to the hole application and that the half recommended rate was equally ineffective.

6.5 Effect of <u>A. besseyi</u> control by hot water treatment of seeds (54°C for 15 mins following 18 h presoaking) and carbofuran at different stages of rice growth on some aspects of grain yield of a rice cultivar "Bagamoyo"

This study was conducted to assess the effect of carbofuran treatment of seeds and soil at planting, treatment of rice plants with carbofuran at different stages of plant growth and in comparison with hot water treatment of rice seeds on the control of <u>A. besseyi</u> and on the grain yield of rice cultivar "Bagamoyo".

#### 6.5.1 Materials and methods

The investigation was conducted using a field experiment designed as a randomised block with four replications. The following eight treatments were used:

- 1. Control (seeds untreated and unsoaked).
- Soil treated at booting (soil at the planting station was treated when 50% of the rice plants had reached booting stage).
- 3. Seeds soaked in water (seeds presoaked in water for 24 h at room temperature and dried).
- 4. Soil treated at tillering (soil at the planting stations treated with carbofuran when 50% of the rice plants had reached tillering stage).
- 5. Soil treated at flowering (soil at the planting stations treated with carbofuran when 50% of the rice plants had reached flowering stage).
- 6. Soil treated at planting (soil in the planting holes treated with carbofuran at planting).
- 7. Hot water treated seeds (seeds soaked in water for 24 h at room temperature, followed by hot water treatment at 54°C for 15 mins, followed by drying).
- 8. Seeds treated with carbofuran (seeds soaked in water with carbofuran for 24 h followed by soaking in water for a further 24 h).

Carbofuran 3G treatment of soil was done at the rate of 20 kg/ha. Seed treatment was done at the rate of 1.5 kg carbofuran

3G to 44 kg of rice seed to which water was added as soaking medium. Seeds were first soaked in water at laboratory temperature for 24 h followed by soaking in water containing corbofuran for further 24 h. Treated seeds were removed, dried and stored.

Soil treatment with carbofuran was done at planting, when 50% rice plants had reached tillering, booting and flowering stages respectively. Precalculated amounts of carbofuran were applied to seeding holes or next to the plants around the root system. Nitrogen and phosphate fertilizers were applied at the rate of 100 kg N/ha and 50 kg P/ha respectively. Half the nitrogen was applied together with the phosphate (TSP) at planting and the rest during booting stage.

Hot water treatment of seeds was done at 54°C for 15 min following a 24 h presoaking in water at room temperature and subsequent drying. Seed soaking treatment was done in water under laboratory temperature for 24 h followed by drying. Control seeds were neither soaked nor treated. Seeds used in this experiment were from a nematode infested lot from a previous experiment.

Nematode assessment of seeds to be planted was done for control, carbofuran treated seeds, seeds that were only soaked in water and the seeds that had been hot water treated (Table 6.9). Nematode assessment was based on fifty seeds per treatment. Single seed nematode assessment following a 24 h presoaking was used as described in the Materials and Methods Section of the thesis. Data was collected on grain yield, some characteristics of grain and on the incidence of nematodes in the harvested seeds.

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Table 6.9	Nematode assessment data from seeds used in the
	experiment to assess the effect of
	different methods of carbofuran
1	application on the grain yield of a rice
	cultivar "Bluu" (from 50 seed samples)

	Control	Carbofuran treated seeds	Water soaked seeds	Hot water treated seeds
% Seed infestation	20	8	16	0
Mean No. nematodes/ infested seed	9.2	3.5	2.9	0
Highest No. of nematodes/seed	24	5	9	0

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Harvested seeds were sampled and assessed for their proportions of ripe, unripe, filled and partially filled grains, respectively. Seeds with a straw colour or orange-light brown colour were considered ripe and those green-looking or those with a greenish colour were considered unripe. Nematode assessment and germination tests were done for the filled and partially filled seeds of the combined carbofuran treatments, hot water treatment and control.

#### 6.5.2 Results and discussion

Summary of analysis of variance for various aspects of yield studied are presented on Appendix 6.4. Tables 6.10 and 6.11 show the mean grain yield and weight of 200 grains and their respective standard errors and levels of significance. The analysis of variance for the grain yield did not show significant differences (Appendix 6.3). However, contrasts of treatment means for the grain yield showed that carbofuran treatment at booting stage of rice had significantly higher grain yield over the control (Table 6.12). Hot water treatment of seeds did not give significantly better yields compared to other treatments. However, yield from hot water treated seeds was 10.5% more than that of the control. Grain yield from soil treated with carbofuran during booting stage was 25% more than the control.

Weight of 200 grains was significantly different for treatments (Appendix 6.4). Table 6.11 shows ranked treatment means. Hot water treatment produced significantly bigger grains than all the other treatments. Treatments where carbofuran was used to treat

	Replications					
Treatment	1	2	3	4	Means	
Soil treated at planting	302.70	352.00	293.30	375.70	330.93	
Seeds soaked in water	265.10	235.50	231.00	304.90	259.13	
Soil treated at tillering	256.30	283.40	306.60	351.70	299.50	
Soil treated at booting	334.90	366.60	433.40	320.60	363.88	
Seeds hot water treated	215.30	375.80	missing	373.80	321.63	
Untreated soil and			•			
untreated seeds (control)	276.10	294.80	301.90	292.00	291.20	
Soil treated at flowering	208.20	261.40	212.20	382.10	265.98	
SE (unadjusted) 23.10	CV(%) 15.2	LSD(9 68.9	5%) (for 91 excl	compariso uding tro	ons eatments	

<b>Table 6.10</b>	Grain yield (g/24 plants) of a rice cultivar "Bagamoyo" aft	er
	using carbofuran to control <u>A.</u> <u>besseyi</u> in seed, in	
	the soil, and using hot water treated seed	

Table 6.11Weight of 200 seeds(g) of a rice cultivar "Bagamoyo",<br/>harvested from an experiment to assess the effect<br/>of controlling A. besseyi using carbofuran and hot<br/>water treated seeds, on the yield of a rice<br/>cultivar "Bagamoyo"

	Replications					
Treatment	1	1 2		4	Means	
Hot water	4.29	4.15	4.20	4.13	4.19	
Soil treated at planting	3.93	4.22	4.13	3.99	4.07	
Soil treated at booting	4.10	4.02	3.94	3.90	3.99	
Seeds soaked in water	4.05	4.00	3.91	3.94	3.98	
Soil treated at flowering	3.90	4.10	4.01	3.80	3.95	
Untreated seeds (control)	3.84	3.72	3.70	4.00	3.82	
Seeds treated with carbofuran	3.74	3.86	3.85	3.75	3.80	
Soil treated at tillering	3.70	3.73	3.66	3.80	3.72	
SE	 CV(%	) LSD	(5%)			
0.05	2.6	0.3	15			

Key:

Means not covered by the same line are significantly different.

# Table 6.12Contrasts of means for the grain yield (g/24 plants) obtained<br/>from an experiment with carbofuran to controlA. besseyiin seed, in the soil, and hot<br/>water treated seed

	F	Significance
Control vs soil treated at planting (F)	1.48	NS
Control vs seeds soaked in water	0.96	NS
Control vs soil treated at tillering (F)	0.06	NS
Control vs soil treated at booting	4.95	
Control vs seeds hot water treated	0.73	NS
Control vs soil treated at flowering	0.60	NS
Carbofuran vs control	0.855	NS
Hot water vs control	0.064	NS
Seeds hot water treated vs soil treated at planting	0.005	NS
Seeds hot water treated vs seeds soaked in water	3.07	NS
Seeds hot water treated vs soil treated at tillering	0.40	NS
Seeds hot water treated vs soil treated at booting	1.40	NS
Seeds hot water treated vs soil treated at flowering	2.43	NS

F(1, 17, 5%) = 4.45

where: 4.45 is the F-value at 5% level of significance 1, 17 are degrees of freedom

soil at the tillering stage yielded almost the same weight as the untreated seeds (Table 6.10). There were 64.5, 52.5, and 33.5% ripened grains for hot water, carbofuran and control treatments respectively (Table 6.13).

The mean number of harvested seed with nematode infestation was significantly different for the hot water treatment, control and for the carbofuran treatments (Table 6.14). There were significantly more infested seeds in the carbofuran treatments compared with the control.

There were significant differences in the mean number of nematodes per seed between treatments and between filled and partially filled grains in the harvested seed (Table 6.15). There were more nematodes in grains from carbofuran treatments compared with those from the control. Filled seeds had significantly more nematodes compared with the partially filled seeds, and filled grain from the control and carbofuran treatments had significantly higher mean numbers of nematodes compared with their respective partially filled grains (Table 6.16). Grain harvested from hot water treatment had no nematodes.

Germination results of seeds harvested from the experiment are shown in Table 6.17. There were no significant differences in the mean percentage germination of seeds harvested from the experiment by treatment. However, partially filled seeds germinated significantly lower than fully filled seeds. Since more seeds were partially filled in the control than in the hot water and carbofuran treatments, it can be concluded that the latter two treatments significantly improved germination performance of the progeny of the

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Seeds	Hot Water	Treatments Carbofuran	Control
Number of ripe grain	129	105	67
Number of unripe grain	71	95	133
Total number of grain	200	200	200
% ripe grain	64.5	52.5	33.5

## Table 6.13Results of assessment of grain ripening from harvested ricefrom carbofuran, hot water and control treatments

## Table 6.14Mean number of A. besseyi infested seed harvested from rice<br/>cultivar "Bagamoyo" following hot water<br/>and carbofuran treatment

		Hot Water	Control	Carbofuran
		0	6.75	7.88
SE	= 0.363			
LSD(5%)	= 1.0927			
CV(%)	= 21			

	Levels	- 0	Treatment	
	1 	2	means	
Hot water	0.000	0.000	0.000 5	SE = 0.0570
Control	2.087	1.768	1 <b>.</b> 933 I	.SD(5%) = 0.1718
Carbofuran	2.768	1.485	2.126	
Lev means	1.622	1.084	1.353 = 0	Frand Mean
SE	0.0465			
LSD(5%)	0.1402		CV(%) = 1	.1.9
Key:				
Lev = Level	1 - Filled 2 - Partially fi	lled grai	ns	
Lev = Level Table 6.16 Con rej	<pre>1 - Filled 2 - Partially fi trast of mean numl plicated four time     partially f   (using the LSD</pre>	lled grai ber of ne s) from f illed gra for compa	ns matodes (mear filled versus hins hrisons)	n of 15 seeds
Lev = Level Table 6.16 Con rej	<pre>1 - Filled 2 - Partially fi trast of mean numl plicated four time partially f (using the LSD Filled grai</pre>	lled grai	ins matodes (mear filled versus tins trisons) fially filled grains	n of 15 seeds
Lev = Level Table 6.16 Con rej	<pre>1 - Filled 2 - Partially fi trast of mean numl plicated four time partially f (using the LSD Filled grai 2.10</pre>	lled grai	Ins matodes (mean filled versus lins urisons) fially filled grains 1.77	n of 15 seeds Significance
Lev = Level Table 6.16 Con rej  Control Carbofuran	<pre>1 - Filled 2 - Partially fi trast of mean numl plicated four time partially f (using the LSD Filled grai 2.10 2.77</pre>	lled grai	ins matodes (mear filled versus hins urisons) fially filled grains 1.77 1.49	n of 15 seeds Significance
Lev = Level Table 6.16 Con rej Control Carbofuran Hot water	<pre>1 - Filled 2 - Partially fi trast of mean numl plicated four time partially f (using the LSD Filled grai 2.10 2.77 0.00</pre>	lled grai	ins matodes (mean filled versus uns urisons) fially filled grains 1.77 1.49 0.00	n of 15 seeds Significance * ** NS
Lev = Level Table 6.16 Con rej Control Carbofuran Hot water LSD(5%) = 0.2429	<pre>1 - Filled 2 - Partially fi trast of mean numl plicated four time partially f (using the LSD Filled grai 2.10 2.77 0.00</pre>	lled grai	matodes (mean Filled versus mins mrisons) Fially filled grains 1.77 1.49 0.00	n of 15 seeds Significance * ** NS

