Control of *Pythium* wilt and root rot of hydroponically grown lettuce by means of chemical treatment of the nutrient solution.

by

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SUMMARY:

Hydroponic production was initially explored as an alternative to field production due to the ease of plant growth control and the hopes of preventing the majority of disease causing agents known to be present in general soil environments. Of primary concern in terms of pathogens are the water-borne and water-motile zoosporic fungi (especially *Pythium* spp.) which are able to spread easily throughout the system and cause root-rot and wilting. Few pesticides are currently registered for use in hydroponic systems due to the high costs of registration, while registered pesticides carry a high cost to the grower. Recent legislative moves by numerous countries are also resulting in a trend towards the re-use of hydroponic nutrient solution. As a result such hydroponic solutions require a greater level of disinfection to prevent disease outbreaks but without resulting in chemical buildup of phytotoxic and environmental concern.

Sanitiser formulation has seen significant changes over the last few years resulting in sanitisers being used in many new areas and in a more environmentally friendly nature. Although sanitisers are not designed to have specific action against micro-organisms (as is the case with fungicides and anti-microbial agents such as antibiotics), most sanitisers are able to act on cell membranes due to the inherent surfactant properties.

This study attempted to determine the suitability of various sanitisers and chemicals as alternate means of control of *Pythium* in recirculating gravel hydroponic systems by:

1). Exposing *Pythium* zoospores in a water suspension to the sanitisers Actsol®, Agral 90®, Fitosan®, Prasin®, Purogene®, TecsaClor®, Sporekill® and copper (as copper (I) sulphate) which all managed to eliminate 80% or more of the viable inoculum within a 10 minute exposure time at relatively low concentrations.

2). Testing the above sanitisers for phytotoxicity effects on cucumber plants in a static hydroculture system under laboratory conditions and lettuce plants in a gravel bed hydroponic system under greenhouse conditions. Purogene® and TecsaClor® exhibited a slight growth promotion effect at low concentrations, yet still caused negative phytotoxic effects when dosed at high concentrations. All other sanitisers exhibited some measure of phytotoxicity, observed as growth retardation and leaf discolouration, with phytotoxic effects increasing with increasing concentrations. Copper sulphate was found to be the most phytotoxic chemical tested.

3). Addition of the sanitisers to a small scale hydroponic system (greenhouse), as well as to a semi-commercial scale (field) gravel bed hydroponic system artificially infested with *Pythium* and cultivated with lettuce. The sanitisers were also compared to a commercially available fungicide, Phytex®. Only Phytex® and Purogene® managed to effectively reduce disease incidence and promote growth over an untreated, *Pythium* infested control.

The results indicated that Purogene® was the most effective for application into a gravel bed hydroponic system cultivated with lettuce, while no sanitiser treatment was able to equal the improved growth and disease control recorded with treatment of the commercial fungicide Phytex®. Although all the sanitisers were able to reduce levels of *Pythium* inoculum in the hydroponic nutrient solution, this beneficial effect did not translate into increased yields, due to the growth retardation due to phytotoxic effects.

CONTENTS

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DECLARATION

I, the undersigned, declare that the dissertation, which I hereby submit for the degree of Master of Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this, or any other, university

Name: _____ Signature: _____

Date: _____

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Hydroponics and soilless cultivation systems of plant production are used worldwide to grow flower, foliage, bedding and vegetable crops (Carruthers, 2002; Song *et al.*, 2004). Certain crops cultivated in this manner are of significant economic importance (Paulitz *et al.*, 1992). Plants are grown using nutrient solutions, with or without solid substrates for root growth (Song *et al.*, 2004). The nutrient solution can either be re-circulated in a closed system or drained after use in an open system. Hydroponic systems have become popular over the last 20 years all over the world for the growth of high-value crops in glasshouses (Savvas *et al.*, 2002).

Use of hydroponic cultivation systems in greenhouses offers a unique situation that may make conditions more favourable for diseases. A hydroponic culture system is easily infected by soil-borne pathogens such as *Fusarium* and *Pythium* spp. (Schwarz and Grosch, 2003). Pathogens cannot be completely excluded from the greenhouse environment. In hydroponic systems, *Pythium* zoospores are released from infected roots into the nutrient solution, where they are dispersed throughout the hydroponic system (Paulitz *et al.*, 1992; van West *et al.*, 2003). Airborne spores enter through doors and screens, soil-borne pathogens enter through dust or contaminated soil on shoes, tools, or equipment, and some pathogens are introduced on seeds or contaminated propagating materials (Paulitz, 1997; Schwarz and Grosch, 2003). Fungal gnats have also been reported as probably the most important vector of root pathogens (Stanghellini *et al.*, 1996)

Methods of control include the application of systemic pesticides via the nutrient solution in closed soilless culture systems (Wood and Laing, 1992, as cited in Karras *et al.*, 2006). Other methods include the use of mono-potassium phosphates (Reuveni *et al.*, 2000). However, the pathogen resistance to most pesticides makes this solution temporary. Current methods of sterilization such as ozonation and the use of ultra-violet light are costly, difficult to manage and can lead to a buildup of toxic compounds (Carrillo *et al.*, 1996; Monarca *et al.*, 2000).

Stanghellini and Miller (1997) have shown that surfactants can exhibit a lytic activity against zoospores. Surfactants can be used to control root-infecting zoosporic plant pathogens in hydroponic systems (Stanghellini *et al.*, 1996). Synthetic surfactants also have potential to control leaf-attacking zoosporic plant pathogens such as white rust (Irish, 2002, as cited by De Jonghe, 2005).

In the current study a range of sanitisers were evaluated for their efficacy in controlling *Pythium* infestation in hydroponics by means of treatment of the nutrient solution. Some additional compounds, such as copper sulphate, the surfactant Agral 90® and the commercial fungicide potassium phosphonate (Phytex®), were included for comparison.

1.2 Motivation for study

Although hydroponic cultivation is able to exclude many soil-borne pathogens, and thus reduce disease variety, a number of water-borne and water-disseminated pathogens (Schwarz and Grosch, 2003) are still able to infest these systems and cause severe crop devastation, to an extent worse than what would be experienced in soil-cultivated crops. This requires that attention be paid to the disinfection of the re-circulating nutrient solution in order to eliminate or reduce disease pressure and associated crop losses.

There is now a greater consumer awareness of agrochemical problems, such as the negative impact on the environment from the use of harmful and liberally applied pesticides and harsh chemical treatments (Saba and Messina, 2003).
After the initial work by Stanghellini *et al.* (1996) on disease control using surfactants, various products with similar formulations or activity have been identified as having comparable effects.

Previous studies have generally been limited in either target pathogen or sanitiser selection and have dealt minimally with possible phytotoxic effects. The current study includes a wide variety of commercially available sanitisers against three vastly different pathogens, while also assessing possible negative effects due to phytotoxicity.

1.3 Aim of the Study

Establishing whether any of the sanitisers under investigation would be suitable for application in hydroponic systems to limit yield losses of lettuce crops due to infection caused by *Pythium* spp.

1.4 Objectives

1. To determine *in vitro* efficacy of a range of sanitisers and the most appropriate dosage rates of each sanitiser against three selected plant pathogens, including *Pythium* spp.
2. Establish whether sanitisers are phytotoxic on both cucumber (rapid assay) and lettuce (hydroponic system).
3. Evaluate the benefits of application of sanitisers at the pre-determined dosages (as established in Points 1 and 2, above) into a *Pythium* infested hydroponic system, at both a greenhouse level and a semi-commercial field system.

1.5 References

Carrillo, A., Puente, M.E. and Bashan, Y. 1996. Application of diluted chlorine dioxide to radish and lettuce nurseries insignificantly reduced plant development. Ecotoxicology and Environmental Safety 35: 57-66.

Carruthers, S. 2002. Hydroponics as an agricultural production system. Practical Hydroponics & Greenhouses. Issue 63. (Mar/Apr) pp1-4.

De Jonghe, K., De Dobbelaerea, I., Sarrazynb, R. and Höfte, M. 2005. Control of brown root rot caused by *Phytophthora cryptogea* in the hydroponic forcing of witloof chicory (*Cichorium intybus* var. *foliosum*) by means of a nonionic surfactant. Crop Protection 24: 771-778.

Irish, B.M., Correll, J.C., Morelock, T.E. 2002. The effect of synthetic surfactants on disease severity of white rust on spinach. Plant Disease 86: 791–796.

Karras, G., Savvas, D., Patakioutas, G. and Pomonis, P. 2007. Fate of cyromazine applied in nutrient solution to a gerbera (*Gerbera jamesonii*) crop grown in a closed hydroponic system. Crop Protection 26: 721–728.

Monarca, S., Feretti, D., Collivignarelli, C., Guzzella, L., Zerbini, I., Bertanza, G., and Pedrazzani, R. 2000. The influence of different disinfectants on mutagenicity and toxicity of urban wastewater. Water Research 34: 4261-4269.

Paulitz, T.C., Zhou, T. and Rankin, L. 1992. Selection of rhizosphere bacteria for biological control of *Pythium aphanidermatum* on hydroponically grown cucumber. Biological Control 2: 226-237.

Paulitz, T.C. 1997. Biological control of root pathogens in soilless and hydroponic systems. HortScience 32: 193-196.

Reuveni, R., Dor, G., Raviv, M., Reuveni, M. and Tuzun, S. 2000. Systemic resistance against *Sphaerotheca fuliginea* in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate. Crop Protection 19: 355-361.

Saba, A. and Messina, F. 2003. Attitudes towards organic foods and risk/benefit perception associated with pesticides. Food Quality and Preference 14: 637-645.

Savvas, D., Manos, G., Kotsiras, A. and Souvaliotis, S., 2002. Effects of silicon and nutrient-induced salinity on yield, flower quality and nutrient uptake of gerbera grown in a closed hydroponic system. Journal of Applied Botany 76: 153-158.

Schwarz, D. and Grosch, R. 2003. Influence of nutrient solution concentration and a root pathogen (*Pythium aphanidermatum*) on tomato root growth and morphology. Science Horticulture 97: 109-120.

Song, W., Zhou, L., Yang, C., Cao, X., Zhang, L. and Liu, X. 2004. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. Crop Protection 23: 243-247.

Stanghellini, M.E., Kim, D.H., Rasmussen, S.L. and Rorabaugh, P.A. 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. Plant Disease 80: 1113-1116.

Stanghellini, M.E. and Miller, R.M. 1997. Biosurfactants. Their identity and potential efficacy in the biological control of zoosporic plant pathogens. Plant Disease 81: 4-12.

van West, P., Appiah, A.A. and Gow, N.A.R. 2003. Advances in research on oomycete root pathogens. Physiological and Molecular Plant Pathology 62: 99-113.

Wood, A.R. and Laing, M.D., 1992. The control of fungal root pathogens of ornamental foliage plants in hydroculture. In: Proccedings of the Eighth International Congress on Soiless Culture. ISOSC, Wageningen, The Netherlands, pp. 513–526.

CHAPTER 2

LITERATURE REVIEW

The following text is intended as a brief overview to elucidate the rationale of the current study and provide background on the concepts discussed during this study and is not intended as an exhaustive and in-depth review.

2.1 Hydroponics

2.1.1 Overview

Hydroponics is the science of growing plants in a soilless (non-nutritive) substrate (Song *et al.*, 2004), with nutrition being supplied artificially, most commonly in the water supply, directly to the roots (Stanghellini and Rasmussen, 1994), while foliar feeding can also be used. The usual design of the hydroponic systems is such that the plant roots are exposed to the nutrient solution (liquid systems) (Stanghellini and Rasmussen, 1994), or the nutrient solution is directly applied to the root zone (aggregate systems) (Neiderweiser, 2001). More recent developments sometimes include application of the fertigants to the leaf surfaces to supplement plant nutrition by foliar application.

The word hydroponics originates from the Greek *hydro*, meaning water, and *ponos*, meaning work or labour (Harris, 1976), to indicate that the main "work" for growth is provided by the water in which the plants are grown.

Originally hydroponics was defined as the growth of plants without soil, or alternatively in water, and was used primarily in the more recent past by scientists to achieve greater control of environmental conditions in small-scale trials (Fresh Produce Hydroponics, 2002). A more current definition which is more applicable to commercial cultivation is: "Hydroponics or soil-less culture is the production of crops isolated from the soil, either with or without a medium, with their total water and nutrient requirements supplied by the system" (Hanger, 1993; Jensen, 1999).

The practice of growing plants in a hydroponic system in various, basic forms has been utilised by farmers since several hundred years B.C. This is specifically seen in hieroglyphs and drawings from ancient Egyptian history (Fresh Produce Hydroponics, 2002). The Egyptians, Inca Indian tribes, the Aztecs, and the Babylonians are examples of ancient civilizations which practiced hydroponic gardening without even realizing it, long before the word "hydroponics" was ever thought of (Deutschmann, 1998). It is quite possible that the most primitive form of hydroponics was the suspension of plants in a thin soil and water mixture which provided the basic nutrients required.

Hydroponics has seen more widespread commercial use since the mid-1930's, with Western Europe leading this trend (Zinnen, 1988). This commercial interest was primarily due to the scientific development of specifically designed fertiliser mixes for use in hydroponics, and these mixes subsequently becoming more readily available to the commercial growers. Other aspects aiding the development of hydroponics included: The use of plastics (Fresh Produce Hydroponics, 2002) which allowed more cost effective and less labour intensive production of the physical facilities; New types of inert substrates such as rockwool, perlite and vermiculite being introduced and used as a growth substrates

(Niederweiser, 2001; Gul *et al.*, 2005); and lastly, the research, and subsequent development of, more refined hydroponic growth systems such as the Nutrient Film Technique and ebb-and-flow systems where the plants are not continuously immersed in a static solution (Harris, 1976). These developments have also greatly expanded the variety of crops which can now be cultivated in modern hydroponic systems.

Hydroponic cultivation is split into two broad categories, namely liquid systems where no inert substrate is present, and aggregate systems where an inert (non-nutritive) substrate such as sand, gravel or rockwool is used (Stanghellini and Rasmussen, 1994). The purpose of the substrate is to provide a physically supportive structure to enable the plant to remain upright.

Hydroponic systems can further be divided into closed and open systems (Stanghellini and Rasmussen, 1994; Niederweiser, 2001) – where closed and open refer to the water supply. Closed systems refer to those where the nutrient solution is collected and re-used after treatment and adjustment for nutrient losses and then re-supplied to the plant roots. This type of system is becoming the method of choice (Carruthers, 2002) due to reduction of constant input costs and thus improving the economic efficiency of the fertigants, while also preventing environmental pollution such as contamination of sub-surface water sources. The Dutch government has already passed laws which enforce the use of only re-circulating systems (Runia, 1994; Runia, 1995; Fresh Produce Hydroponics, 2002) to prevent damage to the environment and it is presumed that other countries will follow this trend in the future.

In open systems the water is allowed to drain freely as waste-water or is collected and used for an alternate purpose such as irrigation. This type of system is usually seen in bag-type production systems where collection of waste-water is far more of a logistic problem than in nutrient-flow based production (Niederweiser, 2001).

The advantage of hydroponic production was initially explored as an alternative to field production due to the ease of plant growth control and the hopes of preventing the majority of disease causing agents known to overwinter or be present in general soil environments (Zinnen, 1988; Stanghellini and Rasmussen, 1994).

Further advantages of hydroponic systems were soon realized in that crops can be grown in areas where there are problems with soil suitability, in non-arable or borderline areas (Savvas, 2003), or where environmental factors such as temperature or winds prevent acceptable yields as well as areas where slope of land prevents ploughing (Paulitz *et al.*, 1992). The environmental conditions can be overcome since much of the hydroponic systems are under some form of covering such as shade net, plastic tunnels or greenhouse (multispan) complexes. In environmentally controlled greenhouses crops can also be grown year round with the same yields obtained during summer and winter (Cornell CEA Homepage, 2002), while a minimal form of environmental consistency can be obtained under shade net and in plastic tunnels by means of fans, heaters and specialised mist systems.

Hydroponics in the current form is extremely beneficial in a commercial sense as plant growth is more controlled and uniform, and up to eight crops (in the case of lettuce) can be cultivated in a 12-month cycle (Zinnen, 1988; Cornell CEA Homepage, 2002), compared

to a maximum of six crops or less when grown under regular open field conditions. Furthermore a higher yield per area is obtained from hydroponic production due to lesser spacing requirements necessary between plants, and a consistent growth is achieved between crops as the nutrient supply remains constant throughout the year, in contrast to fields becoming more nutrient deficient and requiring expensive agricultural inputs between crops (Savvas, 2003). A more consistent growth is also achievable due to minimised seasonal variations of light and temperature when cultivation occurs under a controlled or partially-controlled environment.

A trial greenhouse at Cornell University was able to achieve lettuce yields equivalent to 460-470 tons of lettuce per acre per annum, whereas typical yields under field conditions are only 15-20 tons per acre per annum (Cornell CEA Homepage, 2002). Due to the control of environmental conditions and the supply of all essential and required nutrients in the water supply, hydroponic plant growth is more rapid and a very good uniformity is obtained across the entire planting. This is very beneficial for commercial farmers who are required to supply a specifically sized plant at a certain time. Thus planning and supply become known factors and mechanisation in large greenhouses is also possible (Vanachter, 1995), making hydroponic crop production very cost effective in labour terms.