Table 6.15 Mean number of nematodes (<u>A. besseyi</u>) in hot water and carbofuran treatments by seed filling levels in rice cultivar "Bagamoyo"

#### Key:

\* = significant at P = 0.05
\*\* = significant at P = 0.01
NS = No Significant Difference

# Table 6.17Percent germination results for harvested grain (filled and<br/>unfilled) from an experiment to assess the effect<br/>of controlling A. besseyi using carbofuran and hot<br/>water treated seeds on the grain yield of a<br/>rice cultivar, "Bagomoyo"

				Replications				
Level	Trea	tments		1	2	3	4	Means
	1. Hot	water t	reated	65.0	0 91.5	0 90.50	89.50	84.13
	2. Trea	ted at	booting	94.5	0 84.0	0 71.00	79.50	82.25
	3. Trea	ted at	flowering	75.0	0 51.0	0 83.50	80.00	72.38
Partially	4. Carb	ofuran	treated					
filed	seed	S		48.0	0 65.0	0 90.00	90.50	73.38
	5. Trea	ted at	planting	83.0	0 84.0	0 87.00	86.00	85.00
	6. Untr	eated s	oil and					
	untr	eated s	eeds	89.5	0 64.0	0 88.00	75.50	79.25
	7. Soak	ed seed	8	88.5	0 85.0	0 84.00	80.00	84.38
	1. Hot	water t	reated	85.0	0 96.0	0 99.50	99.00	97.38
	2. Trea	ted at	booting	95.0	0 96.0	0 99.00	99.50	97.38
D:11-4	3. Trea	ted at	flowering	95.0	0 98.5	0 95.00	99.50	97.00
Filled	4. Carb	ofuran	treated					06 60
	seed	8		92.5	0 95.5		99.50	90.03
	5. Trea	ted at	planting	94.5	0 97.5	0 97.00	97.00	90.50
	o. Untr	eated s	oll and					97 25
	7 Sook	eated s		93.0	0 95.5	0 99.50	99.00	94.63
	7. SUak	eu seeu	5	03.0	0 52.5	J 37.30	, 53.00	34.00
Partially	Filled	Means		74.6	4 74.9	3 84.86	83.00	80.11
Filled	Means			93.7	9 95.93	3 98.07	98.93	96.68
	Treatme	nt mean	5					
	1	2	3	4	5	6	7	
	90.76	89.80	84.70	85.00	90.8	<b>88.3</b> 0	89.50	
	a	a	a	а	a	а	а	
	SE of +		<b>-</b>	_	3 0406			
	SE OI L	reatmen	LS F V levele	-	4.3063			
				-	1.6263			
	LSD(5%)	CVCID		-	4.646			
	$CV(\mathscr{C})$			_	9.7			
Key:	( <i>/</i> //			_				
							<b>-</b> -	
Means mark	ed with	differe	nt letters	are s	significa	antly dif	ferent.	

1 to 7 represents treatments 1 to 7.

rice plant grown from treated seeds. The better germination obtained from seeds harvested from rice plants grown from hot water treated and carbofuran treated seeds was due to seed quality improvement resulting from the latter two treatments.

However, carbofuran treatments had significantly higher nematodes compared to the control, suggesting that carbofuran did not control <u>A. besseyi</u>. These results reflect the effect of controlling <u>A. besseyi</u> at different stages of the rice plant and the fact that nematode assessment was done for combined samples from all carbofuran treatments where certain growth stages might have had greater nematode population resulting in inflated overall numbers.

Nematode assessment results showed that filled seeds harboured significantly higher nematode population than unfilled seeds for both carbofuran and control treatments. The fact that some of the partially filled seeds were not fully ripe may have contributed to the fewer number of A. besseyi in them, reflecting that A. besseyi populations in those seeds had not had as much time to multiply as of the populations found in filled (ripe) seeds. The second possibility is that, unripe seeds at harvest were those that were formed later on in the season and by then, the optimum period for rice development and probably that of the pests of rice such as Therefore, A. besseyi the nematode A. besseyi was past. multiplication and development would probably be at its lowest ebb. Indeed, seeds found still unripe during the months of June, July and August in Morogoro would have been subjected to cool weather which is not conducive to rice production or A. besseyi biology as a whole. Therefore, one or both of these factors could have

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contributed to the lower populations observed in partially filled grains compared to filled grains.

Although, there were no significant differences in the overall grain yield, contrasts of mean yield from the carbofuran treatments showed that soil treated with carbofuran at booting stage yielded significantly higher grain than from the other times of treatment with carbofuran. This result seems to tie up with the significant result found for inoculation with <u>A. besseyi</u> during booting stage which is described in Chapter VII of the thesis. The result again suggests that booting stage is a critical period for the control of <u>A. besseyi</u> to improve grain yield.

Although, hot water treatment and the other treatments did not give significantly different results for the grain yield (except carbofuran at booting), hot water treatment did improve yield over the control by 10.5%. Weight of 200 grains was significantly higher for the hot water and carbofuran treatments compared with the other treatments. The 10.5% improvement in the yield by hot water treatment over the control was due to increased weight in the unit grains rather than in the overall increase in number of grains formed.

Therefore, hot water treatment significantly improved the weight of individual grains and considerably increased the number of grains that ripened compared to control and carbofuran treatments (Plate 6.1). The quality of grain was much better in the hot water treatments compared to the control, where the majority of seeds categorised as partially filled were longitudinally cracked, separating the lemma from the palea. There were more distorted and



Plate 6.1 Effect of different treatments on ripening of seeds of rice cultivar "Bagamoyo".

discoloured grains in the control than in the hot water and carbofuran treatments. The results seem to suggest that the effect of <u>A. besseyi</u> on the yield of the rice cultivar under study was an important reduction in the grain quality and unit grain weight. Although germination in petri dishes was not significantly different for the treatments, an investigation into vigour of seeds and seedlings might have given a different picture for unripe and the poorer quality seeds as indicated by the, significantly lower germination of the partially filled grains.

It is noteworthy that the overall percentages of ripe grains were considerably lower than expected (Plate 6.1). This is probably because of the weather and the lateness of the season for rice.

It can be concluded that, since there was a high degree of nematode infestation in the rice grains from harvested paddy, A. besseyi had passed on from infested seed to harvested seed. The effect of hot water treatment resulted in a total elimination of A. besseyi from planted seed and from harvested paddy due to the latter treatment. The yield from the hot water treatments was not significantly higher than the control or other treatments. However, it was 10.5% greater than that of the control and the grains were significantly bigger than most of the treatments except from carbofuran treatment applied at planting. The quality of harvested seed was much better with 32 % and 12 % more ripe grains over the control and carbofuran treatments respectively. The control resulted in poorly shaped grains, most of which were unripe, and/or partially filled and, cracked. Therefore, it can be concluded that A. besseyi caused reduction in unit grain weight and grain quality; properties

which can be significantly improved by hot water treatment of seed at 54°C for 15 mins following presoaking in water for 18 h.

These results agree with Atkins (1974) who stated that "white tip" disease reduces yield and quality of grain, panicle size, numbers of fertile spikelets and the total numbers of rice grain resulting in grains frequently abnormal in shape. The results also agree with Ueda and Matsumo (1975) who pointed out that "white tip" disease caused yield reduction through lowering of grain weight caused by lowered stem weight and plant height and, withered tips of leaves, which resulted in increased numbers of immature grains, lowered grain weight and a reduction in actual numbers of grains formed.

#### 7. THE EFFECT OF A. BESSEYI ON RICE YIELD

#### 7.1 Introduction

Stansel (1975) described three components of rice yield as; panicles per unit area, grain per panicle and the weight of grains. These components, he reckoned, are influenced by physiological processes during the three stages of development, namely; vegetative stage, reproductive stage and maturation stage. Stansel (1975) described the reproductive stage of rice as involving panicle initiation, panicle differentiation, panicle development and flowering, grain filling and grain maturation.

Panicle differentiation is said to mark the end of vegetative stage and the beginning of reproductive stage. This stage is considered critical in plant development. Panicle development begins when all nodes of the stem have been formed. At this stage, the panicle becomes the most active area of growth, such that cultural inputs and climatic conditions become critical for the yield factor.

The period from panicle differentiation to heading (known as panicle development) is described by Stansel (1975) to be the most vulnerable phase in the life and growth of the rice plant. During this stage panicle doubles in size every three days and plant exhibits high sensitivity to adverse conditions, at the same time requiring large quantities of energy. The panicle differentiation and development stage, before heading can be termed booting stage. The number of grains per panicle is determined by the number of florets produced and the number of them that are pollinated.

Grain filling and maturation begins when 50% flower heads

have appeared out of the leaf sheath of the flag leaf and ends when the average grain moisture is 21%. When 15% panicles have emerged from the leaf sheaths (a process of heading), pollination and fertilisation of embryos also begins and when 50% florets are pollinated, grain filling and maturation stage begins.

Thus, appreciating the importance of the reproductive and maturation stages of rice in the determination of grain yield, it was important to examine relationship between this phase of rice development and infection with the rice pest <u>A. besseyi</u>.

7.2 An investigation on the effect of inoculating rice plants with <u>A. besseyi</u> during booting stage on some aspects of yield of a rice cultivar "Sindano"

#### 7.2.1 Introduction and objectives

Infection of rice flowers by <u>A. besseyi</u> has been reported to cause abnormally developed grains with distorted glumes and, in some cases, sterile flowers (Todd and Atkins, 1958; Yoshii and Yamamoto, 1950a; Uebayashi <u>et al</u>, 1971). Huang and Huang (1972) studied the bionomics of white-tip nematode <u>A. besseyi</u> in rice florets and developing grains. The latter workers' population analyses showed high levels of second-stage larvae in florets before anthesis, indicating an active reproduction by the nematodes during booting. The same authors stated that there was high correlation between the numbers of second stage larvae and the loss of moisture in maturing seed. Booting stage is considered to be the panicle development stage which includes panicle differentiation. This stage ends with the emergence of the panicle from the flag leaf sheath.

First sign of booting was the time when the panicle within the leaf sheath could be discerned just as it was being pushed up by the elongating top internode. For the practicability. the experiment was conducted in a glasshouse. The boot before the flower opens was considered the most appropriate stage to inoculate when all developing florets were within the sheath enclosure in close proximity. Furthermore, booting stage was considered appropriate because of the sensitivity of the rice plant to adverse conditions during this stage and also because this is the stage when most of yield attributes are determined (Stansel, 1975), and when A. besseyi are said to migrate to developing panicles and enter inflorescences and multiply abundantly before anthesis, feeding on stamens, lodicles and embryos (Fortuner and Orton Williams, 1975).

The objective of this study, therefore, was to assess whether inoculating rice plants with <u>A. besseyi</u> during booting would affect any of the studied yield parameters of rice cultivar "Sindano". It was also intended to investigate the time of inoculation in relation to the effect of <u>A. besseyi</u> on the grain yield.

#### 7.2.2 Materials and methods

This was a pot experiment conducted in a glasshouse. The design used was a factorial in a randomised block layout with 10 replications, where each potted plant represented a replication.

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Factors involved and the treatment combinations were as follows:

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#### Factors Involved:

a)	Inoculations:	T = Control (where all tillers were inoculated with distilled water).
		T <sub>2</sub> = All tillers inoculated with nematodes (All Tillers T).
		T <sub>3</sub> = Half the tillers inoculated with nematodes (half tillers T).
		T <sub>4</sub> = Half the tillers inoculated with distilled water (Half tillers control ()
b)	Time of Inoculation:	Time At first sign of booting (at 1st sign).
		Time On 3rd day from the 1st sign of booting.
		Time <sub>3</sub> On 5th day from the 1st sign of booting.
		Time On 7th day from the 1st sign of booting.

#### Treatment Combinations:

		Inoculations				
Time of	Inoculation	<sup>T</sup> 1	(Control)	<sup>T</sup> 2	<sup>т</sup> з	т <sub>4</sub>
Time 1		x		x	x	x
Time 2		-		x	x	x
Time <sub>3</sub>		-		x	x	x
Time 4		-		×	x	x
<u>Key</u> : - (	dash) = (inocula tillers Time <sub>4</sub> .	ation was	n with dist not done f	illed wa or Time	ater fo 2 <sup>, Time</sup>	or all and
- C i	)ther details of .n Appendix 7.1.	the	treatment	combinat	tions a	are given

Soil used for this experiment was pretreated with dazomet in a manner described in the Materials and Methods Section of the thesis. Seeds used in the experiment were from progeny of a hot water treated sample that were observed to be nematode free. These seeds were themselves hot water treated to ensure that they were completely nematode free. Hot water treatment was done at 54°C for 15 minutes without presoaking in order to minimise seed damage. Plants were inoculated with an average of 100 nematodes as they reached the required stage. Nematodes were extracted from a mixture of infested rice cultivars that served as a source of inoculum.

Inoculation was done using a special micro-pipette after blowing five times to bring the nematodes into suspension. Nematodes in suspension were gingerly pipetted on to the axils of the nearest leaves to the flower boots. Volumes of water containing aliquots calculated to contain 100 nematodes per millilitre were pipetted during inoculations, while occasionally samples of equal volume were also taken to assess nematode concentration.

Two to three inoculations were done the same day to give the required nematode count. Time was allowed for each inoculated droplet to disappear before another inoculation was done.

At the end of the series of inoculations, the rice plants were left to grow and the grains to mature. At grain maturity, length of panicles were measured, numbers of spikes, spikelets per panicle were counted. For the grain weight the moisture content was adjusted to 12%.

From the harvested seed, samples of 15 seeds per treatment were randomly picked and individually assessed for presence of nematodes, using single seed nematode assessment method described in the Materials and Methods Section of the thesis.

Analyses of variance were conducted for the various

parameters followed by F-tests of comparisons of the inoculation treatments and the inoculation times. The data was analysed twice. One analysis involved all 13 treatments and the other analysis was done for each parameter as a  $4 \times 3$  factorial design which excluded the control.

At 1st sign of booting the control was compared with the rest of the inoculations, subsequent comparisons were made between all tillers and half tillers inoculated with nematodes, and half tillers inoculated with distilled water. The following comparisons were subsequently made:

- All tillers inoculated with nematodes compared with half tillers inoculated with nematodes and water respectively, at all the booting stages studied.
- Half tillers inoculated with nematodes compared with half tillers inoculated with distilled water at all booting stages respectively.

#### 7.2.3 Results and discussion

From the analyses of variance, it was observed that there were significant differences in the treatments for all the studied parameters. Table 7.1 shows the mean values for all parameters, standard errors, the least significant difference and co-efficient of variation values after the main analyses of variance.

Table 7.2 shows the results of an F-test to compare the control with all other treatments at the first sign of booting. There were no significant differences for the parameters studied.

	W	of Dow		
	In t	(mean of	10 plants)	68
Inoculation Treatments	Panicl <b>es</b> (cm)/ plant	Spi <b>kes/</b> plant	Spikelets/ plant	Grain wt (g)/ plant
Control (all tillers				
with water)	17.43	6.90	39.0	0.85
All tillers T. (1st sign)	18.48	7.07	45.6	1.00
All tillers T. at 3rd day	17.07	7.36	43.4	1.03
All tillers T. at 4th day	16.02	6.58	35.9	0.76
All tillers T. at 7th day	17.00	7.21	37.8	0.87
½ tillers T. at 1st sign	18.52	7.45	37.3	0.82
½ tillers T. at 3rd day	17.59	7.43	46.9	1.15
½ tillers T. at 5th day	18.78	7.97	42.6	1.17
炎 tillers T. at 7th day	16.27	7.04	30.2	0.70
Control ½ at 1st sign	18.04	6.52	38.2	1.05
Control ½ at 3rd day	18.33	7.30	49.1	1.22
Control ½ at 5th day	20.17	8.87	52.7	1.26
Control ½ at 7th day	15.30	7.03	40.1	0.90
SE	0.873	0.445	4.646	0.1186
LSD (5%)	3.236	1.247	13.0	0.3327
CV%	15.7	19.3	35.5	38.1

# Table 7.1Effect of inoculating rice plants with A. besseyi during<br/>booting stage on some aspects of yield of<br/>rice cultivar "Sindano"

## Table 7.2Table of comparisons for the control against the restof treatments at the 1st sign of booting

Treatmen	t M	leans
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Parameters measured	Control	All tillers treated	tillers treated	え tillers with water	F-va	lues
X Grain wt	0.853	1.00	0.821	1.054	1	ns
X Length of panicle (cm) /plant	17.43	18.48	18.42	18.04	1	ns
X No of spikes /plant	6.90	7.07	7.45	6.52	1	ns
X No of spike- -lets/plant	39.0	45.6	37.3	38.2	1	ns

Tim <b>e</b> of Inoculation	Inoculation Comparisons	F-values	Signif- icance
At 1st sign of booting	<ul> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> </ul>	0.03	ns
On 3rd day	<ul> <li>(C).</li> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> </ul>	0.14	ns
	(C).	0.34	ns
On 5th day	<ul> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> </ul>	9.77	**
	(C).	1.19	ns
On 7th day	<ul> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> </ul>	1.21	ns
	(C).	0.58	ns

## Table 7.3Comparisons of inoculation means for the length of<br/>panicles/plant

Time of Inoculation	Inoculation Comparisons	F-values	Signif- icance
At 1st sign of booting	<ul> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> </ul>	1	ns
On 3rd day	<ul> <li>(C).</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> </ul>	2.18 1 1	ns ns ns
On 5th day	<ul> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> <li>(C).</li> </ul>	10.52 1.90	** ns
On 7th day	<ul> <li>(a) all tillers T. vs half tillers</li> <li>T. and vs half tillers</li> <li>Control (C)</li> <li>(b) half tillers T. vs half tillers</li> <li>(C).</li> </ul>	1 1	ns ns

.

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## Table 7.4Comparisons of inoculation means for the number of<br/>spikes/plant
Inoculation Comparisons	F-values	Signif- icance
(a) all tillers T. vs half tillers T. and vs half tillers	1 02	
(b) half tillers T. vs half tillers (C).	0.02	ns
(a) all tillers T. vs half tillers T. and vs half tillers	0.62	ne
(b) half tillers T. vs half tillers (C).	0.11	ns
(a) all tillers T. vs half tillers T. and vs half tillers	. 10	
Control (C) (b) half tillers T. vs half tillers (C).	4.10 2.27	* ns
(a) all tillers T. vs half tillers T. and vs half tillers		
Control (C) (b) half tillers T. vs half tillers (C).	0.21 2.18	ns ns
	<ul> <li>Inoculation Comparisons</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers (C).</li> <li>(b) half tillers T. vs half tillers T. and vs half tillers (C).</li> <li>(c) half tillers T. vs half tillers (C).</li> </ul>	Inoculation Comparisons F-values          (a) all tillers T. vs half tillers         T. and vs half tillers         Control (C)       1.83         (b) half tillers T. vs half tillers         (C).       0.02         (a) all tillers T. vs half tillers         T. and vs half tillers         (C).       0.63         (b) half tillers T. vs half tillers         T. and vs half tillers         Control (C)       0.63         (b) half tillers T. vs half tillers         (C).       0.11         (a) all tillers T. vs half tillers         T. and vs half tillers         (C).       0.11         (a) all tillers T. vs half tillers         (C).       2.27         (a) all tillers T. vs half tillers         (C).       0.21         (b) half tillers T. vs half tillers         (C).       0.21         (b) half tillers T. vs half tillers         (C).       0.21         (b) half tillers T. vs half tillers         (C).       0.21         (b) half tillers T. vs half tillers         (C).       0.21         (b) half tillers T. vs half tillers         (C).       0.21

# Table 7.5Comparisons of inoculation means for the number of<br/>spikelets/plant

Inoculation Comparisons	F-values	Signif- icance
(a) all tillers T. vs half tillers T. and vs half tillers Control (C)	0.18	 ne
<ul><li>(b) half tillers T. vs half tillers</li><li>(C).</li></ul>	1.89	ns
(a) all tillers T. vs half tillers T. and vs half tillers		
Control (C) (b) half tillers T. vs half tillers (C).	1.08 0.19	ns ns
(a) all tillers T. vs half tillers T. and vs half tillers		
Control (C) (b) half tillers T. vs half tillers (C).	9.70 0.26	** ns
(a) all tillers T. vs half tillers T. and vs half tillers		
Control (C) (b) half tillers T. vs half tillers	0.25	ns
	<ul> <li>Inoculation Comparisons</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers T. and vs half tillers Control (C)</li> <li>(b) half tillers T. vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers (C).</li> <li>(b) half tillers T. vs half tillers T. and vs half tillers (C).</li> <li>(a) all tillers T. vs half tillers (C).</li> <li>(b) half tillers T. vs half tillers</li> <li>(c) tillers T. vs half tillers</li> </ul>	Inoculation ComparisonsF-values(a) all tillers T. vs half tillers T. and vs half tillers Control (C)0.18(b) half tillers T. vs half tillers (C).0.18(a) all tillers T. vs half tillers (C).1.89(a) all tillers T. vs half tillers Control (C)1.08(b) half tillers T. vs half tillers (C).0.19(a) all tillers T. vs half tillers (C).0.19(a) all tillers T. vs half tillers (C).0.19(a) all tillers T. vs half tillers (C).9.70(b) half tillers T. vs half tillers (C).0.26(a) all tillers T. vs half tillers (C).0.26(a) all tillers T. vs half tillers (C).0.25(b) half tillers T. vs half tillers (C).0.25(b) half tillers T. vs half tillers (C).0.25

Table 7.6 Comparisons of inoculation means for grain weight (g)



Fig. 7,1 Effect of inoculation of rice plants with <u>A besseyi</u> during booting on the mean length of panicles/plant.