Although the majority of crops are grown by providing nutrients only to the roots, additional nutrition can be supplied via a foliar application. This method of nutrient application also has the advantage of aiding temperature and humidity control. This misting does unfortunately add an increase in cost, logistics and general management, and as it is not currently widely used in South Africa this method was not included in the scope of this research project.

Even though hydroponic plant production has numerous benefits (Paulitz *et al.*, 1992), some of which have been discussed above, and commercial hydroponic crop production worldwide has increased to approximately 50 000 acres producing crops worth $6 billion per annum (2002 estimate) (Carruthers, 2002; Fresh Produce Hydroponics, 2002) there are certain inherent difficulties:

Since hydroponic crop production is an intensive monoculture in relatively humid environments (due to the abundant presence of water) these crops are thus extremely prone to devastation by a small number of diseases (Zinnen, 1988; Stanghellini and Rasmussen, 1994; van West *et al.*, 2003). Coupled to this is the fact that due to the intensive crop production methodology, the plants are cultivated at the maximal possible rate. The result is that the crop becomes very prone to stress should the environmental conditions change or the nutrient supply cease for even a short period (personal observation). During these stress conditions susceptible seedlings having survived early infection, can rapidly develop full-blown disease leading to serious outbreaks, plant deaths and yield losses (Wakeham *et al.*, 1997), while plants can also become more susceptible to pathogens present in the nutrient supply or develop disease from sub-clinical infections (Stanghellini and Kronland, 1986; Schwarz and Grosch, 2003; van West *et al.*, 2003).

2.1.2 Diseases in hydroponic systems

The move to re-circulating hydroponic systems, although positive for economical and environmental reasons, could result in serious yield losses due to disease (Zinnen, 1988; van West *et al.*, 2003).

It was soon realized that the move to hydroponics would not prevent soil-borne diseases as initially hoped, as a variety of pathogens can and do infect hydroponic crops (Stanghellini and Rasmussen, 1994), yet the growth of plants in greenhouses can have the benefit of establishing an integrated crop management strategy (Van Assche and Vangheel, 1989; Savvas, 2003) which can aid in preventing pest and disease damage.

Of primary concern in terms of pathogens are the water-borne and water-motile zoosporic fungi (specifically *Pythium* spp.) which are able to spread easily throughout the system (Stanghellini and Rasmussen, 1994; van West, 2003) and cause root-rot and wilting. Due to the intensive cropping and monoculture practices in hydroponic production, infection can lead to severe losses, in many cases without the usual visible root-rot or wilt symptoms of infection yet with yields being reduced by up to 54% by this sub-clinical infection. (Stanghellini and Kronland, 1986; Stanghellini and Rasmussen, 1994; Schwarz *et al.*, 2003).

In recirculating systems each plant becomes a “near neighbour” of every other plant supplied by the same batch of nutrient solution. One infected plant can thus result in every plant becoming infected (Zinnen, 1988) and leading to devastating losses if disease develops fully. Conversely as each plant is affected in the same way, in the case of sub-clinical infections the yield loss is hardly ever noticed as all the plants are equally reduced while appearing healthy (Stanghellini and Kronland, 1986; Schwarz and Grosch, 2003). Once recirculating hydroponic systems become infested, the entire system has to be stopped, drained and thoroughly disinfected (Stanghellini *et al.*, 1996) before being put into economically viable production again.

Ralstonia is another soil-borne pathogen of concern in hydroponic systems, specifically on long-term crops such as tomatoes, peppers and cucurbits (Lemay *et al.*, 2003; Guo *et al.*, 2004) usually grown in open-bag systems. *Ralstonia* causes vascular wilt and soft-rot infection of the stalk at "ground level" (soil or water / air interface) and primarily causes a blockage of water transport up the xylem resulting in a devastating wilt. The motile nature of this bacterium also aids in the spread between plants (Lemay *et al.*, 2003; Guo *et al.*, 2004; Agrios, 2005).

2.2 Pathogens

The three pathogens selected for the current study were *Pythium*, *Fusarium* and *Ralstonia* and their selection criteria are discussed below.

2.2.1 Pythium

Pythium belongs to the Pythiaceous group of fungi which has a motile zoospore stage in its life cycle (Kucharek and Mitchell, 2000). This zoospore is especially well adapted to aqueous environments making it a severe pathogen in waterlogged or over-watered fields and especially devastating in hydroponic systems which rely heavily on water. *Pythium* also has a very broad host range and has been known to infect a large proportion of hydroponically cultivated crops (Stanghellini and Rasmussen, 1994; Kucharek and Mitchell, 2000). The motile zoospore is attracted to the root zone of plants by electrical fields (van West *et al.*, 2003) and infects the roots, causing root decay which can initially manifest as a root rot – the first noticeable symptom (Stanghellini and Kronland, 1986).

During this time the infected root can release millions of new zoospores each day which infect surrounding roots and spread by their motility to nearby plants (Kucharek and Mitchell, 2000). As the level of infection of a plant increases, the plant root function is severely impacted, preventing adequate uptake of nutrients and water and leading to the second noticeable symptom of general plant wilt. Once the plant has reached this stage of infection, recovery generally appears to be impossible (Personal observation).

Pythium infestation was also shown in lettuce plants where no observable symptoms were noted, yet a reduction in yield (Stanghellini and Kronland, 1986; Schwarz and Grosch, 2003; van West *et al.*, 2003) was demonstrated when compared to a non-infected control. This sub-clinical infection is also a problem in hydroponic crop production, although it is not yet recognised as such.

Pythium can also overwinter in plant (root) debris left in the substrate of hydroponic systems, causing rapid re-infection of new seedlings planted in the following cultivation cycle (Kucharek and Mitchell, 2000). The level of infective material increases with each growth cycle, resulting in an increased disease pressure at the initiation of the next growth cycle and a possible higher level of sub-clinical infection (Stanghellini and Kronland, 1986; Schwarz and Grosch, 2003).

Although *Pythium* causes serious hydroponic diseases in the form of root rot and wilt, and once plants are infected there are few curative methods available, it is hypothetically easily prevented as the thin-walled zoospore stage should be very susceptible to control by means of chemicals (Stanghellini and Tomlinson, 1987).

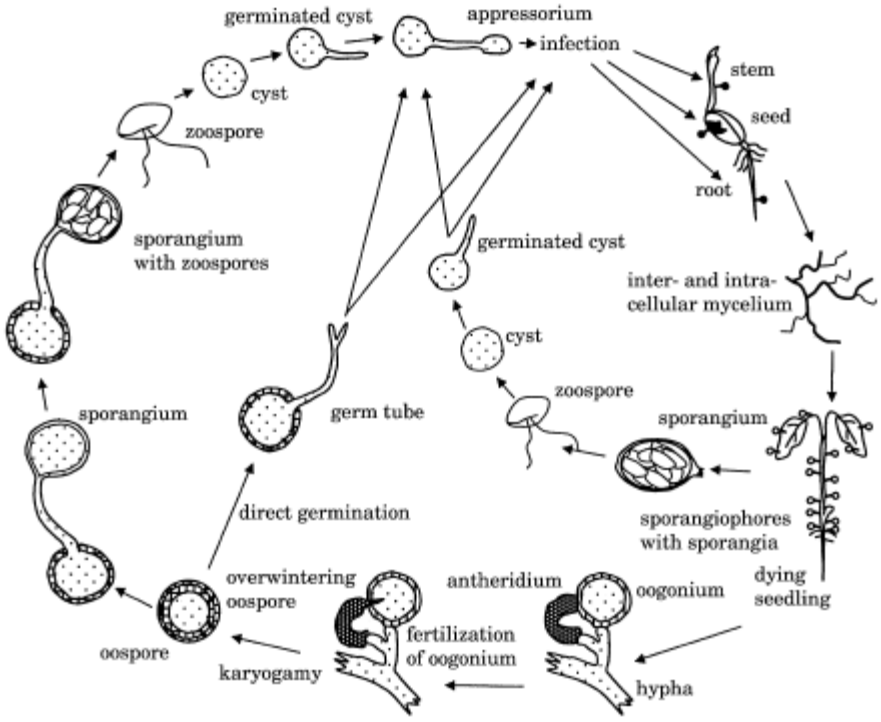


Figure 1: Typical disease cycle of a *Pythium* spp. (van West *et al.*, 2003).

2.2.2 Ralstonia

Ralstonia solanacearum (Smith) Yabuuchi *et al.* is a motile Gram negative bacterium, previously classified as *Pseudomonas solanacearum* (Smith) Smith. The gram negative characteristics of this bacterium hypothetically make it more resistant to the effects of sanitisers due to the complex boundary of a cell wall and cell membranes which have to be overcome by the sanitisers, while it is also considered a model organism for plant pathogenicity (Agrios, 2005).

This bacterium is a general, yet severe, soil-borne plant pathogen which affects a large range of hosts (Guo *et al.*, 2004). Infection occurs at the roots after which the xylem vessels of the plant become clogged with bacterial growth causing a rapid and devastating wilt (Lemay *et al.*, 2003).

This organism is also of great concern in hydroponically grown tomatoes and cucurbits where it is able to devastate entire crops in minimal time due to the motile nature of the organism allowing cross infection between plants, as well as the environmental conditions being ideal for infection (Lemay *et al.*, 2003; Guo *et al.*, 2004).

2.2.3 Fusarium

Fusarium solani (Mart.) Sacc. is a common soil-borne plant pathogenic fungus (Fravela and Larkin, 2004) which forms thick-walled micro- and macro-conidia. These conidia are highly resistant structures resulting in the fungus being able to overwinter successfully as well as aiding in making *Fusarium* one of the most fungicide resistant fungi (Agrios, 2005). *Fusarium* conidia were specifically selected for this study due to their environmental and chemical resistance characteristic, as well as the fact that the cell membrane is enclosed by the thick cell wall, possibly making *Fusarium* more resistant to sanitisers which are theorised to cause disruption of cell membranes (Buck *et al.*, 2002)

Fusarium diseases are common and destructive in many hydroponic systems where the fungus attacks the roots and causes damping off, especially in young seedlings (Fravela and Larkin, 2002; Song *et al.*, 2004). Most commonly affected are tomato and cucurbit plants such as cucumbers (Song *et al.*, 2004).

2.3 Sanitisers

As with hydroponic development, sanitiser formulation has seen significant changes over the last few years resulting in sanitisers being used in many new areas and in a more environmentally friendly nature (Nalecz-Jawecki *et al.*, 2003; Monarca *et al.*, 2004). These formulations have thus seen sanitisers introduced into the food industry specifically in plant-product and fresh fruit packaging processes to prevent post-harvest diseases and also on ready-to-eat products to reduce or prevent contamination by human pathogens (Do Socorro *et al.*, 2005; Allende *et al.*, 2006).

New forms of sanitisers, termed water sanitisers, are efficient products which, when added to contaminated water supplies at low concentrations, effect a high level of sanitation of the water to allow the water to be used without contaminating the downstream products and processes (Radziminski *et al.*, 2002; Lee *et al.*, 2004).

A further benefit of many sanitisers is their ability and effectiveness in biofilm control (Simoes *et al.*, 2005), which can rapidly accumulate in piping used in hydroponic systems due to the high nutrient-salt content and organic plant exudates and debris released into the re-circulated hydroponic nutrient supply. This microbial polymer layer packs onto the internal walls of pipes creating an organic and inorganic biofilm layer, which both blocks pipes and sprayers and is a prime area for pathogens and other micro-organisms to lodge and reproduce or overwinter (Chen and Stewart, 2005).

Although sanitisers are not designed to have specific action against micro-organisms, as with fungicides and anti-microbial agents such as antibiotics, most sanitisers are able to act on cell membranes due to the inherent surfactant properties (Stanghellini *et al.*, 1996).

This causes a disruption of the cell membrane and the resulting lysis and subsequent death of the cell. The action of sanitisers on more resistant structures such as *Fusarium* conidia is more complex and not understood as yet.

It has also been shown that certain surfactants and sanitisers are rapidly broken down after being introduced into hydroponic systems, while initial antimicrobial efficacy is still maintained (Garland *et al.*, 2000; Garland *et al.*, 2004). This indicates that the antimicrobial effect is attained rapidly on addition of the surfactants. A further benefit of this is that the environmental hazard risk of using these products is also minimal.

Thus adding water sanitisers to a hydroponic nutrient supply could have a possible three-fold benefit namely biofilm formation is minimised while the nutrient solution is continually sanitised of the major plant pathogenic propagules, resulting in re-circulated water being less infectious. Lastly the release of toxic chemicals into the environment would also be minimised.

The sanitisers selected for use in this study (Table 1) have active ingredients with known activity against micro-organisms with many products being recommended for agricultural use. Fitosan®, Sporekill® and Prasin® are based on quaternary ammonium compounds which are widely used for disinfection in medical and food environments (Sundheim *et al.*. 1998) and have been shown to have activity against *Pythium* (O'Neill, 1995). Fitosan® and Prasin® also contain guanidines which have been shown to have antifungal and antibacterial activity (Hudson *et al.*, 1986). Purogene® and TecsaClor® have chlorine dioxide as an active ingredient which has also been well described as having antimicrobial activity (Latshaw, 1994; Foschino *et al.*, 1998) as well as having an effect on *Pythium*

(O'Niell, 1995). Purogene® has also specifically been shown to have activity on bacteria (Harakeh, 1988). Actsol® is based on the electrochemical activation (ECA) of water and a brine solution to obtain a solution containing a broad range of mixed oxidising radicals which has been demonstrated to have both antibacterial and antifungal activity including activity against micro-organisms of concern in agriculture (Casteel *et al.*, 2000; Buck *et al.*, 2002). Agral 90® is a non-ionic surfactant containing alkaryl polyglycol ether. Agral 90® was demonstrated by Stanghellini and Tomlinson (1987) to have activity against *Pythium* zoospores, while various surfactants, including Agral 90® were shown to have activity against zoospores of *Olpidium brassicae* (Woronin) P. A. Dang. (Tomlinson and Faithfull, 1980). Pesticides based on copper as an active ingredient have seen widespread use over many years (de Oliveira-Filho *et al.*, 2004). Copper (II) sulphate was selected as the chemical compound providing a source of the basic form of copper used in this study. During the greenhouse and field evaluations the commercially available systemic fungicide Phytex® was selected as a standard treatment due to it being commercially registered for use against pythiaceous fungi. The active ingredient of Phytex® is phosphorous acid which has been demonstrated to have an effect against *Pythium* (Fenn and Coffey, 1984).

Table 1: Detailed information on sanitisers selected for the current study.

Name used	Active ingredient	Type of product	Supplier	Notes & Formulation	Referenced in
Agral 90®	90% $m.m^{-1}$ alkaryl polyglycol ether	Agricultural surfactant	Kynoch chemicals	Nonnionic, SL	3.4.6
Actsol®	Mixed oxidant & metastable species e.g hypochlorous acid, hypochlorite, chlorate, perchlorate (180$mg.l^{-1}$ total)	Electro chemically activated water	Radical Waters	Anionic, SL	3.4.1; 4.4.1.1; 4.4.2.1; 5.4.1.1; 5.4.2.1; 5.4.2.2
Copper sulphate	Copper (II) sulphate pentahydrate supplying Cu^{2+}	Chemical	Merck		3.4.7; 4.4.1.6
Fitosan® (F10 Agricultural)	Quaternary ammonium & biguanide (5.8%)	Agricultural sanitiser	Health & Hygiene	Cationic, SL	3.4.5; 4.4.1.5; 5.4.2.1; 5.4.2.2
Phytex® (marketed as Phytex 200SL)	Potassium phosphonate (200$g.l^{-1}$)	Fungicide	Horticura	SL	5.4.2.1; 5.4.2.2
Prasin® (marketed as Prasin Agri®)	Polymetric biguanide hydrochloride & quaternary ammonium (7%)	Agricultural sanitiser	SIDL cc	Cationic, SL	3.4.2; 4.4.1.2; 4.4.2.2; 5.4.1.2; 5.4.2.1; 5.4.2.2
Purogene® (with activator)	Chlorine dioxide (3$g.l^{-1}$ max)	General & agricultural sanitiser	BTC products & services	Nonionic, SL	3.4.3; 4.4.1.3; 4.4.2.3; 5.4.1.3; 5.4.2.1; 5.4.2.2
Sporekill®	N,N-Didecyl N,N-dimethyl ammoniumchloride (12%)	Agricultural sanitiser	Hygrotech Seed	Nonionic, SL	3.4.8; 4.4.1.7
Tecsa Clor®	Chlorine dioxide (2-3$g.l^{-1}$)	General & agricultural sanitiser	BTC products & services	Nonionic, SL	3.4.4; 4.4.1.4; 4.4.2.4; 5.4.1.4; 5.4.2.1; 5.4.2.2

2.4 References

Agrios, G.N. 2005. Plant Pathology. 5th Ed. Elsevier Academic Press, UK.

Allende, A., Tomas-Barberan, F.A. and Gil, M.I. 2006. Minimal processing for healthy traditional foods. Trends in Food Science and Technology 17: 513-519.