Fig. 7.2 Effect of inoculating rice plants with <u>A</u> <u>besseyi</u> during booting on the mean number of spikes/plant.





Fig.:7.3 Effect of inoculating rice plants with <u>A besseyi</u> during booting on the mean number of spikelets/plant



Days after 1st sign of booting

Fig..7,4 Effect of inoculating rice plants with <u>A</u> <u>besseyi</u> during booting on the mean grain weight (g)/plant.

Tables 7.3 to 7.6 show results of all other inoculation comparisons at all the tested booting stages of the rice plant. Comparisons between all tillers inoculated with nematodes with other two treatments (half tillers (T) and half tillers control) were consistently significant for all parameters on the 5th day after the first sign of booting. There were no significant differences for the other days of inoculations for all the parameters. Similarly comparisons between half tillers treated with nematodes (half tillers T) and half tillers inoculated with distilled water (half tillers control) at all inoculations days gave no significant differences for all parameters.

Figures 7.1 to 7.4 are graphical representations of the effects of the various treatments at different booting stages on the respective studied variables.

Table 7.7 shows contrasts between the control as a whole (all or half tillers inoculated with distilled water) and inoculated treatments for of nematodes extracted from harvested seeds at the end of the experiment. There were significant differences between the inoculated and the control samples for all tested nematode parameters.

The results of the F-test to compare and contrast the effect of the control against the rest of the treatments to all tillers and half the tillers demonstrated that inoculation at first sign of booting in any manner for all the studied parameters did not make any significant difference (Table 7.2). Therefore, at first sign of booting, it did not make much difference whether or not inoculation with nematodes or with water was done to all or half tillers.

The results of the F-test to compare the rest of the

Parameter	Control	(Nematodes inoculated treatments)	F-value	Significance
Mean number of nematodes	0.00	6.66	3 706.0	**
Highest number of nematodes	0.00	40.22	442.93	**
Number of infected seeds out of 15	0.00	6.71	770.71	**

Table 7.7 Comparisons of the control (as a whole) and the nematode inoculated treatments for nematode parameters in harvested rice seeds

treatments as presented on Tables 7.3 to 7.6 demonstrated that during the seven days from the first sign of booting, only the 5th day was significant. These results were consistent for all parameters as shown by the means for all studied parameters on Table 7.1 and figures 7.1 to 7.4. For the other inoculation days the results were consistently non-significant for all parameters Half tillers inoculated with nematodes compared with half tillers inoculated with distilled water (control) at all other inoculation times gave no significant results for all parameters. It can be concluded from these results that, of all comparisons made for the various inoculation times, only the 5th day after the first sign of booting was important. Results on Figures 7.1 to 7.4 and Tables 7.3 to 7.6 show that, on the 5th day after first sign of booting, the control was significantly better than all tillers treated with nematodes. Half tillers treated with nematodes performed significantly better compared to all tillers treated with nematodes.

The findings of the present study confirm the statement by Stansel (1975) that booting is a vulnerable stage of rice growth which is also when yield of rice is determined. The results of present study also demonstrated that the critical period during the booting stage is the 5th day after the first sign of booting when the length of panicles, numbers of spikes and spikelets and the weight of grain were adversely affected. Infection with <u>A. besseyi</u> at booting stage enabled the nematode to get to the developing florets; during a period when the physiological conditions of the host are known to be conducive for the nematode reproduction and development (Huang and Huang 1972). During this period, the nematode invasion coincided with the vulnerable stage of rice (Stansel, 1975). The 5th day after first sign of booting is therefore, the period when the intensity of parasitism, coincided with the sensitive stage of rice panicle development and the determination of yield, resulting in significant adverse effects on the yield. The adverse effects resulted in shorter panicles, less spikes and spikelets, and consequently less grain yield.

For all comparisons made for various inoculations days, only that of the 5th day after first sign of booting was consistently significant for all parameters, with all tillers inoculated with nematodes yielding significantly less for all the studied parameters compared to control. For the same inoculation day (5th day), half tillers inoculated with nematodes yielded significantly better in all studied parameters compared with where all tillers were inoculated with nematodes. Therefore half tillers infestation could be considered below the threshold for significant adverse effects from <u>A. besseyi</u>. However, half tillers inoculated with nematodes yielded comparably less than the control, although not significantly (Figures 7.1 to 7.4).

Results of nematode assessment at the end of the experiment showed that inoculations on the first sign of booting and on the 7th day after the first sign of booting resulted in more nematodes compared to similar inoculations done on the 3rd and 5th days after first sign of booting. Therefore, it seems the presence or absence of nematodes was only important on the 5th day, while on the other days whether or not there were more nematodes on all or half tillers did not matter very much. The nematode populations in harvested seeds is similar to that of the experiment with variety Bagamoyo where damaged, poorly filled and cracked seed had less nematode

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populations than fully filled seeds. These results are contrary to those of Nandakumar <u>et. al.</u> (1975) who reported more nematodes from poorly filled and chaffy spikelets. The results suggest that, either the nematodes from damaged spikelets multiply to a point of population check or they move from badly damaged spikelets.

This study seem to suggest that there were some physiological conditions enabling the rice plants to tolerate nematode infection at other inoculation stages and that on the 5th day after the first sign of booting, the plants were less tolerant to nematode infection. The results of the F-test from the analysis of variance for the nematode assessment data from harvested seeds showed that there were significantly higher nematode infestations in the nematode inoculated treatment compared to the control (P = 0.001). Similarly comparisons of the control (as a whole) and the nematode inoculated treatments (Table 7.7) demonstrated that there were highly significant differences between the control and the nematode inoculated treatments. Therefore, it was experimentally shown that the inoculations with distilled water were, as expected, nematode free, while inoculations with nematodes were successful in infesting the developing panicle resulting in infested harvested seed.

7.3 Effect of different levels of <u>A.</u> <u>besseyi</u> in seed on the grain yield of a rice cultivar "Bluu"

The investigation involved a field experiment which was designed to assess the effects of different levels of <u>A. besseyi</u> in seed on the grain yield of a rice cultivar, "Bluu". The study was also intended to examine possible threshold levels of infestation for significant loss of grain yield.

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### 7.3.1 Materials and methods

Rice cultivar "Bluu" was used in this study because it had a high level of nematode infestation (77.75 mean nematodes per seed). The experiment was designed as a randomised block with three replications. Treatments involved were as follows:

 $L_0 = 200$  seeds all hot water treated  $L_{1/4} = 150$  seeds hot water treated and 50 seeds untreated  $L_{\chi} = 100$  seeds hot water treated and 100 seeds untreated  $L_{3/4} = 50$  seeds hot water treated and 150 seeds untreated  $L_{4/4} = 200$  seeds all untreated

Two hundred seeds were planted in 1,5 x 1 m plots with a 15 x 15 cm within row and 20 x 20 cm between row spacings. Plants were thinned to one per hole at the onset of tillering and thinned plants were left inside the plot to maintain nematode population.

Ten plants were randomly chosen for individual grain yield assessment. Grain weight was assessed at 12% moisture content. Nematode assessment was done for 15 seed samples per treatment per replication. Grain from each treatment was thoroughly mixed before a random sample of 15 seeds was taken for nematode assessment. Seeds were individually assessed for nematodes following the method described in the Materials and Methods Section of the thesis.

#### 7.3.2 Results and discussion

Means for the yield variables and the mean number of nematodes per infested harvested seed are presented on Table 7.8. The grain yield and the weight of 200 grains did not show

Treatments	X grain wt(g)/ plant (out of 10 plants	X wt(g) of 200 seeds )	X No of infested (harvested) seeds (out of 15)	X No of nemas per infested (harvested) out of 60 seeds)
L <sub>0</sub> = 200 seeds treato (hot water)	ed 19.84a	5.708a	0.00a	0.00a
L <sub>3/4</sub> = 150 seeds treate (and 50 seeds) untreated	ed 21.44a	5.722a	2.75ab	4.23ab
L <sub>1/2</sub> = 100 seeds treate (and 100 seeds) untreated	ed 21.66a	5.740a	2.25a	7.33ab
L <sub>1/4</sub> = 50 seeds treate (and 150 seeds) untreated	ed 16.99a	5.613a	5.50bc	12.49bc
L <sub>4/4</sub> = 200 untreated (Control)	17.27a	5.670a	7.50c	16.44c
SE	1.520	0.050	0.472	1.48
LSD (0.1%)	10.834	.305	2.88	8.41
CV (%) Key:	13.5	1.8	26.2	36.5
Column means followed b	y the same lette	er are not	significantly d	lifferent.

## Table 7.8 Mean for the different variables studied in the experiment to study the effect of different levels of <u>A.</u> <u>besseyi</u> in seed on the grain yield of a rice cultivar "Bluu"

significant differences between treatments. However, where one-quarter and half of the seeds were untreated, there was comparably greater grain weights and weight of 200 grains than from the control and hot water treatment.

Where all or three quarters of the seeds planted were untreated, the yields were 12.95 and 14.36% less than where all seeds planted were hot water treated (Table 7.8). However, where three-quarters or half of the seeds planted were hot water treated the yields were 8.06 and 9.17% greater than where all seeds planted were hot water treated, suggesting that both parameters were similarly affected by the treatments. However, the increases and decreases in the weight of both the yield and 200 grains were not statistically significant.

The increases in yield and the weight of 200 grains resulting from the lower levels of nematode infestation show that the half and one-quarter untreated seeds as well as having less nematode infestation (Table 7.8), also had possible advantage of having less seeds debilitated by the hot water treatment of presoaked seeds. Hence their performance were better than where all seeds were hot water treated or where three-quarters or all seeds were untreated. The latter two treatments having higher nematode populations that adversely affected the yield while the former treatment had seed vigour reduced by hot water treatment of presoaked seeds.

The results demonstrated that <u>A. besseyi</u> can adversely affect yield of rice and that hot water treatment of presoaked seeds can affect yield performance of plants grown from seeds thus treated. The adverse effects of hot water treatments at 54°C for 10

minutes following presoaking on the vigour of some of the seeds that may manage to germinate was demonstrated in experiments presented in Chapter V of the thesis. The results agree with Johnson (1975) who stated that seed vigour can be used to predict the potential field performance of resultant plants, and by Heydecker (1972) who stated that seed vigour can show its effects in the survival ability of the seeds in storage, in the ability of the resultant seedlings to emerge in the field, in the establishment of subsequent plants and in the ability of those plants to produce full yield. The latter author further stated that all the above stages can be affected by a range of factors acting on the seed, and that vigour is an essential attribute of the seed that affects its ability to deliver its potential at any stage of the plant life cycle. Since vigour is one of the parameters that were demonstrated to be significantly adversely affected by hot water treatment at 54°C for 15 minutes, following presoaking (see Chapter V of the thesis) it is concluded that the yield resulting from all treated seeds, though it was increased due to elimination of A. besseyi, the increase was depressed by the adverse effects of this thermotherapy. Hence, where there were some untreated seeds (half and one-quarter) and hence lower nematode numbers, there were better performance of those plants. Consequently the combination of lower nematode numbers and better performance of plants gave greater yield than in either the control or where all seeds were hot water treated.