Buck, J.W., van Iersel, M.W., Oetting, R.D. and Hung, Y.C. 2002. In vitro fungicidal activity of acidic electrolyzed oxidizing water. Plant Disease 86: 278-281.

Carruthers, S. 2002. Hydroponics as an agricultural production system. Practical Hydroponics & Greenhouses 63: 4-7.

Casteel, M.J., Sobsey, M.D. and Arrowood, M.J. 2000. Inactivation of *Cryptosporidium parvum* oocysts and other microbes in water and wastewater by electrochemically generated mixed oxidants. Water Science and Technology 41: 127-134.

Chen, X. and Stewart, P.S. 2005. Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. Water Research 39: 1809-1817.

Cornell CEA Homepage. 2002. http://www.bee.cornell.edu/extension/CEA/index_research.htm. Accessed: 20/06/2002.

de Oliveira-Filho, E.C., Lopes, R.M. and Paumgartten, F.J.R. 2004. Comparative study on the susceptibility of freshwater species to copper-based pesticides. Chemosphere 56: 369–374.

Deutschmann, G.V. 1998. The history of hydroponics. http://www.archimedes.galilei.com/raiar/histhydr.html. Accessed: 05/12/2006.

Do Socorro, M., Bastos, R., de Fatima Ferreira Soares, N., de Andrade, N.J., Arruda, A.C. and Alves, R.E. 2005. The effect of the association of sanitizers and surfactant in the microbiota of the cantaloupe (*Cucumis melo* L.) melon surface. Food Control 16: 369-373.

Fenn, M.A. and Coffey, M.D. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-al and phosphorous acid. Phytopathology 74: 606-611.

Foschino, R., Nervegna, I., Motta, A. and Galli, A. 1998. Bactericidal activity of chlorine dioxide against *Escherichia coli* in water and on hard surfaces. Journal of Food Protection 61: 668-672.

Fravela, D.R. and Larkin, R.P. 2002. Reduction of *Fusarium* wilt of hydroponically grown basil by *Fusarium oxysporum* strain CS-20. Crop Protection 21: 539-543.

Fresh Produce Hydroponics. 2002. Produce Marketing Association. http://www.pma.com/ii/mem_only/hydroponics.pdf. Accessed: 20/06/2002.

Garland, J.L., Levine, L.H., Yorio, N.C., Adams, J.L. and Cook, K.L. 2000. Graywater processing in recirculating hydroponic systems: Phytotoxicity, surfactant degradation, and bacterial dynamics. Water Research 34: 3075-3086.

Garland, J.L., Levine, L.H., Yorio, N.C. and Hummerick, M.E. 2004. Response of graywater recycling systems based on hydroponic plant growth to three classes of surfactants. Water Research 38: 1952-1962.

Gul, A., Erogul, D. and Ongun, A. R. 2005. Comparison of the use of zeolite and perlite as substrate for crisp-head lettuce. Scientia Horticulturae 106: 464-471.

Guo, J., Qi, H., Guo, Y., Ge, H., Gong, L., Zhang, L. and Sun, P. 2004. Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. Biological Control 29: 66-72.

Hanger, B. 1993. Hydroponics: The world, Australian, and South Pacific islands scene. In: Commercial Hydroponics in Australasia, A Guide for Growers. Australian Hydroponics Association Inc. Pro-Set Pty Ltd, Hobart, NZ.

Harakeh, S., Illescas, A. and Matin, A. 1988. Inactivation of bacteria by Purogene. Journal of Applied Bacteriology 64: 459-463.

Harris, D.A. 1976. Hydroponics: The Gardening Without Soil. Purnell, Cape Town, South Africa.

Hudson, H.R., Ojo, I.A.O. and Pianka, M. 1986. Guanidines with antifungal (and antibacterial) activity – a review. International Pest Control 28: 148-155.

Jensen, M.H. 1999. Hydroponics worldwide. In: Proceedings international symposium on growing media and hydroponics, Ontario, Canada 19-26 May 1997, Ed. Papadopoulos, A.P. Acta Horticulturae 481: 719-729.

Kucharek, T. and Mitchell, D. 2000. Diseases of agronomic and vegetable crops caused by *Pythium*. Plant Pathology Fact Sheet pp-53. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Christine Waddill, Dean. http://plantpath.ifas.ufl.edu/takextpub/FactSheets/pp0053.pdf.

Latshaw, C.L. 1994. Chlorine dioxide: effective, broad-spectrum biocide for white water systems. Tappi Journal 78: 163-166.

Lee, S., Gray, P.M., Dougherty, R.H. and Kang, D. 2004. The use of chlorine dioxide to control *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples. International Journal of Food Microbiology 92: 121-127.

Lemay, A., Redlin, S., Fowler, G. and Dirani, M. 2003. Pest Data Sheet: ***Ralstonia solanacearum*** race 3 biovar 2. USDA/APHIS/PPQ, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory, Raleigh, NC. http://www.aphis.usda.gov/ppq/ep/ralstonia/ralstoniadatasheet_CPHST.pdf.

Monarca, M., Zani, C., Richardson, S.D., Thruston Jr, A.D., Moretti, M., Feretti, D. and Villarini, M. 2004. A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water. Water Research 38: 3809-3819.

Nalecz-Jawecki, G., Grabinska-Sota, E. and Narkiewicz, P. 2003. The toxicity of cationic surfactants in four bioassays. Ecotoxicology and Environmental Safety 54: 87-91.

Niederweiser, J.G. 2001. Guide to Hydroponic Vegetable Production. Agricultural Research Council, Roodeplaat, South Africa.

O'Niell, T.M. 1995. Evaluation of disinfectants against a *Pythium* spp. and *Thielaviopsis basicola*. Test of Agrochemicals and Cultivars, Annals of Applied Biology 126 (Supplement): 22-23.

Paulitz, T.C., Zhou, T. and Rankin, L. 1992. Selection of rhizosphere bacteria for biological control of *Pythium aphanidermatum* on hydroponically grown cucumber. Biological Control 2: 226-237.

Radziminski, C., Ballantyne, L., Hodson, J., Creason, R., Andrews, R.C. and Chauret, C. 2002. Disinfection of *Bacillus subtilis* spores with chlorine dioxide: A bench-scale and pilot-scale study. Water Research 36: 1629-1639.

Runia, W. 1994. Disinfection of recirculation water from closed cultivation systems with ozone. Acta Horticulturae 361: 388-396.

Runia, W. 1995. A review of possibilities for disinfection of recirculation water from soilless cultures. Acta Horticulturae 382: 221-229.

Savvas, D. 2003. Hydroponics: A modern technology supporting the application of integrated crop management in greenhouse. Food, Agriculture and Environment 1: 80-86.

Schwarz, D. and Grosch, R. 2003. Influence of nutrient solution concentration and a root pathogen (*Pythium aphanidermatum*) on tomato root growth and morphology. Scientia Horticulturae 97: 109-120.

Simoes, M., Pereira, M.O. and Vieira, M.J. 2005. Action of a cationic surfactant on the activity and removal of bacterial biofilms formed under different flow regimes. Water Research 39: 478-486.

Song, W., Zhou, L., Yang, C., Cao, X., Zhang, L. and Liu, X. 2004. Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. Crop Protection 23: 243-247.

Stanghellini, M.E. and Kronland, W.C. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by *Pythium dissotocum*. Plant Disease 70: 1053-1056.

Stanghellini, M.E. and Rasmussen, S.L. 1994. Hydroponics : A solution for zoosporic pathogens. Plant Disease 78: 1129-1138.

Stanghellini, M.E., Rasmussen, S.L., Kim, D.H. and Rorabaugh, P.A. 1996. Efficacy of nonionic surfactants in the control of zoospore spread of *Pythium aphanidermatum* in a recirculating hydroponic system. Plant Disease 80: 422-428.

Stanghellini, M.E. and Tomlinson, J.A. 1987. Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. Phytopathology 77: 112-114.

Sundheim, G., Langsrud, S., Heir, E. and Holck, A.L. 1998. Bacterial resistance to disinfectants containing quaternary ammonium compounds. International Biodeterioration and Biodegradation 41: 235-239.

Tomlinson, J.A. and Faithfull, E.M. 1980. Studies on the control of lettuce big-vein disease in recirculated nutrient solutions. Acta Horticulturae 98: 325-332.

Van Assche, C. and Vangheel, M. 1989. Plant protection in hydroponics. Acta Horticulturae 260: 363-375.

van West, P., Appiah, A.A. and Gow, N.A.R. 2003. Advances in research on oomycete root pathogens. Physiological and Molecular Plant Pathology 62: 99-113.

Vanachter, A. 1995. Development of *Olpidium* and *Pythium* in the nutrient solutions of NFT grown lettuce, and possible control methods. Acta Horticulturae 382: 187-196.

Wakeham, A.J., Pettitt, T.R. and White, J.G. 1997. A novel method for detection of viable zoospores of *Pythium* in irrigation water. Annals of Applied Biology 131: 427-435.

Zinnen, T.M. 1988. Assessment of plant diseases in hydroponic culture. Plant Disease 72: 96-99.

CHAPTER 3

IN VITRO EFFICACY OF WATER SANITISERS AGAINST *PYTHIUM* ZOOSPORES IN AQUEOUS SUSPENSION

3.1 Abstract

Although the use of re-circulating hydroponic systems has its advantages, it is also prone to infestation by pathogens such as *Pythium*. Current methods of sterilization of hydroponic nutrient solution, such as chlorination and ozonation, are costly and difficult to manage. Several sanitisers are now available that are consumer friendly and environmentally safe and these were tested for their efficacy in controlling *Pythium* zoospores in a water suspension. Testing was performed by addition of various concentrations of these sanitisers into a volume of water containing *Pythium* zoospores, allowing a specific exposure time and then determining the viable zoospores remaining. Two other plant pathogens (*Fusarium* and *Ralstonia*) were also tested for comparison. All the sanitisers were made up at the recommended rates. Actsol® demonstrated very good efficacy against all the test organisms and eradicated *Pythium* from the test suspension at all the concentrations tested, and with the shortest exposure time of 10 min. Prasin® (5mg.l^{-1}) and TecsaClor® (25mg.l^{-1}) achieved the desired 80% kill of *Pythium* zoospores at 10 and 30min exposure times respectively. *Pythium* zoospores were effectively eliminated within 10min when exposed to a Fitosan® concentration of 7.5mg.l^{-1}. Agral 90® was able to achieve the desired kill rate of 80% at 1mg.l^{-1} and 10min exposure time. Exposure to Sporekill® gave a percentage kill of above 80% of *Pythium* zoospores at a concentration of 5mg.l^{-1} with a 10min exposure time. For most of the sanitisers, *Fusarium* and *Ralstonia* exhibited a typical dose-response where the kill rates increased with increased exposure time. However, these two pathogens proved to be more resistant to the sanitisers than *Pythium*. This data shows that the addition of the above products to a *Pythium*-infested water supply would effectively eliminate *Pythium* and also greatly reduce *Fusarium* and *Ralstonia* inoculum levels, indicating possible use for disinfection of recirculating hydroponic nutrient solutions.

3.2 Introduction

The use of surface- and water-sanitisers have seen increased usage as new chemical formulations are developed which are less harmful in terms of human health and environmental concerns than the "toxic" sanitisers such as chlorine, which have also been shown to produce mutagens and carcinogens (Andrews *et al.*, 2002). Thus the new sanitisers are a more consumer and environmentally friendly alternative as well as being more effective at lower concentrations, resulting in both reduced risk and reduced cost, while meeting stricter standards for effluents (Adler *et al.*, 2003).

The use of sanitisers also does not carry the same stigma as specifically formulated chemicals (anti-microbial and antibiotics) for use in control of pathogens and pests. This meets consumer demands for products that are grown under conditions where pesticides are not liberally applied (Saba and Messina, 2003). Additionally there is also a reduced risk of the pathogens developing resistance to these products.

Although primarily used for sanitation and disinfection of fixed surfaces (Peng *et al.*, 2002), numerous sanitisers and detergents, when applied in water, are able to effect sanitation, or even total sterilisation, of the water volume (Lee *et al.*, 2004). Due to their reasonably safe nature and use at low concentrations, sanitisers have also seen widespread use in postharvest cleaning of fruits and also fresh-cut vegetables to both remove pathogens and spoilage organisms (Singh *et al.*, 2002; De Socorro *et al.*, 2005)

Current methods of sterilisation of recirculated hydroponic nutrient solution, such as chlorination, ozonation, iodination and ultra-violet (UV) sterilisation are costly, difficult to manage and often ineffective due to high organic load and concentration of salts (Runia, 1994; Runia, 1995). Chlorination, although relatively cheap and easy to apply, is not widely used due to the phytotoxic nature of chlorine. Additionally all the previously mentioned controls have, in certain instances, been shown to produce toxic by-products or degradation products (Monarca *et al.*, 2004).
Due to these setbacks, it is thought that the sanitisers, such as those under investigation in this study, will be more effective in sanitising water, with fewer harmful side-effects in recirculating hydroponic systems.

The life-cycles of many *Pythium* species include a motile stage where flagellated zoospores are produced (Roux and Botha, 1997). These flagellated zoospores have been implicated as the major agents in disease spread (Stanghellini *et al.*, 1996; Stanghellini and Miller, 1997). Since zoospores only have thin cell membranes, which are easily disrupted by surfactants (Stanghellini and Tomlinson, 1987; Stanghellini *et al.*, 1996; De Jonghe *et al.*, 2005), zoospores were thus identified as the most vulnerable target of water sanitiser activity. In hydroponic systems, zoospores are released from infected roots into the nutrient solution, where they can then be dispersed throughout the hydroponic system, resulting in rapid disease spread and increase in disease pressure (Kucharek and Mitchell, 2000).

Thus elimination or inactivation of plant pathogens (in this case specifically *Pythium* zoospores), or even total sterilisation of the nutrient solution, is of importance, especially with regards to recirculating hydroponic systems, where rapid increase in inoculum can occur, as inoculum is continually being added and recirculated.

The primary aim of this study was to determine whether selected sanitisers are effective in killing *Pythium* zoospores in hydroponic nutrient solutions. To achieve this, the sanitisers were tested for efficacy against three pathogens (*Pythium* spp. zoospores, *Fusarium solani* (Mart.) Sacc. conidia and *Ralstonia solanacearum* (Smith) Yabuuchi *et al.* planktonic cells) in aqueous suspension using sterile water. The results of this exposure would determine whether viable control, or elimination, of the pathogens could be achieved, as well as the lowest concentration at which this could be achieved.

3.3 Materials and Methods

3.3.1 Maintenance of cultures

Initial experiments were performed using naturally infested runoff water taken from a commercial hydroponic system known to have *Pythium* infestation. Although positive results were obtained from these experiments, the variation in the results indicated that consistency could not be maintained between experiments. A novel method of obtaining fresh *Pythium* zoospores was developed, which allowed for consistent results, as well as the elimination of other infectious propagules and organic matter which may have affected the experimental outcome.

Pythium cultures were maintained on V8 juice agar (De Jonghe *et al*., 2005) as well as the *Pythium* selective media BNPRA (Roux and Botha, 1997). *Fusarium* cultures were maintained on Potato Dextrose Agar (PDA) (Biolab C100, Merck, South Africa), and *Ralstonia* on Nutrient Agar (NA) (Biolab C150, Merck, South Africa) as well as the selective media TZC (2,3,5-Triphenyltetrazolium chloride) (Merck, South Africa)(van Broekhuizen, 2002).
For each experiment fresh cultures of each organism were grown from a stock culture with the average age of cultures during the experiments being six days.

3.3.2 Inoculum preparation

Pythium zoospores were obtained from an artificially infested static hydroponic system where 5l containers were planted with Butter lettuce (*Lactuca sativa* L. var *capitata* L. cv Nadine) seedlings in sterile tap water. The containers were then inoculated with macerated 7d old *Pythium* Group F cultures on V8 agar medium (De Jonghe *et al*., 2005). This culture was previously isolated from a commercial hydroponic system and stored in an internal culture collection as UP 92/00, later deposited at the National Mycological Herbarium (Agricultural Research Council, Vredehuis, Pretoria, South Africa) culture collection with reference number PPRI 7078. Maceration was done by placing three V8 agar plates containing the *Pythium* growth into 800ml sterile water in an alcohol-sterilised kitchen blender and pulsing for 0.5s followed by a 3s standing period until a visually homogenous suspension was obtained. This suspension was then added to the static hydroponic system at a rate of 200ml per 5l container. Regular *Pythium* baiting (Grimm and Alexander, 1973) was carried out on this water to ensure the consistent presence (an incidence rating of 70% or greater) of zoospore inoculum.

Fusarium solani (isolated from citrus roots in a previous study) conidia were harvested by pouring 5ml sterile deionised water over a fresh culture on PDA media and brushing lightly with a sterile etaleaur. The resulting conidial suspension was removed and a spore count was done using a haemocytometer.

Ralstonia solanacearum Biovar 3 (isolated from tomato plants by van Broekhuizen, 2002) cells were harvested by pouring 5ml sterile water over a fresh culture on TZC media and brushing lightly with a sterile etaleaur. The resulting cell suspension was removed from the Petri dish and cells counted using a Petroff-Hauser counting chamber.

3.3.3 Sanitiser preparation

Prasin® (SIDL, South Africa), Fitosan® (Health & Hygiene, South Africa), TecsaClor® (BTC Products, South Africa), Agral 90® (Kynoch Chemicals, South Africa) and Sporekill® (Hygrotech Seeds, South Africa) were provided by the various manufactures and used undiluted.

Fresh Purogene® (BTC Products, South Africa) was generated for each experiment according to the label instructions (addition of one part supplied activator to ten parts Purogene®). This was allowed to react for 5min before use.

Fresh Actsol® was generated for each experiment using an ECA (ElectroChemical Activation) device provided by Radical Waters (Midrand, South Africa) and freshly prepared brine solution [2.5g NaCl (Merck, South Africa) per litre water] to achieve an Actsol® solution of average pH 7.2 and ORP 800mV. This freshly prepared solution was used in all the experiments.

Copper (II) sulphate crystals (Merck, South Africa) were used to provide copper ions when dissolved in water and diluted to the final volume of water. Details of each sanitiser are provided in Appendix I: B. Contact details of each supplier can be found in Appendix I: C. The above sanitisers were tested at a range of concentrations and exposure times, as described in Table 1 below.

Table 1: Concentration and exposure time of chemicals tested in the current study.

Product	**Product concentrations**	**Exposure times**
Actsol®	1:10, 1:20 and 100%	10, 30, 60 and 120min
Prasin®	5, 7.5, 10, 20, 100, 150, 200, 250 and 500mg.l^{-1}	10, 30 and 60min
Purogene®	5, 10, 25, 50 and 100mg.l^{-1}	10, 30 and 60min
TecsaClor®	10, 25, 50 and 100mg.l^{-1}	10, 30 and 60min
Fitosan®	1, 5, 7.5 and 10mg.l^{-1}	10min
Agral 90®	1, 2.5, 5 and 10mg.l^{-1}	10 and 30min
Copper (II) sulphate	0.5, 1, 2, 5, 10 and 20mg.l^{-1}	10 and 30min
Sporekill®	1, 2.5, 5 and 10mg.l^{-1}	10 and 30min

Concentrations referred to are product concentrations, i.e. concentrations made directly from the stock solutions. Active ingredient concentrations for each product are stipulated in Appendix I: B.

3.3.4 Experimental procedure

For most of the tests, sterilized 500ml Erlenmeyer flasks were filled with 500ml of sterile deionised water. The exceptions were the *Pythium* tests where artificially infested water was used, and the Actsol® tests where the Actsol® solution was diluted with sterile water to give a final volume of 500ml at the test dilution. Each product was then diluted into the Erlenmeyer flasks to give the test dilutions described Table 1. For each organism an untreated control (no sanitiser) was included.

Fusarium inoculum was added to give a final concentration of approximately 10^5 cfu.ml^{-1}, while the *Ralstonia* was diluted to an approximate concentration of 10^7 cells.ml^{-1}.

Pythium infested water from the static hydroculture described in Section 3.3.2 was used as a source of *Pythium* zoospores, having a concentration approaching 10^4 zoospores.ml^{-1}. An adequate sample volume was taken, stirred to ensure a homogenous distribution of zoospores and then divided equally into sterile Erlenmeyer flasks. A sample was also observed microscopically to confirm the presence of zoospores.

Directly after addition of the inoculum, at time 0, a sample was taken from each untreated control, with further samples taken at 5min; 10min; 30min and 60min, and processed as described below. A further control sample was also taken at the maximum time.

For enumeration a 50ml sample of the zoospore suspension and a 20ml sample for *Fusarium* and *Ralstonia* was drawn out of the flasks using a sterile 25ml syringe and filtered through a 25cm syringe filter (Osmonics Acetate Plus, Separations, South Africa) of pore sizes 0.22µm for *Ralstonia* and 1.2µm for *Pythium* and *Fusarium*. The filters of the *Fusarium* and *Ralstonia* samples were then placed in 10ml sterile water in a test-tube and vortexed for 10s, after which a 10x serial dilution of the resulting suspension was prepared.

For *Fusarium* and *Ralstonia* 100µl of each dilution was plated out on PDA and NA respectively, using the spread-plate technique. Plates were then incubated in darkness at 25ºC for 3d, after which colony forming units (cfu) were counted and the cfu/ml calculated.

Pythium was enumerated by baiting the suspension from the vortexed test tube containing the filter according to a modification of the baiting method described by Grimm and Alexander (1976) where citrus leaf discs are floated on the surface of the suspension for

24h as opposed to 48h. After 24h the discs were transferred to the *Pythium* selective medium (BNPRA) and incubated for 3d, after which the leaf discs showing fungal growth were microscopically examined to verify *Pythium* growth. The number of discs rendering *Pythium* were counted and the percentage incidence of the fungus calculated as an indication of the proportion of live zoospores remaining in the suspension. This procedure constitutes a semi-quantitative assessment.

Each experiment was done in duplicate, with two replicate Petri-dishes being used at each step.

3.3.5 Data analysis

For the respective pathogens, percentage kill was calculated according to the following equation:

$$\frac{T_0 - T_x}{T_0} \times 100$$

Where T_0 = sample taken at time = 0 minutes (control)

T_x = sample taken after x minutes.

The data was statistically analysed using the SAS for Windows program (version 8e) applying Duncan's Multiple Range test at $P = 0.05$.

A percentage kill of 80%, or higher, was considered to be a positive result.

3.4 Results

3.4.1 Actsol®

Actsol® demonstrated good efficacy against all the test organisms and totally eradicated *Pythium* from the test suspension at all the concentrations tested, including the shortest exposure time of 10min (Fig. 1a). Actsol® was shown to have adequate efficacy for *Pythium* kill at the highest dilution of 1:20. *Fusarium* exhibited a typical dose-response where the kill rate increased with increased exposure time, and this trend was more noticeable at the lower sanitiser concentration (Fig. 1b). *Ralstonia* demonstrated a similar trend to *Fusarium*, albeit to a lesser extent. No significant (P=0.05) differences were observed between treatments (Fig. 1c). A 100% kill was recorded for all pathogens.

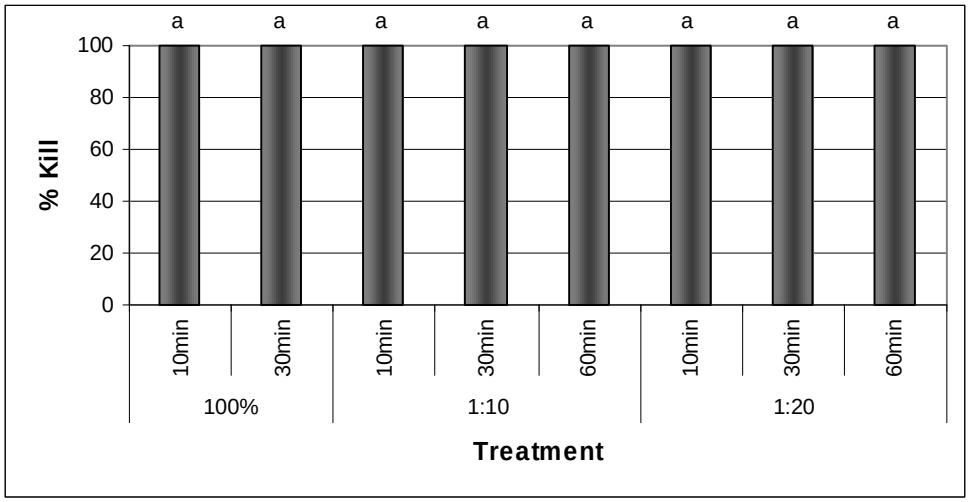


Figure 1a: Efficacy of Actsol® at various concentrations and exposure times on *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

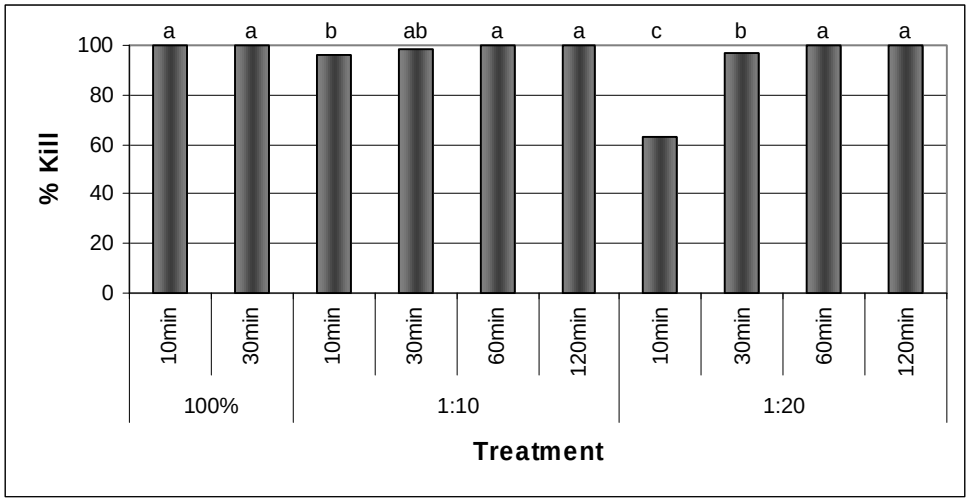


Figure 1b: Efficacy of Actsol® at various concentrations and exposure times on *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

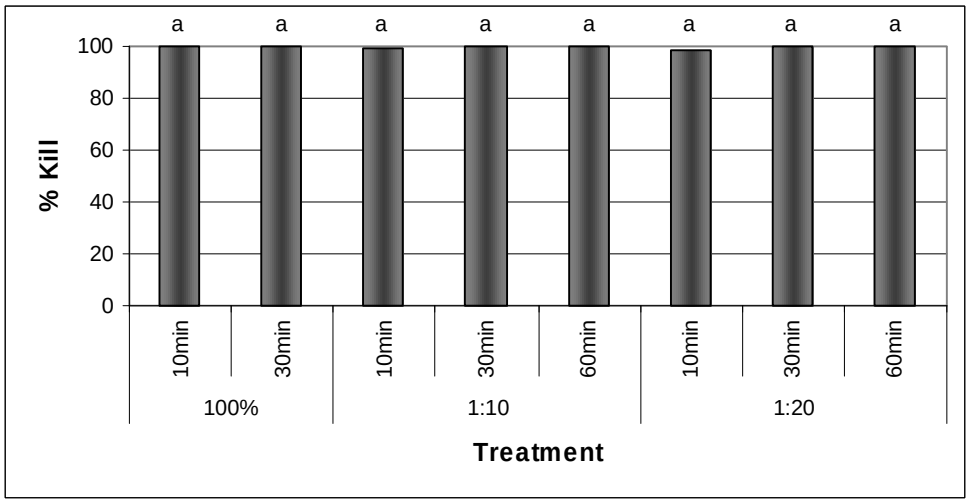


Figure 1c: Efficacy of Actsol® at various concentrations and exposure times on *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.4.2 Prasin®

When exposed to Prasin® at 5mg.l^{-1} *Pythium* exhibited the expected dose response over time. With a 5mg.l^{-1} concentration, Prasin® achieved a greater than 80% kill of *Pythium* zoospores at a 10min exposure time. Higher sanitiser concentrations resulted in 100% kill of *Pythium* zoospores within a 10min exposure time (Fig. 2a).

The same dose response trend was demonstrated for both *Fusarium* (Fig. 2b) and *Ralstonia* (Fig. 2c), although total kill was only achieved after a 60min exposure time at a 100mg.l^{-1} concentration. *Fusarium* conidia were less affected than *Ralstonia* cells at the same concentration and exposure time.

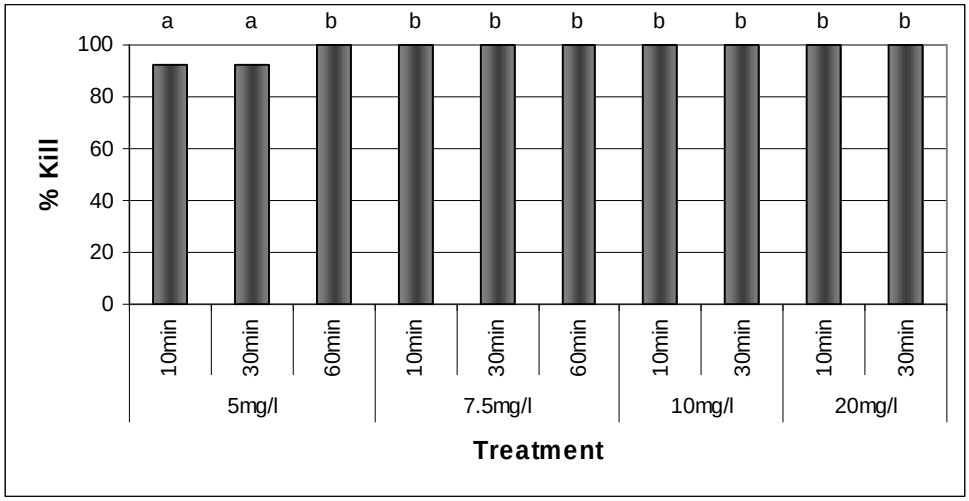


Figure 2a: Efficacy of Prasin® at various concentrations and exposure times against *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

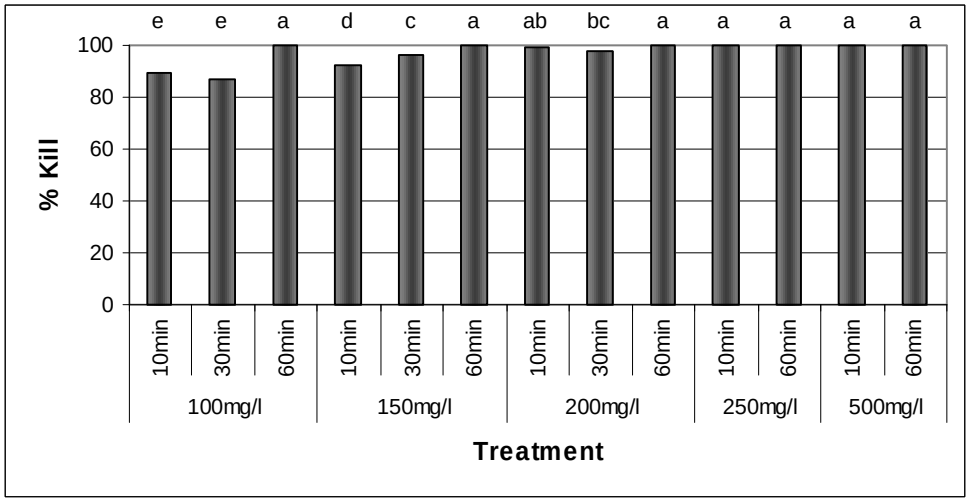


Figure 2b: Efficacy of Prasin® at various concentrations and exposure times against *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

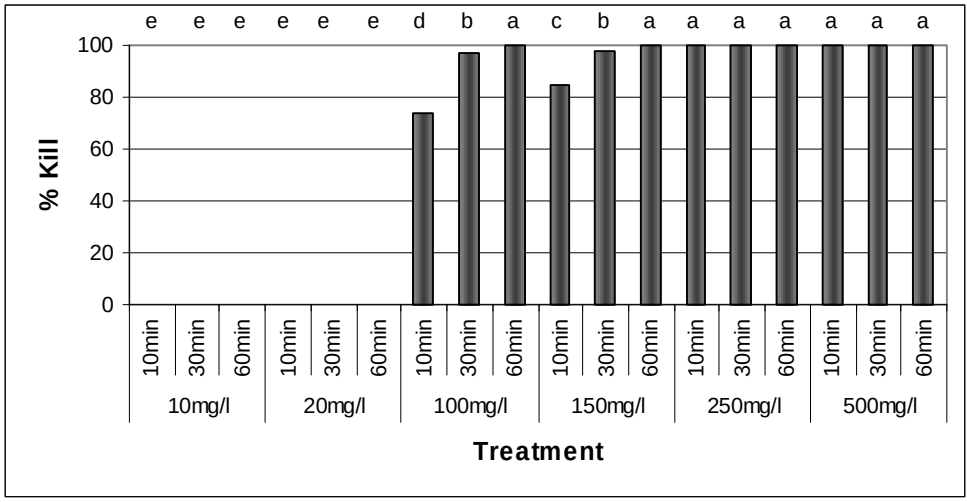


Figure 2c: Efficacy of Prasin® at various concentrations and exposure times against *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.4.3 Purogene®

Exposure of *Pythium* zoospores to Purogene® rendered a typical dose response at a 5mg.l^{-1} sanitiser concentration where an increase in kill was achieved with increasing exposure time (Fig. 3a). A 30min exposure time at this concentration achieved the desired 80% kill. Sanitiser concentrations of 10mg.l^{-1} or above achieved a 100% kill within a 10min exposure time (Fig. 3a).

Fusarium (Fig. 3b) and *Ralstonia* (Fig. 3c) showed similar dose response trends at a 20mg.l^{-1} sanitiser concentration. Similar results were achieved for both organisms at this concentration. A sanitiser concentration of 50mg.l^{-1} or higher achieved a 100% kill within a 10min exposure time.