The results confirm the points raised in the previous experiments presented elsewhere in the thesis that yield increases from seeds treated following presoaking might have been better if the treatment had been done without presoaking, as did the yields of cultivar Mpunga mwepesi.

The results therefore demonstrated that A. besseyi infestation at certain levels, can reduce grain yield and further confirmed previous results that hot water treatment of presoaked seeds, at the tested regime, can completely eliminate seed borne A. besseyi resulting in increased grain yield and unit weight of grains. However, the resultant increases in the yield were lowered by the adverse effects of hot water treatment following presoaking. The adverse effects can be emeliorated by treating a proportion of the infested seeds to reduce the nematodes populations to below the threshold level, and mixing with the treated and untreated seeds in the right proportions. Another alternative would be to treat seed without presoaking at the treatment regimes recommended in this study for the control of seed borne A. besseyi.

The nematode results from harvesed seeds showed that <u>A. besseyi</u> had passed from the seed to the harvested grain in a manner demonstrating the decreasing levels of infestation effected by the mixing of infested (untreated) and treated seeds. The findings are in conformity with the objective of the mixing of the treated and the untreated seed. The control had significantly higher numbers of <u>A. besseyi</u> than all other treatments except where three-quarters of the seed planted were untreated (Table 7.8).

Although the 12.95 percent and 14.36% yield depression by <u>A. besseyi</u> was not statistically significant, to a farmer losses of that magnitude can be economically significant especially considering that they can be avoided by dry seed treatment or mixing treated and untreated seed in the right proportions.

#### 8. DISCUSSION AND CONCLUSIONS

Rice has lately been gaining importance as a food crop in the African continent and its production has increased significantly during the past ten years (FAO, 1986). Pests and diseases are some of the significant factors contributing to the low yields being obtained in Africa.

The rice nematode, Aphelenchoides besseyi Christie, 1942, which causes the "white tip" disease of rice is among the documented rice pests and has been identified on rice in Tanzania. The economic importance of this nematode in Asia, particularly in Japan, has been established (Fukano, 1962; Huang et al, 1972). However, in Africa particularly in Tanzania, studies of that nature have not yet been conducted; hence the extent of the incidence, severity and importance of this pest are not well established. economic Investigations on the occurrence of this nematode on rice in Africa started in the 1960s (Anon, 1965; Hopper and Merney, 1966) and in East Africa, a preliminary survey of A. besseyi on rice was carried out by EAAFRO workers in 1970-71 (Taylor <u>et al</u>. 1972). The latter workers considered their 1970-71 survey a preliminary study and had intended to conduct a further survey and an assessment of crop damage due to this pest. However, the breakup of the East African Community did not allow the intended follow up survey.

The present study therefore, was designed to follow up some of their objectives. A survey of <u>A. besseyi</u> in some rice cultivars grown in Tanzania was conducted followed by assessment of some control methods and studies of crop damage by <u>A. besseyi</u>. Results of the survey while confirming the presence of <u>A. besseyi</u> in

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Tanzania; also highlighted heavy infestations in many of the rice cultivars. The implication of these observations were twofold. Firstly, that it was possible that the low yields obtained by farmers in many parts of Tanzania could be partly attributed to pest activities of <u>A. besseyi</u>. Secondly, the wide incidence of <u>A. besseyi</u> recorded in rice cultivars in Tanzania is probably due to the fact that this nematode's spread is facilitated through the seed where it can survive for considerable periods of time. Consequently, it was deemed necessary to find appropriate means of controlling A. besseyi under Tanzania conditions.

The review of literature, indicated that hot water treatment of A. besseyi-infested rice seeds was the best control method, which need not be repeated every year. In comparison to other control methods, such as chemical control involving large rice fields it was concluded that for Tanzania, control of A. besseyi in the seed treatment. would be more before planting by hot water socio-economically acceptable, practical and feasible. Hence, several hot water treatment experiments were conducted involving both unsoaked seeds (dry seed treatment) and pre-soaked seeds (seed pre-soaked in water for 18 hours before treatment).

It was noted that except for some work done by Yoshii and Yamamoto (1950), observations on the viability of treated seeds by Nandakumar <u>et al</u> (1975) and the more recent studies by Garrity and Ventura (1986) and Ventura and Garrity (1986), inadequate attention has been paid to the important aspect of the effects of hot water treatment on the germination of treated seeds. It was therefore necessary to assess the effects of some of the studied treatments on the germination and emergence of rice seeds of several cultivars. A few of the available chemicals were assessed for their efficacy in controlling <u>A. besseyi</u> and their effect on rice yields. Two experiments were also conducted to assess possible crop damage by <u>A. besseyi</u> and some corollary studies on critical periods for significant reduction of grain yield by <u>A. besseyi</u>. Field experiments using hot water treated seed and, carbofuran seed and soil treatments were also conducted, to assess the effect of the two control methods.

Results obtained from dry seed treatment of rice to control <u>A</u>. <u>besseyi</u> demonstrated, that it was possible to significantly control seed-borne <u>A</u>. <u>besseyi</u> with treatment temperatures of 54, 55, 56, 60 and 62 degrees centigrade depending on the length of exposure time, especially for the lower temperatures such as 54 and 55 degrees centigrade. However, for all the tested dry seed treatment regimes, complete control of the nematode, was not achieved. Furthermore, at the lower dry seed treatment regimes, such as 54, 55 and 56 degrees centigrade for 5, 10 and 15 minutes, more live nematodes were recovered compared to where treatment had been done at 60 and 62 degrees centigrade for 10 or 15 minutes. Similarly, when treatment was done at 54, 55 and 56 degrees centigrade for 5, 10 and 15 minutes.

It was also observed that in dry seed treatment to control <u>A. besseyi</u>, temperature as a factor was secondary in importance to exposure times.

These results agree with the findings of Cralley (1949), Todd and Atkin (1959) and, Muniappan and Sheshadri (1964), who recommended hot water treatment of rice seeds at temperatures ranging from 50°C to 61°C. These workers however, did not explicitly highlight the importance of length of exposure time as a critical factor in the efficacy of dry seed treatment to control seed-borne <u>A. besseyi</u>. Nandakumar <u>et al.</u> (1975), pointed out that in seed treatment, it is essential to get the treatment temperature to the target nematode below the seed coat.

Adequate exposure time is therefore necessary to allow penetration of treatment water through the husk/seed coat to the site of <u>A. besseyi</u> to facilitate the necessary heat transfer to kill the nematode. In dry seeds <u>A. besseyi</u> are in a quiescent state, and are considerably resistant to harsh conditions. Thus, while treatment temperature is important, the exposure time to the treatment temperature seems more crucial in actually facilitating the killing of the nematode.

Higher temperatures such as 60 and 62 degrees centigrade were found to be above the threshold for significant control of <u>A. besseyi</u>, such that with almost all nematodes dead, there were no significant differences between the two temperatures and their tested times (10 and 15 minutes) combinations.

Repeated assessments of treated seeds conducted on the same day of treatment showed 100% <u>A</u>. <u>besseyi</u> kill. However, the latter results proved inaccurate after assessments were conducted some days later (5 days and 1 week). It was concluded that live nematode assessment from treated seeds should be done 5 days or 1 week after hot water treatment.

Irrespective of whether seeds were or were not treated, 24 hours of soaking rice samples to revive <u>A. besseyi</u> was found to be inadequate for the recovery of <u>A. besseyi</u> from anabiosis. However, with treated seeds, the need to extend the soaking period for the revival of <u>A. besseyi</u> from 24 to 48 hours was apparent. It was concluded that for more correct revival results, it was necessary to treat, dry and store seeds for 5 - 7 days before assessing for the control of <u>A. besseyi</u> and that seeds to be assessed should be dehusked and soaked for 18 hours.

The time required to revive more nematodes in both treated and untreated seed was found to be the same, irrespective of cultivar (48 hours). However, the capacity for nematode revival varied with cultivars. This could be due to the condition of seeds of different cultivars, for example their storage history, moisture content, and hence their capacity to support a potentially healthy nematode population. A. besseyi has been shown to have precise activity survival, and limits for narrow environmental multiplication (Tikhonova 1966; Barat et al. 1966; Huang and Huang 1972).

Therefore, given such types of environmental limits, it is possible that the capacity to survive hot water treatment by <u>A. besseyi</u> in seed samples can vary with cultivars. This is more likely to be a reflection of the possible variations in the environments provided by the different rice cultivars, rather than the actual genetic differences. Cralley and French (1952), planted 8, 20 and 32 months old, <u>A. besseyi</u>-infested rice seeds and found that the intensity of "white tip" disease decreased with increase in the age of the seed, suggesting a deterioration in the nematode viability with storage. This would correspond to the deterioration of the seed condition and its viability. The environmental condition provided by the different cultivars can therefore vary resulting in variations in the status of the contained nematodes which will determine their capacity to survive hot water treatment.

Hot water treatment of rice seeds following presoaking was also studied in an endeavour to find out which of the two methods (dry seed treatment and treatment following presoaking) would be most appropriate under Tanzania conditions.

Hot water treatment of presoaked seeds demonstrated that seed-borne <u>A. besseyi</u> can be completely controlled at 54°C and 56°C for 15 minutes. The results agreed with Cralley, (1949) and Nandakumar <u>et al</u> (1975) who stated that presoaking seed facilitated better control of <u>A. besseyi</u> by physiologically activating the nematode and predisposing it to hot water treatment.

Hot water treatment of dry seed was comparatively less effective in controlling <u>A. besseyi</u> because, the nematode in the dry seed will be in a quiescent state, a physiological condition during which the nematode can better withstand more harsh conditions such as may be precipitated by hot water treatment. Furthermore, treatment of dry seed may not effectively allow easy and even entry of treatment water into the seed and hence transfer of the required treatment heat, to the same extend as in the treatment following presoaking. Vergara (1979) stated that presoaking rice seeds in seed quality as determined by their germination and emergence performance without treatment should be taken seriously when considering the ability of rice seeds to resist hot water treatment.

Further studies are necessary to investigate the effectiveness of hot water treatment of rice seeds in conjunction with stress factors such as sub-optimal temperatures for germination and sowing depths, to assess physiological properties of seed quality that affect germination performance.

Hot water treatment of rice seeds following presoaking at 54°C, 55°C and 56°C for 15 minutes respectively destroyed viability of a significant proportion of treated seeds and delayed onset of germination by three days for three out of four studied cultivars in one experiment. A follow up study to assess the effect of hot water treatment of seeds of four rice cultivars treated at 54°C for 15 (following presoaking) using temperature controlled minutes germinators, confirmed the possible serious adverse effects of hot water treatment of seeds following presoaking on the germination of two of the studied cultivars (Nyati and Kihogo) and the resultant significant numbers of abnormally germinated seedlings for cultivar The abnormalities included weak seedlings, seedlings with Kihogo. root or shoot only or seedlings with abnormal root systems. These findings agreed with Ventura and Garrity (1986) and David (1936) who different genotypes or seed types may differ stated that substantially in sensitivity to hot water treatment.

Therefore, it was concluded, that while hot water treatment following presoaking, at much lower temperatures such as 54°C for 15 minutes can completely control seed-borne <u>A. besseyi</u>, it can also significantly destroy seed viability and/or produce abnormal or weak

seedlings in some cultivars. These results agree with the observations made by Nandakumar et al. (1975) that while presoaking seeds before treatment results in better control of A. besseyi compared to dry seed treatment, seeds thus presoaked also became predisposed to the adverse effects of the thermotherapy. This is because presoaking will enable the seeds to imbibe water and become physiologically activated to initiate germination processes. In that physiological condition, subjecting them to hot water treatment would enable easier entry of treatment water into the seed (Vergara 1979); which in turn facilitate easier transfer of the treatment heat to the embryo, enzymes and other important proteins, destroying viability or damaging some important components of the seed. The destruction of viability is probably effected by the denaturation of enzymes and proteins in the seed caused by the treatment heat.

While the treatment regime of 54°C for 15 minutes was tolerated by some rice cultivars, for others the latter treatment significantly adversely affected germination. Tolerant cultivars were only partially affected, resulting in abnormal and/or weak seedlings. The findings agree with David (1936) who stated that some seed types are resistant to treatment at high temperatures, and that the range of thermal tolerance varies with genotypes.

A follow up investigation to study the effect of hot water treatment at 54, 55 and 56°C for 5, 10 and 15 min of presoaked seeds of 22 rice cultivars on the germination, was carried out to further assess the cultivars' seed germination performance.

The experimental results demonstrated that the factors of time, temperature and cultivar were all significantly important in the germination of presoaked rice seeds. The effect of time and temperature was inter-related in such a way that for each temperature, an increase in exposure time resulted in a significant decreas in viability and vigor. The results also showed that the reduction in percentage germination was related to the increase in either ungerminated seeds or weakly germinated seeds or both and that these were in turn significantly positively related to the effects of hot water treatment.

The findings agree with the statement made by Mayer and Poljakoff-Mayber (1978) that seeds have minimum, optimum and maximum temperatures for germination and, with Levitt (1956), who stated that seeds may still be viable after treatment at high temperatures but the subsequent development of their seedlings may be adversely affected. Therefore, treatment following presoaking is much more damaging to the seed in terms of viability and other aspects of seed and plant performance compared to hot water treatment of seeds without presoaking.

Some of the 22 rice cultivars tested were seriously affected while others showed variable levels of tolerance to what could be termed resistance, and yet a few cultivars showed positive germination reaction to hot water treatment. Genetically related cultivars reacted to the thermal treatment in a similar manner and, their germination in terms of normal seedlings, weak seedlings and ungerminated seeds were within the same range. These findings, if further investigated might be useful in assessing genetical relationships of cultivars. Cultivars were tentatively grouped according to their sensitivity to the tested regimes of hot water with suggestions for further studies of this aspect.

Results of an experiment with six cultivars designed to

study the relationship between hot water treatment and abnormally germinated seedlings demonstrated that hot water treatment following presoaking may affect seeds of some cultivars resulting in abnormally germinated seedlings with root or shoot only. The results also demonstrated that the incidence of the abnormality termed "shoot-only" was more frequent than the one termed "root-only", suggesting that the root systems were more sensitive, giving rise to more seedlings with "shoot-only" and no roots compared to those with root and no shoot. The incidence of shoot-only abnormality in genetically related cultivars such as the Afaa series was very close. The latter findings confirmed previous results that showed that genetically related cultivars reacted to hot water treatment in a similar manner. It was concluded that a further study of this phenomenon was necessary involving cultivars known to be sensitive to hot water treatment such as the Afaa Mwanza and Afaa Kilombero series and others known to be tolerant to hot water treatment such as rice cultivar Sindano.

Overall observations point to the fact that the phenomenon of abnormal seedlings, is a result of partial destruction of the seed, which was considered as a stage between a healthy germinated seed and a seed whose viability is totally destroyed. Similarly, the weakly germinated seedlings which do not show any of these root or shoot-only abnormalities, nor any other obvious abnormality, may be a result of comparatively less affected seed than those giving rise to root or shoot-only seedlings.

It is therefore suggested that further studies of the effects of hot water treatment of presoaked or unsoaked seeds should examine the incidence and the nature of seed damage from the anatomical and physiological angles of the rice seeds and seedlings.

Germination studies were also conducted involving seeds which were hot water treated following presoaking or without presoaking and sown at different depths, or sown in soil treated with carbofuran. One of the experiments involved one cultivar, Sindano, and seeds hot water treated at 54°C for 15 minutes following an 18 hour of presoaking in water. The results from the carbofuran treatments did not show significant improvement to suggest any germination enhancement conferred by the chemical. It that the latter chemical does not was therefore concluded significantly improve germination or cause any phytotoxicity. However, it was shown that hot water treatment delayed onset of germination and slowed down the rate of germination. At the deeper sowing level (6cm), hot water treated seeds showed even more delayed germination (11 days compared to less than 6 days for control seeds At the latter depth (6cm) hot water treated seed sown deep). germinated significantly less than in the control treatments. Treated seeds sown shallow attained comparable germination to those of the control treatments at the end of the experiment.

It was concluded, that the significantly less germination in the treated seeds sown deeply could not be all due to failure to germinate (loss of viability) but that it was also partly due to failure of seedlings to emerge (loss of vigour). Therefore, hot water treatment at 54°C for 15 minutes following presoaking significantly reduced total germination of seeds of cultivar Sindano by reducing vigour, resulting in affected seedlings failing to emerge from deeper levels. The results further confirmed previous findings which demonstrated that cultivar Sindano was tolerant to

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hot water treatment at 54°C for 15 minutes following presoaking but that some of its seed were nevertheless affected by this treatment resulting in loss of seed or seedling vigour. The result agrees with Levitt (1956) who stated that hot water treated seeds may still germinate, even though their subsequent growth will have been affected.

It was therefore necessary to conduct a follow up experiment to assess germination and emergence of treated seeds. The experiment was conducted in temperature controlled germination chambers, with seeds of three rice cultivars from a recently harvested crop which was not infested with <u>A. besseyi</u>. One of the cultivars, Sindano, used in the previous two-depth experiment was again included in this study which involved hot water treatment of presoaked and unsoaked seed at 48°C and 60°C for 10 and 20 min respectively and, three sowing depths.

Results showed that the treatment regimes used were two extremes. The one extreme treatment at 48°C for 10 and 20 minutes of • presoaked or unsoaked seeds and treatment at 60°C for 10 and 20 minutes of unsoaked seeds did not adversely affect germination and emergence. However, the other extreme treatment at 60°C for 10 and 20 minutes of presoaked seeds destroyed viability of almost all the seeds. It was therefore concluded that hot water treatment of presoaked or unsoaked seeds at 48°C for 10 and 20 minutes did not seriously affect germination and emergence. Similarly dry seed treatment at 60°C for 10 and 20 minutes did not adversely affect germination, if anything, the latter treatment in particular 60°C for 10 minutes and, treatment of presoaked seeds at 48°C for 20 minutes significantly improved germination and emergence over the

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control.

The failure to emerge by germinated seedlings in this particular experiment was associated with deep seeding and not with hot water treatment suggesting that there were some less vigourous seedlings among seed samples that could not cope with the deep seeding at 7.5cm. These findings agree with Heydecker and Coolbear (1977) who stated that overall seed quality may vary within the same batch of seeds and that this may be reflected in their overall performance in germination, especially under suboptimal conditions or in their capacity to tolerate stressful conditions such as thermal treatment.

The findings also demonstrated that seeds of studied cultivars irrespective of treatment were significantly lowered in germination at the deeper sowing level of 7.5cm, suggesting that rice seeds have an optimum sowing depth. The results agree with Purseglove (1976) who stated that in a dry bed, the optimum sowing depth for rice is 5-6cm in light loam soil but, not more than 2-3cm in heavy clays. The findings also showed that the tested cultivars had different optimum depths for germination or maintenance of viability. Cultivar Sindano's optimum germination depth lay between 1-3cm cultivar Bagamoyo seemed to germinate better or maintain viability better at lower levels. However, at 7.5cm depth, all cultivars were adversely affected.

It was also shown that lower germination by the rice cultivar Sindano when seeded at the deeper level (7.5cm) was not due to destruction of viability, but rather due to failure of some seedlings to emerge, thus further demonstrating the ability of cultivar Sindano to retain viability under stressful conditions.

In conclusion, dry seed treatment was shown to be more

practical and feasible, compared to treatment following presoaking due to the fact that it involves less treatment stages and therefore less risks of damaging or mismanaging the seed. Generally, dry seed treatment with temperatures ranging from 48°C to 60°C for up to 20 minutes showed no serious adverse effect on the germination of a wide range of tested cultivars. On the other hand, hot water treatment following presoaking at comparatively much lower temperature regimes of 54°C for 15 minutes significantly reduced germination of some cultivars by destroying viability and/or reducing vigour or producing abnormal seedlings.

It was shown that even for the more thermal tolerant cultivars, treatment following presoaking resulted in some far-reaching physiological adverse effects that either resulted in reduced vigour or produced abnormal seedlings. The abnormal and weak seedlings become less capable in coping with tougher environmental conditions such as suboptimal temperatures and sowing depths.

Therefore, from the seed health and practical points of view, dry seed treatment can be considered the better of the two studied treatment methods, with possible added advantage of improved seed germination and emergence. The safe treatment for germination of seeds treated without presoaking was found to be 60°C for 20 minutes for a wide range of cultivars compared with 48°C for 20 minutes for presoaked seeds. However, good seed quality and optimum germination and emergence conditions are a pre-requisite in the adoption of any of the two hot water treatment methods.

Comparative field studies on the effects of carbofuran application at different stages of rice plant growth, soil treatment

with dazomet and, hot water treatment to control seed-borne <u>A. besseyi</u> on the growth and/or yield of chosen rice cultivars were conducted.

Positive increases in the growth and yield of a rice cultivar Mpunga mwepesi were achieved from hot water and carbofuran treatments (4.1 and 3.8 times more grain than control) corresponding to zero, 220 and 1189 nematodes per 50 sample spikelets for hot water, carbofuran and control respectively. This combination of hot water treated seeds and carbofuran soil treatment resulted in complete control of nematodes and, 4.6 times more grain yield than the control. The grain yield from the combined hot water and carbofuran treatments were shown to be 10.6 and 17.1% more than the ones obtained in the hot water and carbofuran treatments independently.

It was concluded that the better yield obtained from the combined treatments was due to either the control by carbofuran of the remaining <u>A. besseyi</u> in the seed after hot water treatment and/or, probably the control of other root nematodes and insects pests. There were generally lower numbers of nematode population in the carbofuran treated plants during growth and reproduction phases of rice, compared with the control, corresponding to improvements in the measured growth parameters of rice plants. Therefore, it was shown that both hot water and carbofuran treatments were able to control <u>A. besseyi</u> resulting in improved growth and yield of rice.

Hot water treatment, in particular, dry seed treatment produced comparably better grain yield than carbofuran and can be considered more practical and economical to carbofuran treatment. Rice plants in the dazomet treatments showed vegetative overgrowth

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and delayed flowering and grain ripening compared to all other treatments. The plants had much higher numbers of spikelets compared to other treatments but the grain yield was lowest, only comparable to the control.