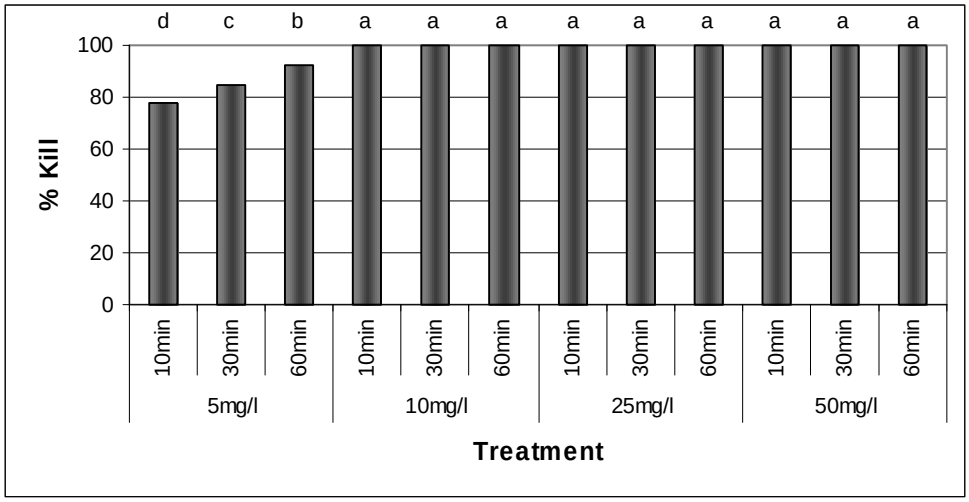


Figure 3a: Efficacy of Purogene® at various concentrations and exposure times against *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

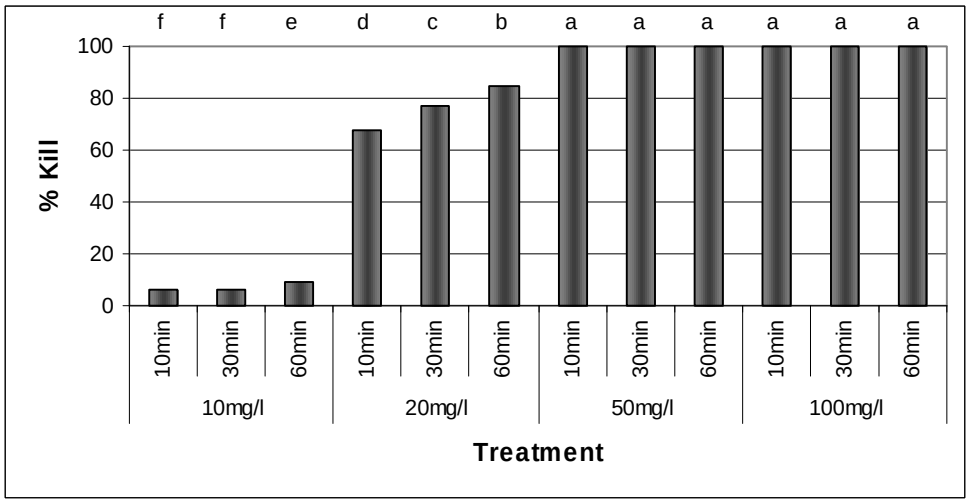


Figure 3b: Efficacy of Purogene® at various concentrations and exposure times against *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

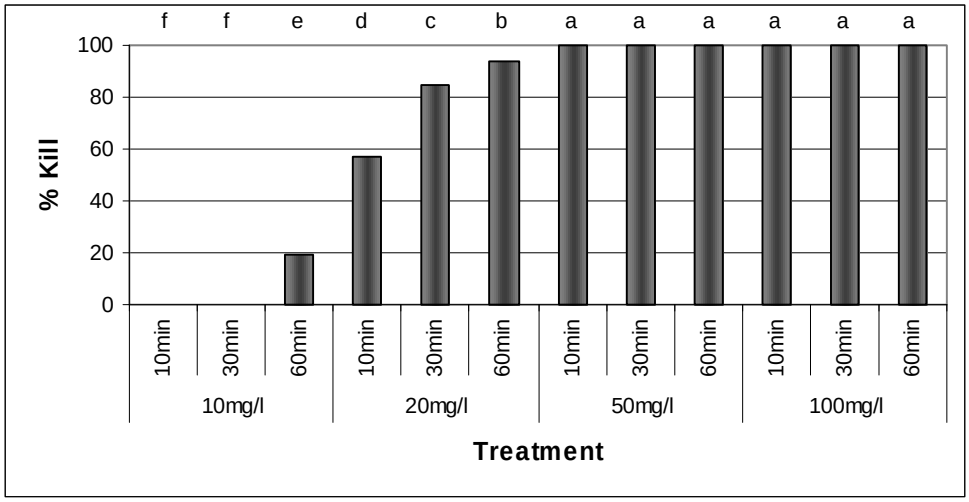


Figure 3c: Efficacy of Purogene® at various concentrations and exposure times against *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.4.4 TecsaClor®

Exposure of *Pythium* zoospores to TecsaClor® at 10mg.l^{-1} did not result in a typical dose response with increasing time, although a total kill of zoospores was achieved within a 10min exposure to a 25mg.l^{-1} TecsaClor® concentration (Fig. 4a). The typical dose response was observed for *Fusarium* (Fig. 4b) and *Ralstonia* (Fig. 4c) at a 50mg.l^{-1} sanitiser concentration where increased percentage kill was observed with increasing exposure time. *Ralstonia* cells also showed a slightly higher sensitivity than *Fusarium* conidia at this concentration, with a higher level of kill achieved with *Ralstonia* at the same concentration and time exposure.

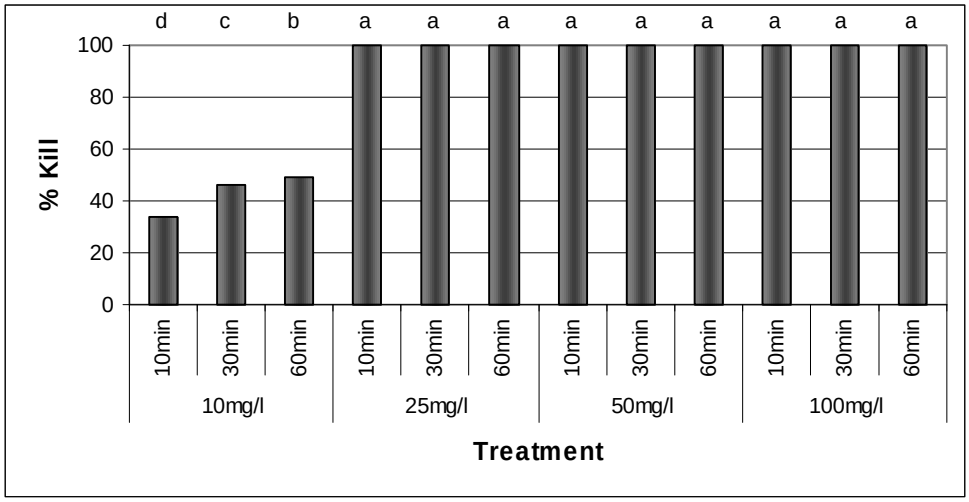


Figure 4a: Efficacy of TecsaClor® at various concentrations and exposure times against *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

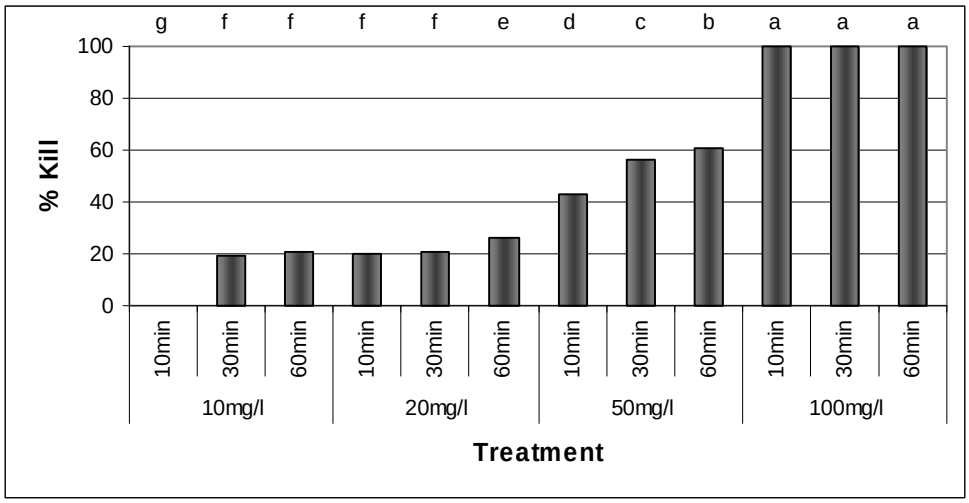


Figure 4b: Efficacy of TecsaClor® at various concentrations and exposure times against *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

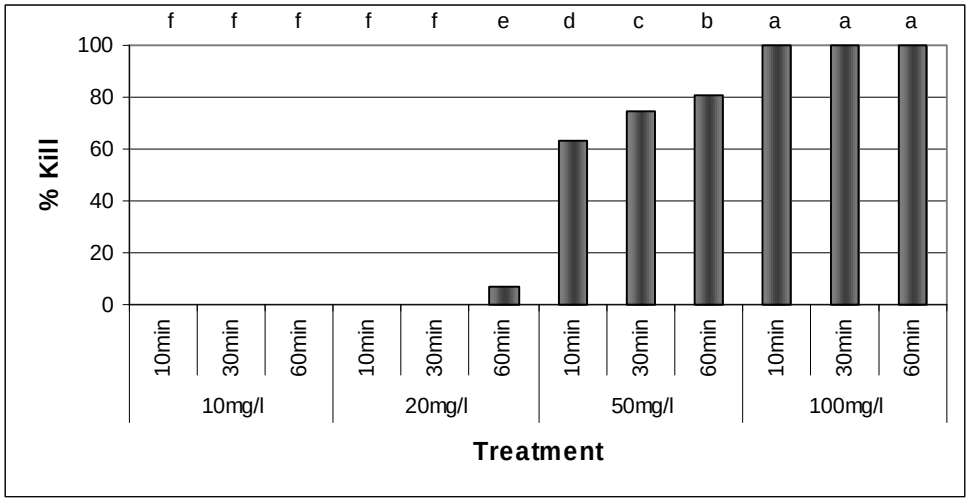


Figure 4c: Efficacy of TecsaClor® at various concentrations and exposure times against *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test with *P*=0.05.

3.4.5 Fitosan®

Fitosan® was only tested against *Pythium* zoospores for a 10min exposure time, where a classic dose response was observed with a steady increase in zoospore kill being obtained with increasing sanitiser concentration (Fig. 5). Total kill of zoospores was achieved at 7.5mg.l^{-1} and 10mg.l^{-1} concentrations.

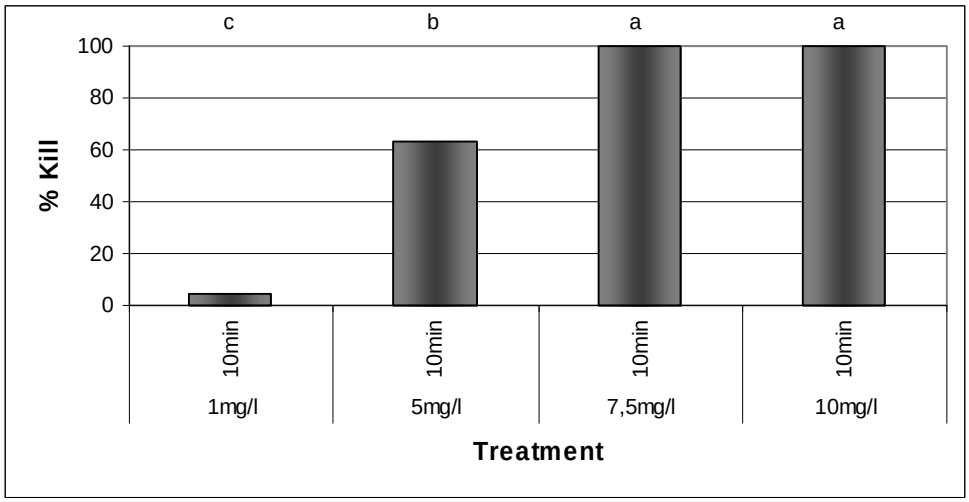


Figure 5: Efficacy of Fitosan® at various concentrations and a 10min exposure time on *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.4.6 Agral 90®

Pythium zoospore survival showed an atypical trend when exposed to increasing Agral 90® concentrations at a 10min exposure time (Fig. 6a). This observation showed a trend which is effectively an inverse of the expected dose response, where a decreased efficacy was noted with an increased sanitiser concentration.

This trend was also demonstrated by *Fusarium* where a 10mg.l^{-1} concentration for both a 10min and 30min exposure time showed a lower level of efficacy than a 1mg.l^{-1} or 5mg.l^{-1} concentration at the same exposure times (Fig. 6b). *Ralstonia* cells showed high levels of tolerance to Agral 90® with the desired 80% kill level not being achieved in the tested concentration range and exposure time (Fig. 6c). These results showed a similar trend to those observed with the *Pythium* and *Fusarium* tests.

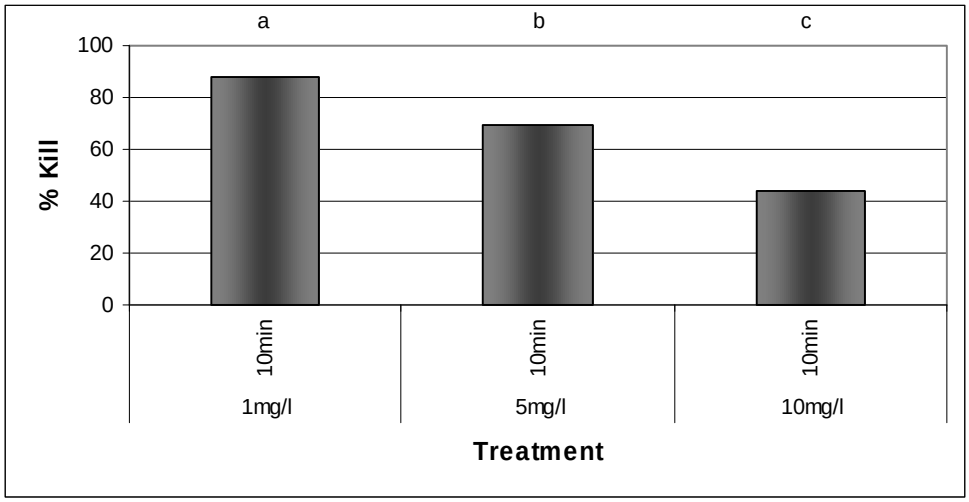


Figure 6a: Efficacy of Agral 90® at various concentrations at a 10min exposure time on *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

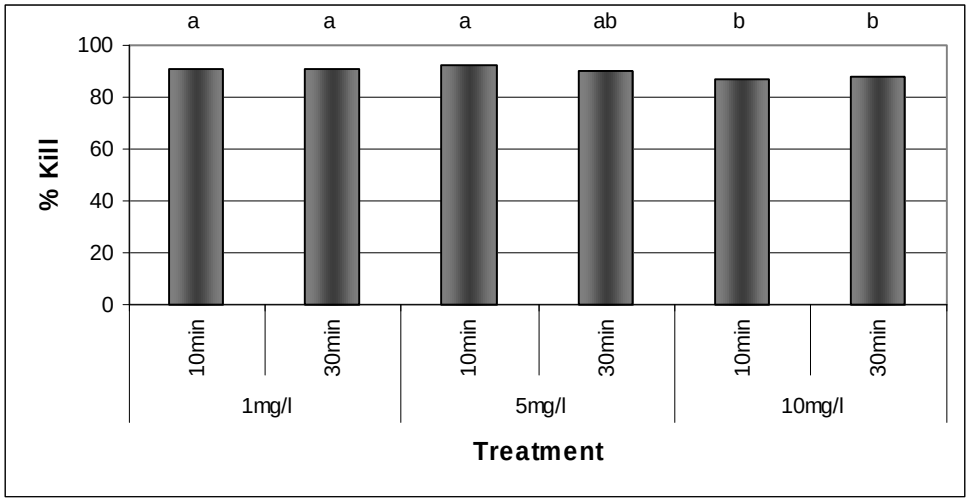


Figure 6b: Efficacy of Agral 90® at various concentrations and exposure times on *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

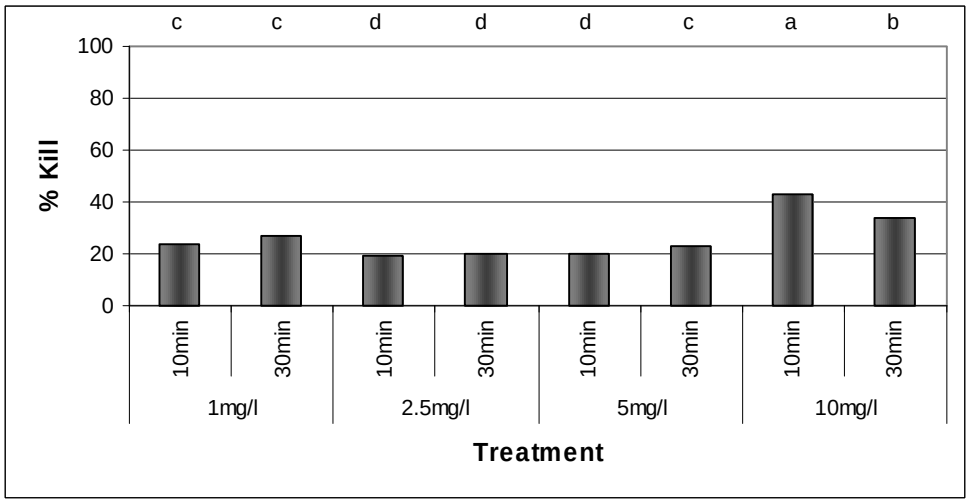


Figure 6c: Efficacy of Agral 90® at various concentrations and exposure times on *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.4.7 Copper sulphate

Pythium zoospores, when exposed to increasing levels of copper ions for 10min, demonstrated a regular dose response with higher levels of kill being achieved with an increase in copper ion concentration (Fig. 7a).