When the treated, untreated soil and, the chemical dazomet were analysed for available nitrogen, the results showed that the chemical contributed considerable amounts of nitrogen to the soil. It was concluded that the vegetative overgrowth observed for the rice plants from the dazomet treatments were due to excessive available nitrogen which made the results of the latter treatment difficult to explain in terms of the effect of <u>A. besseyi</u>. It can be concluded that hot water treatment of dry seeds was the better of the two tested methods giving better yield, being practically more feasible, less expensive and environmentally safe. Dry seed treatment was also found to have other beneficial effects on the germination and emergence of the treated seeds contributing to improved grain quality and greater grain yield.

The results of one other field experiment conducted to study the effects of hot water treatment of presoaked seeds (at 54°C for 15 minutes following presoaking for 18 hours) and of carbofuran soil treatment at planting on the grain yield of a rice cultivar Meli demonstrated that hot water treatment of presoaked seeds at 54°C for 15 minutes completely controlled seed-borne <u>A. besseyi</u> and increased grain yield by 27% over the control and by 11.6% over carbofuran treatment. On the other hand, carbofuran treatment had achieved 11.0% increase in grain yield over the control. However, the increase in yield was not statistically significant.

The weight of 200 grains showed that carbofuran treatment

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significantly improved the unit grain weight over the control. The increase in grain weight observed in the hot water treatment over the control and carbofuran treatments was, due to both increased unit weight of grain and more grain.

It is possible that the adverse effects of hot water treatment following presoaking on seed germination and emergence may also affect the final yield performance of plants growing from such seeds. It is therefore likely that some of the plants from treated seeds (following presoaking) did not perform optimally. Therefore, the 27% yield increase obtained with treatment of presoaked seeds could have been exceeded with dry seed treatment as demonstrated by the experiment with cultivar M'punga mwepesi.

It should be noted that, from a practical and economic point of view, a 27% increase in yield to a farmer can be considerable, especially if the increase has resulted from a low-cost technology. Consequently, results of this nature, should be viewed not only from a statistical point of view but also from the farmer's practical and economic situation.

A field experiment was also conducted to study the effects of carbofuran treatment at different stages of rice growth and of hot water treatment of presoaked seeds on the yield of a rice cultivar, Bagamoyo.

Results of this study demonstrated that hot water treatment of presoaked seeds completely controlled seed-borne <u>A. besseyi</u>. Carbofuran seed treatment considerably reduced both percentage seed infestation and the total infestation levels but did not achieve complete control. Soaking seeds in water (for 18 hours) without subsequent hot water treatment reduced nematode infestation but, to significantly improved grain yield by increasing the number of grains formed. The results agree with Stansel (1975) who stated that booting stage of rice is a very sensitive and vulnerable stage in the life of a rice plant, during which the numbers of buds formed to become florets (grains) are determined.

The results of an experiment conducted to assess the best method of applying carbofuran to improve yield showed that, grain yield increased from carbofuran half rate to the recommended rate when applied to holes and, decreased from half to recommended rate for furrow application. It was also shown that, with increased rate of chemical application to holes, there was an increase in the grain yield. The chemical was more thinly spread when applied to furrow and therefore, the results of the furrow application were considered as not reflecting the actual effects of carbofuran on the yield.

It was concluded that, hole application of carbofuran at the recommended rate is better than hole or furrow application of the half rate. There was an indication that more yield may be obtained by increasing the rate of chemical applied to holes over the recommended rate, although that kind of increase would be economically prohibitive.

In the present study, yield results were considered from the following perspective: that pest control studies designed to improve yield for the growers should not only consider the technical significance of statistics, but to also evaluate the yield improvements from a farmer's economic and practical situation. The latter aspect was considered to involve the type of crop, the value of which can vary with the market, the production hectarage and the cost of the technology used to improve the yield. These factors among other economic criteria will determine the profitability of the yield increase, which may or may not tally with the statistical significance levels. Furthermore, quality improvements such as those obtained in the present studies with the rice cultivar, Bagamoyo are also considered to be important factors of yield improvement.

Hot water treatment, depending on the cultivar was found to improve yield by increasing the numbers of grains formed or by increasing the unit grain weight and the quality of grains. Except for rice cultivar Bagamoyo, hot water treatment gave comparably more grain yield for all other experiments than did carbofuran. The improvement of grain yield by carbofuran application during booting stage of rice was by increasing the numbers of grain formed, while that of carbofuran applied to seeding holes at planting was by increasing the unit grain weight. The results suggest a possibility of choosing cultivar and/or phase of rice plant growth for the control of <u>A. besseyi</u> by carbofuran or by hot water treatment for the improvement of seed size or to increase numbers of grains formed or both.

The results demonstrated that <u>A. besseyi</u> can reduce rice yields, the magnitude of which can depend on rice cultivar. The yield and seed quality improvements obtained with hot treatment for cultivars M'punga mwepesi, Meli and Bagamoyo were considered important, as they were obtained with a low cost technology that need not be repeated for years, while its benefits continue to accrue.

Inoculation of all or half tillers with <u>A. besseyi</u> at the first sign of booting for all studied parameters did not make any significant difference to the yield. The results also demonstrated that during the seven days from the first sign of booting, for the four inoculation times, only the 5th day was consistently significant for all parameters. The results from the inoculation on the 5th day after the first sign of booting showed that the control was consistently better in all yield attributes studied than all or half tillers inoculated with nematodes. Half tillers inoculated with nematodes yielded significantly better than all tillers inoculated with nematodes. It was therefore concluded that inoculation with <u>A. besseyi</u> to all tillers on the 5th day after the first sign of booting significantly adversely affected all the studied yield parameters.

Infection by <u>A.</u> <u>besseyi</u> during the 5th day after the first sign of booting probably interfered with the determination of numbers of flower buds formed (as that is the time when the latter are said to be determined, Stansel, 1975) and the development of the panicle resulting in shorter panicles and less spikes and spikelets (grains).

These findings isolated the 5th day after first sign of booting as a particularly critical stage within the period of panicle development for the damage by <u>A. besseyi</u> resulting in adverse effects on all studied yield parameters. The results also confirmed the importance of the booting stage in the determination of grain formation and also demonstrated that <u>A. besseyi</u> can significantly reduce rice yield.

The results from a field experiment with a rice cultivar Bluu, demonstrated that, where either 3/4 or 4/4 seeds planted were untreated, the grain yields were lower by 12.95 and 14.36%
respectively than where all seeds planted were hot water treated following presoaking. The same was true for the weight of 200 grains. However, where  $\frac{1}{2}$  or 3/4 of the planted seeds were hot water treated, the yields were 8.06 and 9.17% greater than where all seeds planted had been hot water treated and even greater than in the control (where all (4/4) seeds) and 3/4 of the seeds were untreated. These yield increases however were not statistically significant.

Better yields and greater weight of 200 grains obtained from the ½ and 3/4 seed-treated treatments over yields from all seeds presoaked and hot water treated were more likely due to below-damaging levels of A. besseyi within the former treatments coupled with the proportion of treated to untreated seed within them, which more likely offset the debilitating effect of hot water treatment following presoaking. The combination of the two aspects put the two treatments at an advantage over where 3/4 and 4/4 seeds were untreated (with maximum A. besseyi effect) or where 3/4 or 4/4 seeds were presoaked and hot water treated (with maximum thermal treatment effect on seeds) resulting in reduced seedling vigour. Hot water treatment of presoaked and dry seeds at 54°C for 15 min was shown in the previous experiments to significantly affect seed vigour for considerable numbers of rice cultivars, and that seeds thus treated did not germinate/emerge and hence affected the yield. Johnston (1975) stated that seed vigour can be a measure of subsequent field performance of the resultant plants and Heydecker (1972) stated that vigour is an essential attribute of the seed that affects its ability to deliver its potential at any stage of the plant life.

The combination of the below-damaging levels of A. besseyi

in the  $\frac{1}{2}$  and  $\frac{3}{4}$  treated seeds and the proportion of treated to untreated seeds in the latter two treatments probably produced plant populations with better field and yield performance than in the control ( $\frac{4}{4}$  seeds) and in  $\frac{3}{4}$  seeds infested with <u>A. besseyi</u> or where all seeds were hot water treated following presoaking.

The findings substantiate observations made from yield performance of previous experiments with presoaked and hot water treated seeds of cultivars Meli and Bagamoyo and those of dry seed treatment of seeds of cultivar M'punga mwepesi. The former treatments (of presoaked seeds) increased yield over the control by 10-27% while the latter (dry seed treatment) gave 4-5 times more yield than the control.

The complete control of <u>A. besseyi</u> in the seeds planted in the experiment was confirmed in the harvested seeds. The nematode infestation was significantly higher in the control (all seeds untreated) as compared to where all or one quarter or half of the seeds were treated. However, where three quarters of the seeds were untreated, the nematode infestation in the harvested seeds though less than in the control, was not significantly different.

Therefore, the mixing of treated and untreated seeds had successfully graded the nematode infestation levels from zero (all treated seeds) through to the maximum in the control seeds and these levels had been passed on from the planted seed through to the harvested grain. It was therefore concluded that seed-borne <u>A. besseyi</u> can reduce grain yield and unit grain weight and that the extent of yield reduction depends on the levels of <u>A. besseyi</u> in the seeds. It was also concluded that hot water treatment of rice seeds at 54°C for 15 min following presoaking can completely control

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seed-borne <u>A.</u> <u>besseyi</u> with consequential increases in the grain yield and unit weight of grains. It was considered that, grain yield could be greater if hot water treatment is done to seeds without prior soaking or if treated and untreated seeds are mixed in right proportions.

The findings of the whole range of studies conducted to investigate the incidence and control of <u>A. besseyi</u> and, to assess the effect of <u>A. besseyi</u> on the growth and yield of several rice cultivars demonstrated that considerable numbers of cultivars grown in Tanzania harbour high levels of <u>A. besseyi</u> in their seeds.

The latter pest was shown to be contributing to the lower rice yields in Tanzania. Hot water treatment of seeds (without prior soaking) at 60°C for 10 min can significantly control the nematode resulting in better seed germination, emergence and subsequent better field performance and yield. The latter control method is recommended as the best of all tested methods because its less complicated, cost - effective and safe to the seed and the environment. The method can be applied on a wide scale through the national seed distribution system.

## 9. SUMMARY

Aphelenchoides <u>besseyi</u> Christie, 1942, is a plant parasitic nematode of world wide distribution, which causes "white tip" disease of rice. The disease is considered of economic importance in most rice producing countries. In view of the increasing importance of rice (<u>Oryza sativa L</u>) as a food crop in Tanzania and Africa as a whole, investigations were conducted to assess the incidence of <u>A. besseyi</u> in seeds of some rice cultivars grown in Tanzania, its effects on the yield and its control under Tanzania conditions.

Results of the survey revealed that the distribution of <u>A. besseyi</u> in rice in Tanzania is wide with heavy infestations in some cultivars. The wide distribution of this nematode was considered to be associated with its habit of spreading through infested seed, where it can survive for considerable periods.

Having confirmed the prevalance of <u>A. besseyi</u> in seeds of rice cultivars grown in Tanzania, assessed the effect of <u>A. besseyi</u> on the yield of selected cultivars, tested two control measures, namely hot water treatment (HWT) and chemical control; work was carried further to study the effect of HWT on the germination and emergence of seeds of selected cultivars as a means of assessing the latter method's possible practical use for Tanzanian farmers.