Copper ion concentrations of 5mg.l^{-1} and higher achieved a 100% kill of zoospores, while a 1mg.l^{-1} concentration achieved a percentage kill of over 80%.

Both *Fusarium* (Fig. 7b) and *Ralstonia* (Fig. 7c) demonstrated similar dose responses with higher level of efficacy being noted at increased copper ion concentrations and longer exposure times. *Ralstonia* was shown to be less sensitive than *Fusarium*, with lower levels of efficacy observed with *Ralstonia* cells at the same concentration and exposure time.

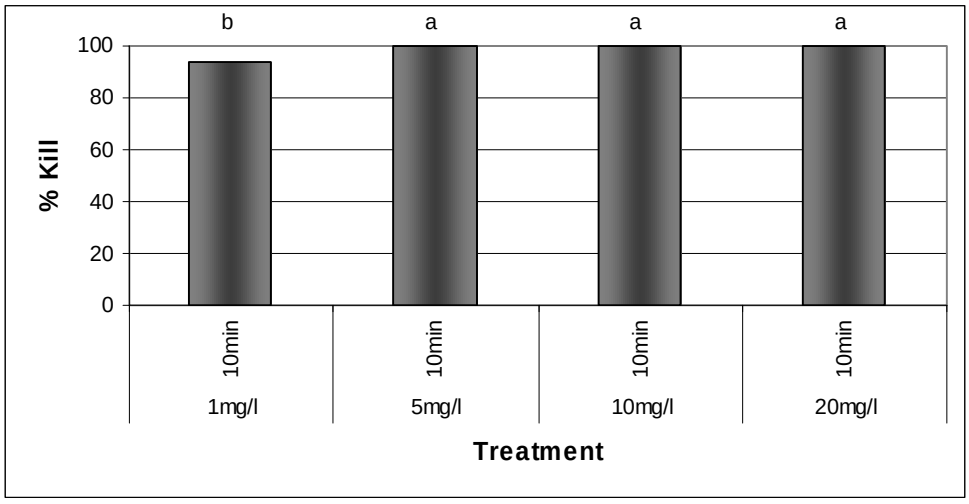


Figure 7a: Efficacy of copper ions at various concentrations at a 10min exposure time on *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

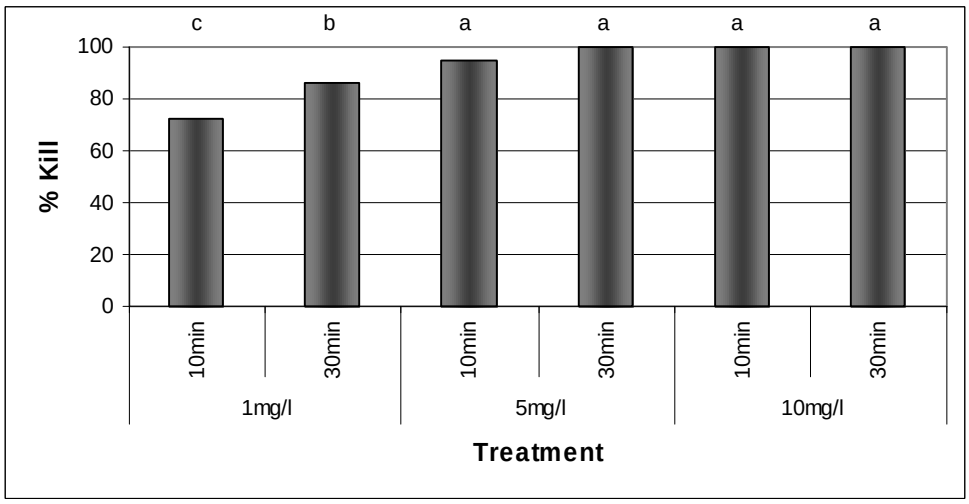


Figure 7b: Efficacy of copper ions at various concentrations and exposure times on *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

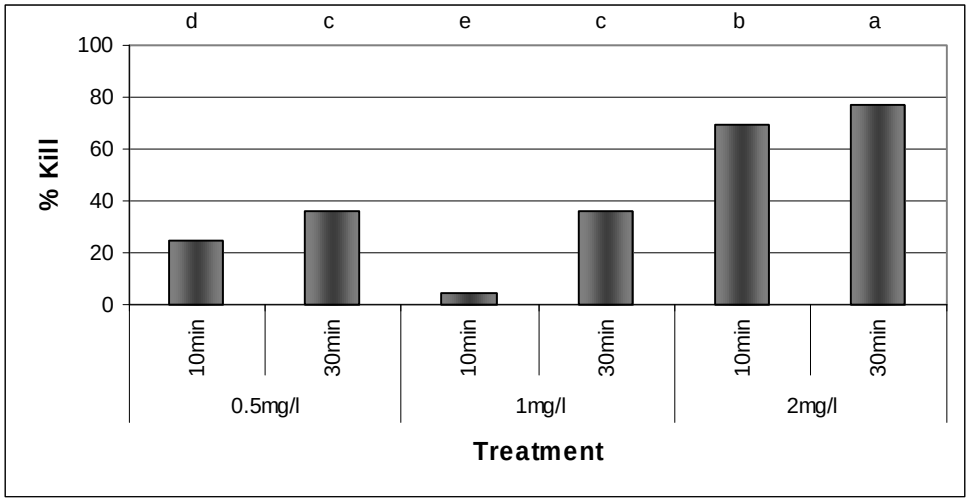


Figure 7c: Efficacy of copper ions at various concentrations and exposure times on *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.4.8 Sporekill®

When exposed to increasing concentrations of Sporekill® for 10min, *Pythium* zoospore survival was decreased at an escalating level (Fig. 8a). At 5mg.l^{-1} a total elimination was achieved. This is the expected dose response.

Fusarium conidia also demonstrated this classic dose response with a near linear increase in efficacy with increasing exposure time or concentration, with the exception of a 30min exposure at 5mg.l^{-1} which showed an unexpected decrease in efficacy, below that expected from the other results (Fig. 8b).

Ralstonia cells initially also demonstrated an expected dose response with the exception of a 10min exposure at 2.5mg.l^{-1} which yielded a result higher than would be expected (Fig. 8c). Excluding this anomalous singularity the other results demonstrated the expected trend.

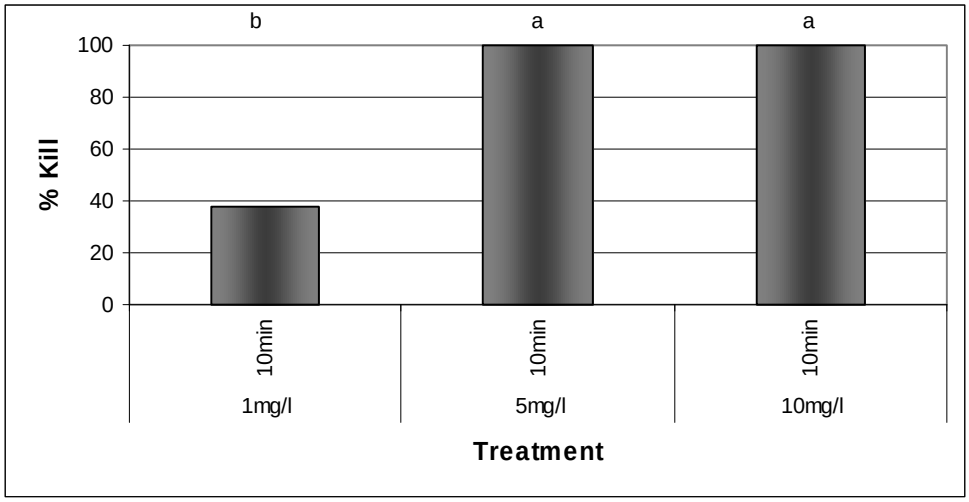


Figure 8a: Efficacy of Sporekill® at various concentrations at a 10min exposure time against *Pythium* zoospores in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

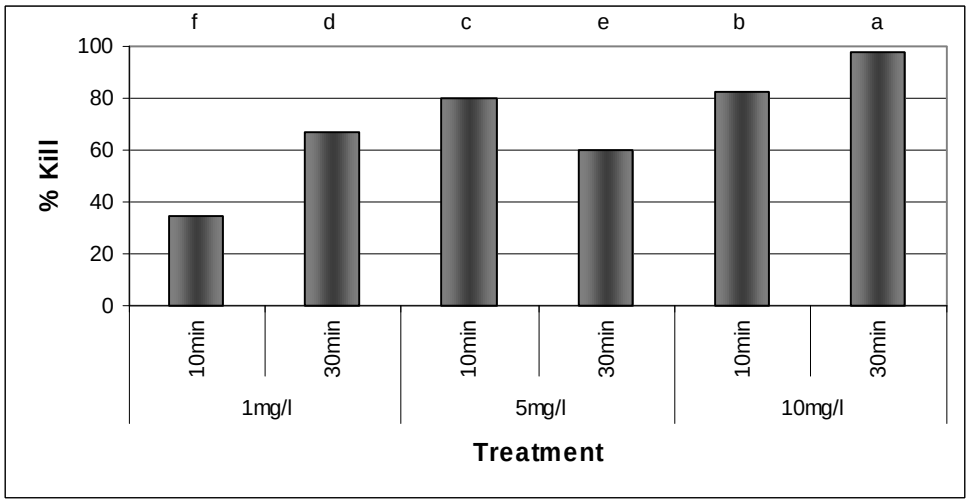


Figure 8b: Efficacy of Sporekill® at various concentrations and exposure times against *Fusarium* conidia in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

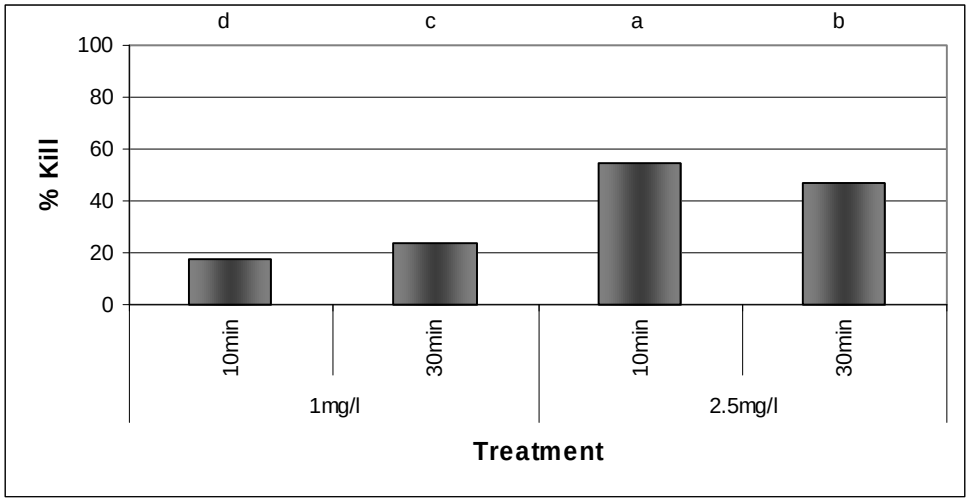


Figure 8c: Efficacy of Sporekill® at various concentrations and exposure times against *Ralstonia* planktonic cells in aqueous suspension. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

3.5 Discussion

All the products were effective against *Pythium* zoospores in an aqueous suspension at relatively low concentrations. Presumably due to the presence of a cell wall and more complex barrier, *Fusarium* and *Ralstonia* required exposure to higher concentrations of the sanitisers before effective reduction in organism survival was noted. This was to be expected since the zoospores with only a simple cell membrane are likely to be more vulnerable to the sanitisers. This was in agreement with results obtained by Stanghellini and Tomlinson (1987) who showed that the non-ionic surfactant Agral 90® was able to cause lysis of *Pythium* zoospores and inhibit root infection and growth.

In parallel trials (described in Chapter 4) the phytotoxic nature of Sporekill® and copper was determined, these chemicals were subsequently tested at lower concentrations against *Ralstonia* to ascertain whether testing at lower concentrations would be indicated. As the lowered concentrations against *Ralstonia* did not exhibit adequate efficacy, these low concentrations were not tested additionally against *Pythium* and *Fusarium*.

From these experiments the most effective dosages for control of *Pythium* in water for the respective compounds at a 10min exposure time were:
Actsol® at a 1:10 dilution (one part Actsol® to ten parts water); Prasin® at a concentration of 5mg.l^{-1}; Purogene® at a concentration of 10mg.l^{-1}; TecsaClor® at a concentration of 25mg.l^{-1}; Fitosan® at a concentration of 7.5mg.l^{-1}; Agral 90® at a concentration of 1mg.l^{-1}; copper sulphate at a concentration of 1mg.l^{-1} and Sporekill® at a concentration of 5mg.l^{-1}.

The Agral 90® results was consistent throughout all the experiments, yet did not follow the expected trend, nor did these results concur with those of Stanghellini and Tomlinson (1987) who demonstrated increasing activity with increasing concentration. A concentration of 1mg.l^{-1} did, however, show a similar effect on the zoospores. The anomaly in the current experiment can possibly be explained by the fact that the higher concentrations cause a rapid encystment of the *Pythium* zoospores (van West *et al.*, 2003) with an associated increase in resistance to the sanitiser, while lower concentrations affect the zoospores directly and cause lysis before encystment can occur. This inverse trend is not demonstrated to the same degree in the results of tests conducted on *Ralstonia* and

Fusarium, indicating that the more complex nature of the cell walls and cell membranes of these organisms may aid in the resistance to Agral 90®.

The trends shown by the results of water treatment with the other sanitisers were as expected, where a decrease in inoculum survival was seen with increasing sanitiser concentrations. An increased exposure time generally resulted in minimal increase in kill rate, indicating that the effects of the sanitisers are more of an immediate nature as opposed to a cumulative effect over time.

The results further confirm the hypothesis that the more complex cell wall and cell membrane structures found in *Ralstonia* and *Fusarium* the less effective the sanitiser. This same trend has also previously been reported by Hudson *et al.* (1986), Koponen *et al.* (1992) and Mebalds *et al.* (1997) where organisms with increasing barrier complexity showed decreasing sensitivity to water sanitisers. It is possible that the complex Gram negative structure of the *Ralstonia* cell walls and cell membranes resulted in the greatest resistance to the effects of the sanitisers as well as the destructive effects of the copper ion treatment. This data also indicates that the simple membrane of a *Pythium* zoospore results in this structure being highly sensitive to the effects of water sanitisers.

From this data it can be presumed that the addition of the above products to a *Pythium* infested water supply of a hydroponic system would effectively inactivate or kill *Pythium* inoculum and also greatly reduce *Fusarium* and *Ralstonia* inoculum levels. However, it must be borne in mind that these experiments were conducted in the absence of organic matter and other contaminants which would be present in a commercial system. Therefore, in a recirculating hydroponic system the exposure time is not considered critical since, if effective mixing occurs, the product will remain in the solution until the solution is replaced or the product dissipates as would be the case with the Actsol®, Purogene® and TecsaClor® where the active ingredients will tend to volatilise, or be degraded as would also be expected with Prasin® and Fitosan®.

3.6 References.

Adler, P.R., Summerfelt, S.T., Glenn, D.M. and Takeda, F. 2003. Mechanistic approach to phytoremediation of water. Ecological Engineering 20: 251–264.

Andrews, L.S., Key, A.M., Martin, R.L., Grodner, R. and Park, D.L. 2002. Chlorine dioxide wash of shrimp and crawfish: An alternative to aqueous chlorine. Food Microbiology 19: 261-267.

De Jonghe, K., De Dobbelaere, I., Sarrazyn, R. and Hofte, M. 2005. Control of brown root rot caused by *Phytophthora cryptogea* in the hydroponic forcing of witloof chicory (*Cichorium intybus* var. *foliosum*) by means of a nonionic surfactant. Crop Protection 24: 771–778.

Do Socorro, M., Bastos, R., de Fatima Ferreira Soares, N., de Andrade, N.J., Arruda, A.C. and Alves, R.E. 2005. The effect of the association of sanitisers and surfactant in the microbiota of the cantaloupe (*Cucumis melo* L.) melon surface. Food Control 16: 369–373.

Grimm, G.R. and Alexander, A.F. 1973. Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. Phytopathology 63: 540-541.

Hudson, H.R., Ojo, I.A.O. and Pianka, M. 1986. Guanidines with antifungal (and antibacterial) activity – a review. International Pest Control: 144–155.

Koponen, H., Avikainen, H. and Tahvonen, R. 1992. The effect of disinfectants on fungi in pure culture and on different surface materials. Agricultural Science Finland 1: 587-595.

Kucharek, T. and Mitchell, D. 2000. Diseases of agronomic and vegetable crops caused by *Pythium*. Plant Pathology Fact Sheet PP-53. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Christine Waddill, Dean. http://plantpath.ifas.ufl.edu/takextpub/FactSheets/pp0053.pdf.

Lee, S., Gray, P.M., Dougherty, R.H. and Kang, D. 2004. The use of chlorine dioxide to control *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples. International Journal of Food Microbiology 92: 121–127.

Mebalds, M., Bankier, M. and Beardsell, D. 1997. Water disinfections control pathogens. Australian Horticulture, April 1997: 66-68.

Monarca, M., Zani, C., Richardson, S.D., Thruston Jr, A.D., Moretti, M., Feretti, D. and Villarini, M. 2004. A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water. Water Research 38: 3809–3819.

Peng, J., Tsai, W. and Chou, C. 2002. Inactivation and removal of *Bacillus cereus* by sanitiser and detergent. International Journal of Food Microbiology 77: 11–18.

Roux, C. and Botha, W.J. 1997. An introduction to the Pythiaceae in South Africa. Agricultural Research Council, Plant Protection Research Institute, Roodeplaat.

Runia, W. 1994. Disinfection of recirculation water from closed cultivation systems with ozone. Acta Horticulturae 361: 388-396.

Runia, W. 1995. A review of possibilities for disinfection of recirculation water from soilless cultures. Acta Horticulturae 382: 221-229.

Saba, A. and Messina, F. 2003. Attitudes towards organic foods and risk/benefit perception associated with pesticides. Food Quality and Preference 14: 637–645.

Singh, N, Singh, R.K., Bhunia, A.K. and Stroshine, R.L. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. Food Science and Technology 35: 720–729.

Stanghellini, M.E. and Tomlinson, J.A. 1987. Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. Phytopathology 77: 112-114.

Stanghellini, M.E., Kim, D.H., Rasmussen, S.L. and Rorabaugh, P.A. 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. Plant Disease 80: 1113-1116.

Stanghellini, M.E. and Miller, R.M. 1997. Biosurfactants: Their identity and potential efficacy in the biological control of zoosporic plant pathogens. Plant Disease 81: 4-12.

van Broekhuizen, W. 2002. Detection, characterisation and suppression of *Ralstonia solanacaerum*. Appendix 2, M.Sc Dissertation, University of Pretoria, South Africa.

van West, P., Appiah, A.A. and Gow, N.A.R. 2003. Advances in research on oomycete root pathogens. Physiological and Molecular Plant Pathology 62: 99–113.

CHAPTER 4

IN VIVO ASSESSMENT OF PHYTOTOXICITY OF SANITISERS ON CUCUMBER AND LETTUCE PLANTS

4.1 Abstract

In Chapter Three it was demonstrated that the sanitisers being tested were able to eliminate *Pythium* infestation from a volume of water while also reducing levels of *Fusarium* and *Ralstonia.* The aim of this Chapter was to evaluate the phytotoxic effect of the sanitisers on cucumber and lettuce plants *in vivo*. Two plant models were used to assess phytotoxic effects and establish threshold dosages (in terms of phytotoxicity) of the various sanitisers. These models were a rapid model using cucumber seedlings (*Cucumis sativa* L.) exposed to the sanitisers in a static hydroponic system under controlled conditions, and a model using Butter lettuce (*Lactuca sativa* L.) cultivated in a greenhouse-scale gravel bed re-circulating hydroponic system under controlled greenhouse conditions. The sanitisers that were observed to be highly phytotoxic on cucumber plants were: Actsol®, Copper, Prasin® and Sporekill®, with copper being most phytotoxic at concentrations above 2mg.l^{-1}. Phytotoxicity manifested mainly as stunting of growth and leaf development and a reduction in fresh biomass of both foliar plant parts and roots, when compared to the untreated control. Actsol® and copper treatments resulted in a yellowing of the leaves. An interesting aspect observed in the cucumber model was that the chlorine-dioxide based sanitisers (Purogene® and TecsaClor®) caused a slight growth stimulating effect on the cucumber seedlings and no observable phytotoxic effects, at concentrations lower than 50mg.l^{-1}. In the lettuce model, at lower Actsol® concentrations of 1:50 and 1:100 phytotoxic effects were reduced, while a concentration of 100mg.l^{-1} Prasin® caused an unexpected result in that a lesser reduction in fresh mass was observed when compared with the 7.5mg.l^{-1} treatment. Treatment of the nutrient solution with TecsaClor® did not result in any visible or measurable phytotoxic effects on lettuce plants after a four week exposure time at concentrations up to 100mg.l^{-1}. The final conclusion from the current study is that the sanitisers could further be tested at low concentrations for disease control or yield enhancement in pathogen infested hydroponic systems.

4.2 Introduction

Although it has been shown that many sanitisers can effectively reduce the levels of *Pythium* zoospores in a water suspension (Koponen *et al.*, 1992; Mebalds *et al.*, 1997), this treatment cannot necessarily be applied directly into a hydroponic nutrient solution that feeds hydroponic plant roots since exposure may result in phytotoxic effects on the plants (Nalecz-Jawecki *et al.*, 2003).

An intermediate step is necessary to ascertain whether the sanitisers under investigation have any phytotoxic effects, in terms of growth reduction, discolouration or any other effects, which would disadvantage the marketability of the crop in question, namely Butter lettuce (*Lactuca sativa L.* var *capitata L.* cv Nadine). This study aimed to address this by subjecting two models (discussed below) to the sanitisers at a concentration range centred on the most effective concentrations as established in a previous chapter (Chapter 3 of this study). The two plant models used were: 1) A rapid laboratory model, using a fast growing crop which is known for sensitivity to phytotoxic effects (cucumber) and cultivated under accurately controlled climatic conditions, and 2) A scale model using a slower growing crop (lettuce) grown under greenhouse conditions which approximate field conditions. The aim of this study was to establish the phytotoxicity thresholds of the two crops to each sanitiser, with the aim of establishing whether the effective concentrations could be included in a nutrient solution in a recirculating hydroponic system.

For the rapid model, cucumber (*Cucumis sativa* L.) was selected as this plant is both an important hydroponic crop (Paulitz *et al.*, 1992) and it can be rapidly cultivated to an age where any phytotoxicity effects would be evident. Cucumbers are also susceptible to infection by all the pathogens assessed in the previous chapter (Chapter 3) (Paulitz *et al.*, 1992; Fravela and Larkin, 2002; Lemay *et al.*, 2003). Cucumber plants have been reported as having sensitivity to numerous chemicals and are therefore suitable as monitors of environmental contamination (Migliore *et al.*, 2003). Phytotoxic effects have also been well documented for this crop (Vinit-Dunand *et al.*, 2002; Wang *et al.*, 2002). This data indicated that cucumbers would allow for rapid assessment of even minimal phytotoxic effects.

Hund-Rinke and Kordel (2003) also demonstrated the benefits and increased rate at which phytotoxic effects can be observed with laboratory scale experiments under controlled conditions as a precursor to more lengthy and complicated field-scale experiments.
Butter lettuce was selected as the crop used in the greenhouse hydroponic system as it is a commercially important hydroponic crop and will be the main focus of this study. Butter lettuce is also abundantly available and is less sensitive to phytotoxic effects by at least one of the sanitisers under investigation in this study (Carrillo *et al.*, 1996). Lettuce still remains sensitive enough to phytotoxic effects to be considered an acceptable crop to be used as a monitor of phytotoxic effects (Migliore *et al.*, 2003).

Furthermore, both lettuce and cucumbers are listed as acceptable crops in the Ecological Effects Test Guidelines (1996), which describes procedures for phytotoxicity evaluations on non-target crops. Thus the results obtained from this study would give an indication of the likely effects these sanitisers would have on the majority of crops.

4.3 Materials and Methods

4.3.1 Cucumber model

4.3.1.1 Cucumber variety and germination

Disease-free seeds of a commercial variety (Dalat 22) of a parthenocarpic English cucumber (*Cucumis sativa* L.) were obtained from Hygrotec Seeds (South Africa). The seeds were planted in sterilised vermiculite (autoclaved at 121°C for 15min with an inclusion of 100ml tap water.kg^{-1} vermiculite), which was liberally moistened with sterilised tap water (autoclaved at 121°C for 15min). The seeds were then germinated for 7d in an environmentally controlled growth cabinet (Conviron™) with conditions set at 25°C with 65% relative humidity (RH) and no light.

4.3.1.2 Static hydroculture

Distilled water was used to prepare 1l batches of a standard hydroponic nutrient solution (Appendix I: A, Solution 1). Appropriate volumes of each sanitiser were then added to the nutrient solution to achieve the required test concentrations. The solution was mixed thoroughly by manual agitation for 10s. The resulting solution was then dispensed into clean 250ml plastic containers. Eight containers were prepared for each sanitiser concentration. For each sanitiser concentration range a control was also prepared as above, with no sanitiser added to the nutrient solution. The entire volume of nutrient solution was replaced with freshly prepared and treated nutrient solution after one week of growth to maintain ideal nutrient growth conditions and constant sanitiser concentrations.

Cucumber seedlings of equivalent size and appearance were selected and planted singly into the prepared containers. Seedlings were kept upright by making an incision into the lid of each container and placing the seedling into this incision in such a way that the roots were completely immersed in nutrient solution (Plate 1: A). The stems were supported by a thin strip of foam rubber to prevent damage.

4.3.1.3 Growth conditions

The containers containing the cucumber seedling were placed in a Conviron™ controlled environment growth cabinet set in a cycle of 25°C, 66% RH, with simulated daylight for 12h followed by conditions of 20°C, 60% RH and total darkness for 12h. The plants were visually observed daily for signs of phytotoxicity.

4.3.1.4 Phytotoxicity assessment

After 14d of growth the seedlings were again observed for any visible signs of phytotoxicity such as colour changes in leaves, general leaf size and development and root development. The plants were then harvested, the roots excised and fresh weight of shoots and roots determined by weighing (Vinit-Dunand *et al*., 2002).

4.3.1.5 Analysis

Root and shoot mass data was statistically analysed using Duncan's Multiple Range test at $P = 0.05$, utilizing the SAS for Windows version 8.0e software package.

4.3.2 Lettuce model

4.3.2.1 Lettuce variety and germination

Disease-free Butter lettuce seeds were germinated at a commercial hydroponics grower (Hydrotec, South Africa) under conditions preventing general disease infestation. Clean seedling trays were filled with steam pasteurised vermiculite and peat mixture (80:20) that was used as the germination medium. The seeds were watered every 20min, during daylight hours, by overhead emitters, supplied with pathogen-free water. Seedlings were germinated at environmental conditions under a shade net structure.

4.3.2.2 Small scale gravel bed hydroponic system

A small scale gravel bed hydroponic system (based on the gravel film technique) was assembled in an environmentally controlled greenhouse (Plate 1: B). This system consisted of ten 100l reservoirs, each containing 100l heat pasteurized tap water and hydroponic nutrient mixture (Appendix I: A, Solution 1). Each reservoir supplied nutrient solution to three plastic (PVC) troughs of equal lengths (2.5m) by means of a submersible pump within each reservoir. Each trough was filled to a level of 8cm with washed gravel (crushed dolerite / granite chips of approximately 15mm). Nutrient solution flow was limited to 400mg.min^{-1}.trough^{-1}. The outflow solution was collected at the lower end of each trough due to a gradient and recirculated back into the 100l reservoir by means of gravity flow. The entire volume of nutrient solution in each reservoir was replaced on a weekly basis. Sanitisers were added to each reservoir during this preparation of the nutrient solution at the necessary dosages required to achieve the required test concentration ranges.

Each of the three troughs supplied by a single reservoir were planted with 15 28d Butter lettuce seedlings placed equidistant from each other, resulting in a total of 45 plants per treatment (15 plants per trough) (Plate I: B).

Plants were allowed to grow naturally for a total of 28d and were inspected every 2d for any visible symptoms of phytotoxicity or growth problems.

4.3.2.3 Growth conditions

Environmental conditions were maintained within the greenhouse at an average RH of 65%, an average maximum daytime temperature of 28°C and average minimum nightly temperature of 18°C. Light conditions were as natural and no supplementation was added, resulting in average length of daylight being approximately 13h.

4.3.2.4 Phytotoxicity assessment

After 28d the lettuce plants were observed for any visible signs of phytotoxic effects after which they were harvested. The shoots and roots were separated from each other and their fresh mass determined separately (Migliore *et al.*, 2003). Harvesting and weighing was completed before 10am for each experiment to minimize possible growth-cycle differences.

Selected root samples were analysed for *Pythium* infection by plating 3mm root tip pieces at a rate of five per Petri-dish on a *Pythium*-selective medium (Roux and Botha, 1997) to determine the absence or presence of infection.

4.3.2.5 Analysis

Root and shoot mass data was statistically analysed by means of Duncan's Multiple Range test at $P = 0.05$, utilizing the SAS for Windows version 8.0e software package.

4.3.3 Sanitiser preparation

For both models sanitisers were prepared as follows, with the concentrations tested in each model detailed in Table 1.

Prasin® (SIDL, South Africa), Fitosan® (Health & Hygiene, South Africa), TecsaClor® (BTC Products, South Africa), Agral 90® (Kynoch chemicals, South Africa) and Sporekill® (Hygrotech, South Africa) were used directly from the solution provided by the manufacturer.

Fresh Purogene® (BTC Products, South Africa) was generated for each experiment according to label instructions (addition of one part supplied activator to ten parts Purogene®). This was allowed to react for 5min before use.

Fresh Actsol® was generated for each experiment using an ECA (ElectroChemical Activation) device provided by Radical Waters (Midrand, South Africa) and freshly prepared brine solution [2.5g NaCl (Merck, South Africa) per litre water] to achieve an Actsol® solution of average pH 7.2 and ORP 800mV. This solution was used directly in the experiments.

Copper (II) sulphate crystals (Merck, South Africa) were dissolved in de-ionised water and diluted to the final volume of water. Details of each sanitiser are provided in Appendix 1: B). Contact details of all suppliers are provided in Appendix I: C.

Table 1: Concentrations of sanitisers tested in the cucumber and lettuce models

Product	**Cucumber Model**	**Lettuce Model**
Actsol®	1:10; 1:20; 1:50; 1:100 and 1:200	1:10; 1:20 and 1:50
Prasin®	5mg.l^{-1}; 7.5mg.l^{-1} and 100mg.l^{-1}	2.5mg.l^{-1}; 5mg.l^{-1}; 7.5mg.l^{-1} and 100mg.l^{-1}
Purogene®	2.5mg.l^{-1}; 5mg.l^{-1}; 10mg.l^{-1}; 25mg.l^{-1} and 50mg.l^{-1}	2.5mg.l^{-1}; 10mg.l^{-1} and 50mg.l^{-1}
TecsaClor®	10mg.l^{-1}; 50mg.l^{-1} and 100mg.l^{-1}	25mg.l^{-1}; 50mg.l^{-1} and 100mg.l^{-1}
Fitosan®	1mg.l^{-1}; 2.5mg.l^{-1}; 5mg.l^{-1}; 7.5mg.l^{-1}; 10mg.l^{-1} and 15mg.l^{-1}	Not tested
Copper	1mg.l^{-1}; 2mg.l^{-1}; 5mg.l^{-1}; 10mg.l^{-1} and 20mg.l^{-1}	Not tested
Sporekill®	1mg.l^{-1}; 5mg.l^{-1}; 7.5mg.l^{-1} and 10mg.l^{-1}	Not tested

Concentrations referred to are product concentrations, i.e. concentrations made directly from the stock solutions. Active ingredient concentrations for each product are specified in Appendix I: B.

4.4 Results

4.4.1 Cucumber model

4.4.1.1 Actsol®

Young cucumber plants demonstrated severe phytotoxic effects when exposed to high concentrations of Actsol®, while plant growth (as measured by fresh biomass differences) was significantly reduced (P=0.05) to less than half that observed in the untreated control, with the exception of the 1:200 concentration (Fig. 1).
Stunting and reduced growth was visually observed within 7d after exposure with minimal growth being observed after the initial exposure when subjected to concentrations of 1:10 and 1:20. The trend displayed does follow the expected dose response with increased effects being noted with an increase in Actsol® concentration (Fig. 1), although the effects were more severe than expected.

Actsol® was observed to be highly phytotoxic across the entire range of tested concentrations, exhibiting symptoms such as stunting of growth and leaf development and an associated reduction in fresh biomass of both aerial plant parts (shoot mass) and roots (root mass). General root development was reduced when compared to the untreated control (Fig. 1). No observable discolouration was however noted on leaves.