Hot water treatment studies involved treatment of presoaked and unsoaked seeds to assess the efficacy of the two methods in the control of <u>A. besseyi</u> and the effect of the thermotherapy on the germination and emergence seeds. Dry seed treatment studies demonstrated that, it was possible to significantly control <u>A. besseyi</u> at temperatures ranging from  $54^{\circ}$ C to  $62^{\circ}$ C depending on the length of exposure time where 5 and 10 min were comparably less effective than 15 min, especially at the lower temperatures.

At higher treatment temperatures of 60 and 62°C, exposure times of 10 and 15 min were not significantly different from each other, because at those treatment regimes almost all nematodes were killed.

With dry seed treatment, it was demonstrated that, exposure time was the significant factor in controlling seed-borne <u>A. besseyi</u> and not the treatment temperature and, the two factors did not significantly interact. The effect of hot water treatment of dry seeds on the control of <u>A. besseyi</u> was not influenced by different cultivars. Furthermore, it was demonstrated that 48 hours as opposed to 24 hours of soaking dehusked seed gave maximum revivals of seed borne <u>A. besseyi</u> from quiescence in both treated and untreated seeds, and that to obtain reliable results of nematode kill in hot water treated seeds, assessment should be done several days (1 week) after treatment, as opposed to the same day of treatment.

Results of hot water treatment of presoaked seeds demonstrated that complete control of <u>A. besseyi</u> can be achieved at much lower temperature regimes (54°C for 15 min) compared to dry seed treatment. While hot water treatment of presoaked seeds at 54°C for 15 min did significantly affect germination, vigour and the normal growth of seedlings of some cultivars, higher temperatures such as 56°C for 15 min killed almost all seeds of most cultivars.

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For treatment of presoaked seeds, both temperature and exposure time were significant factors (in the germination of treated seeds)which interacted significantly, such that at each treatment temperature, an increase in the exposure time resulted in a significant increase in the percentage seeds that failed to germinate or germinated into weak seedlings.

Some rice cultivars exhibited great sensitivity to increases in temperature, others reacted positively but moderately, while a few showed negative correlation with temperature. It was observed that genetically related cultivars reacted in a similar manner and within the same range.

Dry seed treatment was considered the better of the two studied hot water treatment methods, as it significantly controlled <u>A. besseyi</u> without deliterious effects on the seed and its subsequent performance in germination, emergence and yield. Dry seed treatment was considered better because it is logistically a less complicated procedure which, if conduceted on seeds of good quality, may even improve germination performance over that of untreated seed.

Hot water treatment of presoaked or unsoaked rice seeds at suitable temperatures and time regimes significantly controlled seedborne <u>A. besseyi</u> and, depending on the rice cultivars, increased grain yield and/or the unit grain weight. In the case of the rice  $\frac{1}{100}$ , Bagamoyo, the quality of rice grains and percentage grain ripening was also considerably improved. Yield increases of 10 -27% were obtained for experiments that involved hot water treatment of presoaked seeds. Although most of the grain increases were statistically non significant, to a farmer, yield improvements of such magnitudes could nevertheless be economical and desirable, especially when the increases are obtained through a low-cost and environmentally safe technology, such as hot water treatment which need not be repeated for some years, meanwhile it's benefits continue to accrue with added grain quality improvements.

It was concluded that seed-borne <u>A. besseyi</u> does reduce yield of rice in Tanzania and that the control of the latter pest results in yield increases, the magnitude of which depends on the cultivar and on whether or not HWT was done to presoaked or unsoaked seeds.

Older rice seeds became more sensitive to hot water treatment suggesting a breakdown in tolerance as seed quality and viability decreased with age.

The adverse effects of hot water treatment following preasoaking included loss of viability, reduction in vigour and above ally production of abnoranlly germinated seedlings. The latter two forms of abnormality were found to be intermediate stages between total loss of viability and normal germination. More sensitive cultivars lost seed viability, tolerant cultivars lost vigour or germinated into abnormal seedlings and, the less vigorous seedlings failed to successfully germinate from suboptimal seedling depths of 6.0 and 7.5 cm, especially seeds treated following presoaking.

Hot water treatment of presoaked seeds delayed onset of, and peak germination, attainment of maximum germination, significantly reduced the actual percentage germination and emergence and resulted in significant increases in debilitated and abnormal seedlings, which failed to perform optimally under stressful germination conditions. It was considered that, seedlings thus debilitated or abnormal, might not recover well enough to yield to their full potential.

Dry seed treatment studies showed that rice seeds of different cultivars were able to germinate and emerge well after treatment with temperatures ranging from 48°C to 60°C for up to 20 min. In fact, dry seed treatment at 60°C for 10 min and treatment of presoaked seeds at 48°C for 20 min significantly improved germination and emergence of studied cultivars beyond that of the control. However, for both dry seed treatment and treatment of presoaked seeds, it was shown that older seeds were significantly adversely affected by hot water treatment.

While dry seed treatment could be tolerated by more rice cultivars over wider and higher treatment regimes, treatment of presoaked seeds was tolerated by fewer rice cultivars at lower temperature and time ranges.

Seed treatment with carbofuran before planting or carbofuran treatment to the soil during tillering and flowering of rice was not effective in controlling <u>A. besseyi</u> or in improving grain yield. However, carbofuran soil treatment at planting and booting stages of rice improved grain yield, especially treatment during booting stage resulted in significant yield improvement. Carbofuran soil treatment at planting increased yield by increasing unit grain weight, while treatment at booting increased the numbers of grains formed. On the whole, carbofuran treatments were surpassed in yield performance by hot water treatment. It was concluded that hot water treatment of unsoaked or presoaked rice seed was better than carbofuran treatment in controlling <u>A. besseyi</u>, as well as in improving rice yield. Hot water treatment is therefore recommended for it s excellence in the control of <u>A. besseyi</u>, in improving yield and seed quality and, for being cost-effective and environmentally safe.

The fifth day after the first signs of booting was shown to be the critical period for infection of developing panicles with <u>A. besseyi</u> adversely affect yield parameters. The latter results demonstrated that <u>A. besseyi</u> can significantly adversely affect grain yield of rice. Investigations of the effects of different levels of <u>A. besseyi</u> in the seeds on the yield of rice demonstrated that, there are levels below and above which yield will or will not be adversely affected by seed borne <u>A. besseyi</u>.

The present study demonstrated that, the white tip nematode of rice, <u>Aphelenchoides besseyi</u> Christie, is an important pest of rice in Tanzania, which should be considered as a constraint in rice production. It can effectively be controlled by hot water treatment of rice seeds to the benefit of Tanzanian rice growers.

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Appendix 6.1 Analysis	of soil,	treated with the c	chemical dazomet, for ava	ilable nitrogen
Analysis for available N:-				
		NH <sup>+</sup> – N	$N - \frac{2}{3} - N$	Total N
Untreated soil (2g sample)	0.7mJ	of 0.01N acid	1.9ml of 0.01N acid	2.6ml of 0.01N acid
Treated soil (2g sample)	1.7ml	of 0.01N acid	2.2ml of 0.01N acid	3.9ml of 0.01N acid
Dazomet (1g sample)	1.5mJ	. of 1N acid	2.1ml of 1N acid	3.6ml of 1N acid
N in sample (mg)		11 acid x N acid x 1 g sample	14	
mgN in 1g dazomet	11	3.6 x 1 x 14 = 50.4n	ත ළ	
dazomet applied	1111	356.4g/800kg soil Lg/2.2kg soil 0.45g/kg soil		
1g dazomet adds 50.4mgN	to kg so	п		
• • 0.45g dazomet adds 22.7m	gN to kg	soil		
Dazomet adds 22.7mg	N to eac	h kg soil		
Extra N in treated soil (mg)	11	N in treated soil -	. N in untreated soil	
	II	(3.9 - 2.6) x 0.01 2	<u>× 14</u> mg/g	
	11 11 11	1.3 x 0.01 x 7 mg/g 9.1 x 0.01 mg/g = C 91mg/kg soil	g soil 0.091 mg/g soil	

68.3mgN/kg soil more than that added by the dazomet itself.

II

. Treatment adds 91 - 22.7

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Appendix 6.2 Summary of Analysis o using carbofuran and h "Weli"	of variance fi hot water trea	com an experi- tment on the	iment to assess th grain yield and we	e effect of co ight of 200 seed	ntrolling <u>A. besseyi</u> Is of a rice cultivar
		Grain	yield	Weight of	200 seeds
source of variation Ariation	DF	SM	Significance level	SM	Significance level
reatment Residual	0 0	0.9689 0.5324	SN	0.09351 0.01574	*
Appendix 6.3 Summary of analysis c application on grain w	of variance f weight and hus	rom an exper k weight of a	iment to assess t nematode infested	he different me rice cultivar	thods of carbofuran 'Bluu"
		Grair	ı yield	Weight o	200 seeds
Source of variation variation	DF	W	Significance level	W	Significance level
Treatment Residual	0 0	97.48 40.92	SN	6.537 5.673	SN

-

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Summary of significance levels for various studied aspects from an experiment to study the effect of controlling <u>A.</u> <u>besseyi</u> using carbofuran and hot water treated seeds on the yield and weight of 200 grains of a rice cultivar "Bagamoyo" are shown Appendix 6.4

a. Grain weight (g) and weight of 200 grains (g)

		Gra	in yield	M	eight o	r 200 seeds
Source of variation variation	DF	SM	Significance level	DF	SM	Significance level
Treatments Residuals	6 17 (1)	5469 2134	NS	7 21	0.0951 0.0103	* *

b. Analysis of mean number of infested harvested seed and mean number of nematodes per seed

		Gra	in yield	Weight	of 200 seeds
Source of variation variation	DF	WS	Significance level	WS	Significance level
Treatments	N	145.125	*	11.05774	* *
Seed filling levels	ч	3.375	NS	1.7334	**
Treatment x levels	2	0.875	NS	0.8870	* *
Residuals	15	1.053		0.0260	

Analysis for the seed germination of filled and unfilled harvested seed :0

Source of variation variation	DF	WS	Significance level
Treatment Seed filling levels Treatment x levels Residuals	90 H 0 6	52.82 3844.57 63.44 74.13	SN * 20 SN * 20

## Appendix 7.1

## Treatment combinations

- 1. Control = all tillers inoculated with distilled water at first sign of booting.
- 2. All tillers at first sign = all tillers inoculated with nematodes at first sign of booting.
- 3. All at third day = all tillers inoculated with nematodes on third day from first sign of booting.
- 4. All at fifth day = all tillers inoculated with nematodes on fifth day from first sign of booting.
- 5. All at seventh day = all tillers inoculated with nematodes on seventh day from first sign of booting.
- 6. Half at first sign = half the tillers inoculated with nematodes at first sign of booting.
- 7. Half at third day = half of the tillers inoculated with nematodes on third day from first sign of booting.
- 8. Half at fifth day = half the tillers inoculated with nematodes on fifth day from first sign of booting.
- 9. Half at seventh day = half the tillers inoculated with nematodes on seventh day from first sign of booting.
- 10. Control half at third day = half the tillers inoculated with distilled water on third day from first sign of booting.
- 11. Control half at third day = half the tillers inoculated with distilled water on third day from first sign of booting.
- 12. Control half at fifth day = half the tillers inoculated with distilled water on fifth day from first sign of booting.
- 13. Control half at seventh day = half the tillers inoculated with distilled water on seventh day from first sign of booting.
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