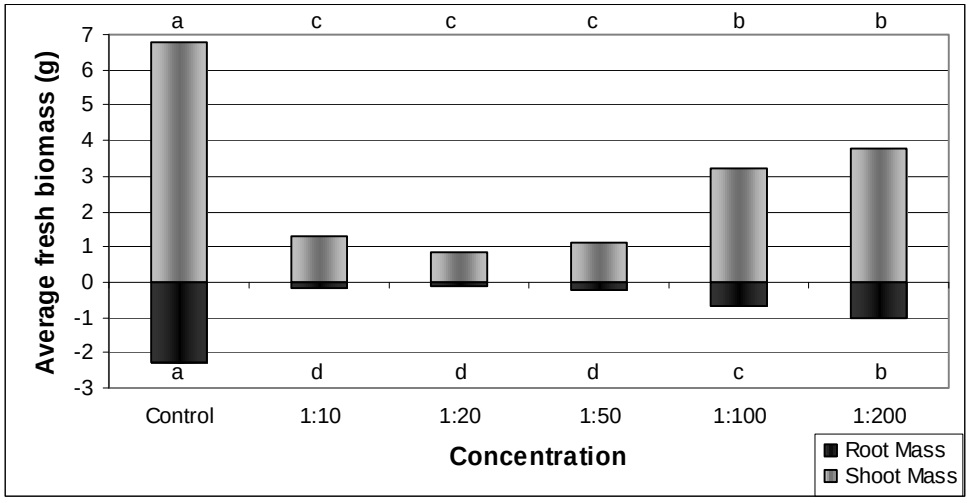


Figure 1: Phytotoxic effects of Actsol® on cucumber seedlings grown for 14d in static hydroponic nutrient solution. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

4.4.1.2 Prasin®

Prasin® demonstrated an expected dose response trend. The cucumber seedlings demonstrated increased phytotoxic effects with an increase in sanitiser concentration (Fig. 2). A concentration of 100mg.l^{-1} prevented all further growth and development over the initial size of the seedlings. The phytotoxic effects manifested as stunting of growth and development of both aerial plant parts (leaf size and formation) and roots (reduced development) and an associated decrease in fresh biomass after two weeks exposure (Fig. 2). No distinguishable discolouration or yellowing of the leaves was noted.

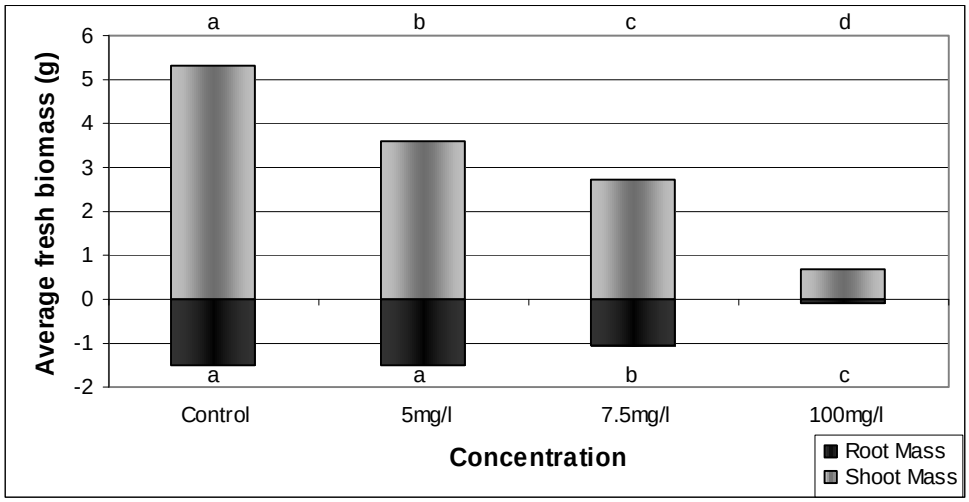


Figure 2: Phytotoxic effects of Prasin® on cucumber seedlings grown in static hydroculture for 14d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

4.4.1.3 Purogene®

After a 14d exposure to various concentrations of Purogene® cucumber seedlings demonstrated no significant (P=0.05) difference to the untreated control, even at a high concentration of 50mg.l^{-1} (Fig. 3). Both leaf and root growth and development was comparable to that of the untreated control, and no visible signs of phytotoxicity were observed.

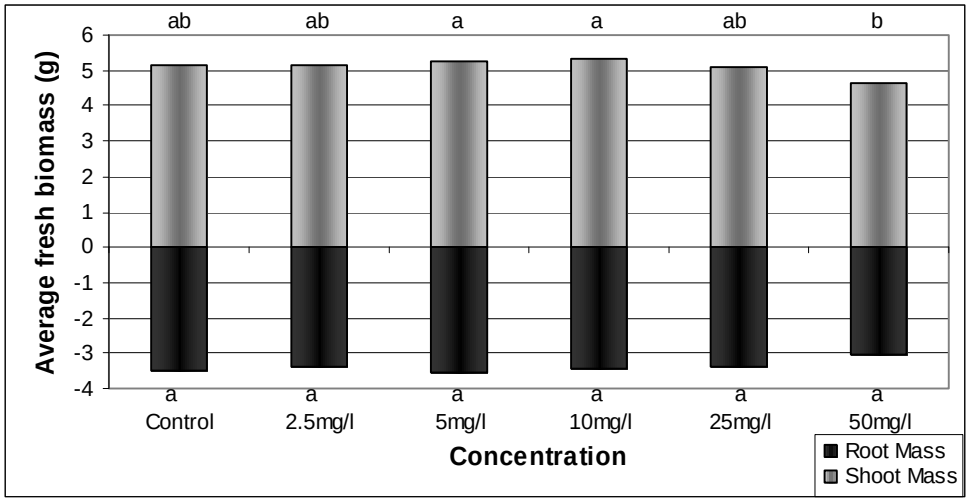


Figure 3: Effects of Purogene® on cucumber seedlings grown in static hydroculture for 14d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

4.4.1.4 TecsaClor®

Cucumber seedlings exposed to TecsaClor® concentrations of up to 100mg.l^{-1} demonstrated no significant (P=0.05) differences between any of the concentrations in fresh root or shoot mass (Fig. 4). Root and leaf growth and development was comparable across all treatments and showed no visible symptoms of phytotoxicity.

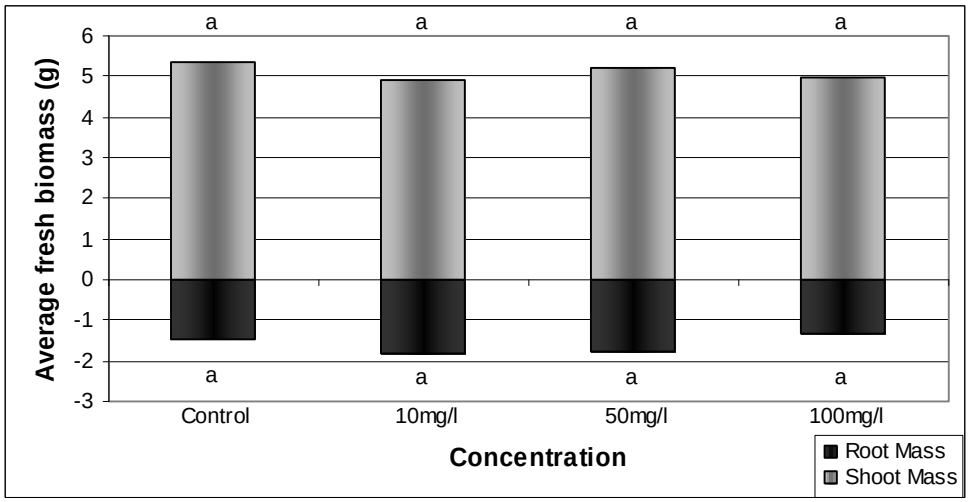


Figure 4: Effects of TecsaClor® on cucumber seedlings grown in static hydroculture for 14d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

4.4.1.5 Fitosan®

After a 14d exposure to Fitosan® concentrations cucumber seedlings exhibited an expected dose response reaction where a decrease in fresh root and shoot biomass was observed with increasing concentration up to a concentration of 7.5mg.l^{-1}, after which no further effects on development, as evaluated by fresh mass, were observed (Fig. 5). A high concentration of 15mg.l^{-1} resulted in a visibly lighter green leaf, and reduced development of the 3rd true leaf.

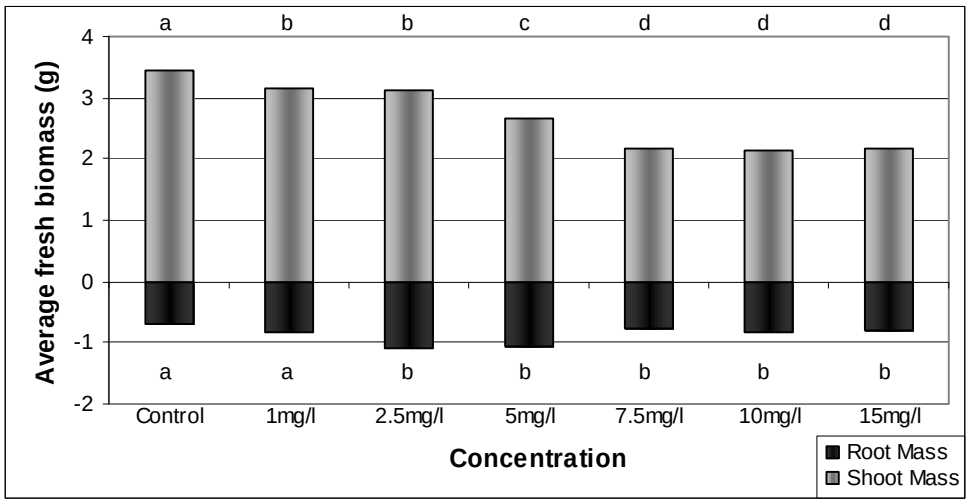


Figure 5: Phytotoxic effects of Fitosan® on cucumber seedlings grown in sterile hydroponic nutrient solution, expressed as a change in fresh biomass after 14d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

4.4.1.6 Copper sulphate

Copper sulphate at concentrations of above 2mg.l^{-1} resulted in visible severe phytotoxic effects on cucumber seedlings after the first week of growth. During the second week of growth no further development was noted at the 5mg.l^{-1} and 10mg.l^{-1} concentrations and plant death was seen at the 20mg.l^{-1} concentration (Fig. 6). Plant development rate was reduced when compared to the untreated control, and a visible lightening in colour of the leaves was observed (Plate 1: C).

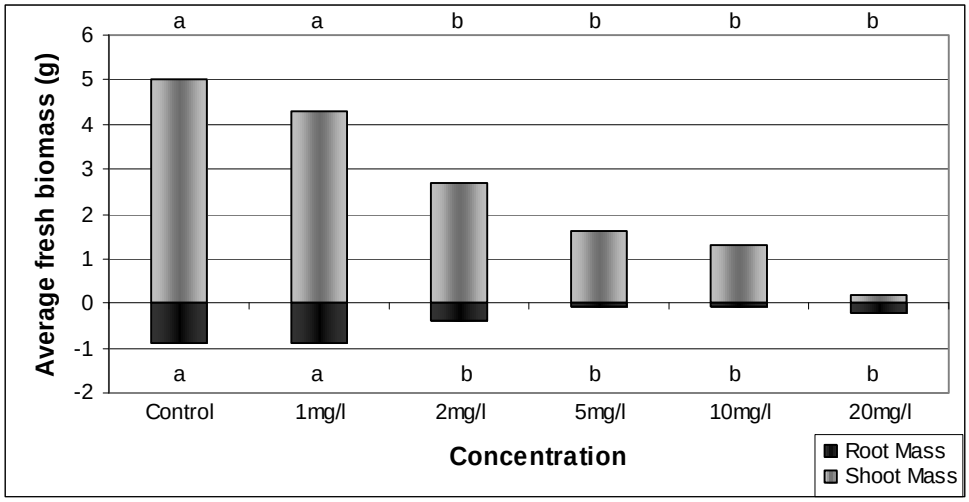


Figure 6: Phytotoxic effects of copper at various concentrations on cucumber seedlings grown in hydroponic nutrient solution after 14d growth. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

4.4.1.7 Sporekill®

An increasing concentration of Sporekill® did not result in either the expected linear or exponential increase in phytotoxic effects on 14d old cucumber seedlings but rather an inconsistent increase in phytotoxic effects (Fig. 7). Concentrations of 1mg.l^{-1} and 5mg.l^{-1} resulted in a significant (*P*=0.05) stunting of plant development, as measured by a reduction in fresh root and shoot mass, both these concentrations yielded similar and insignificantly different results from each other. The higher concentrations of 7.5mg.l^{-1} and 10mg.l^{-1} resulted in further, significant, decrease in plant root and shoot mass over the untreated control but were insignificantly different between them (Fig. 7).

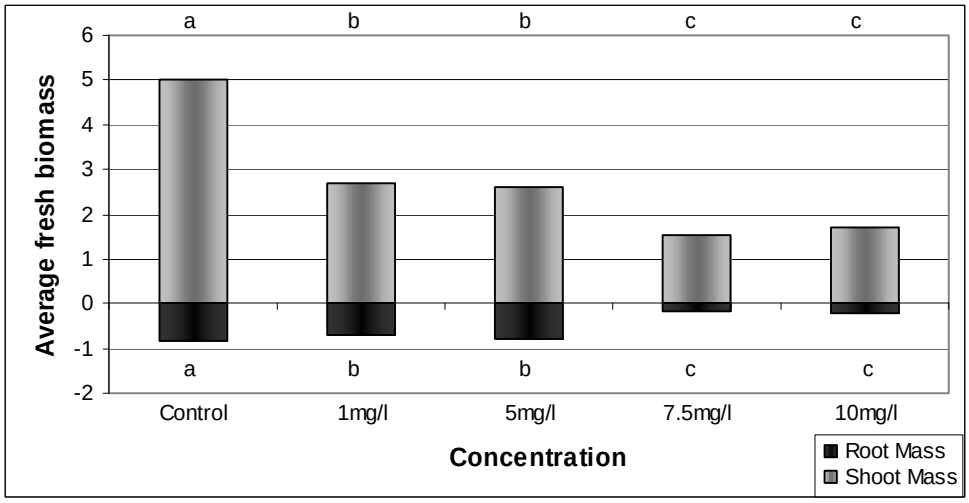


Figure 7: Phytotoxic effects of Sporekill® at various concentrations on cucumber seedlings grown in hydroponic nutrient solution after 14d growth. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

4.4.2 Small scale gravel bed hydroponic system (lettuce model)

4.4.2.1 Actsol®

Lettuce seedlings exposed to a 1:10 concentration of Actsol® showed extreme phytotoxic effects in terms of wilting and leaf discolouration within three days of exposure and total plant death occurred during the second week of exposure. Actsol® concentrations of 1:20 and 1:50 were significantly (P=0.05) less than the untreated control (in terms of fresh leaf plant mass) (Fig. 8). These two treatments gave equivalent results which did not differ significantly.

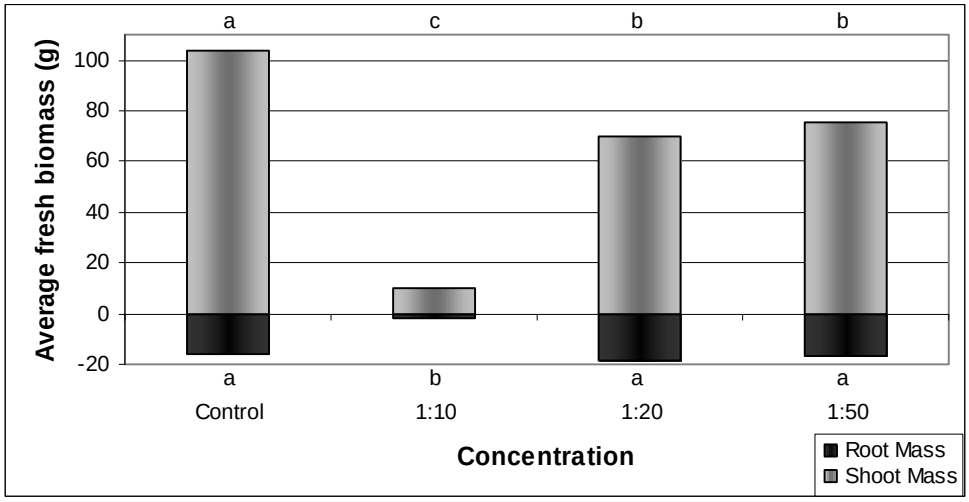


Figure 8: Phytotoxic effects of Actsol® on lettuce plants grown in a gravel bed hydroponic system for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

4.4.2.2 Prasin®

Prasin® treatment of the nutrient solution at increasing concentrations resulted in the lettuce plants exhibiting an expected dose response, with decreasing fresh plant root and shoot mass up to a concentration of 7.5mg.l^{-1} (Fig. 9). A dosage concentration of 100mg.l^{-1} gave an unexpected and anomalous result in that a lower reduction in fresh mass was observed when compared to the 7.5mg.l^{-1} treatment.

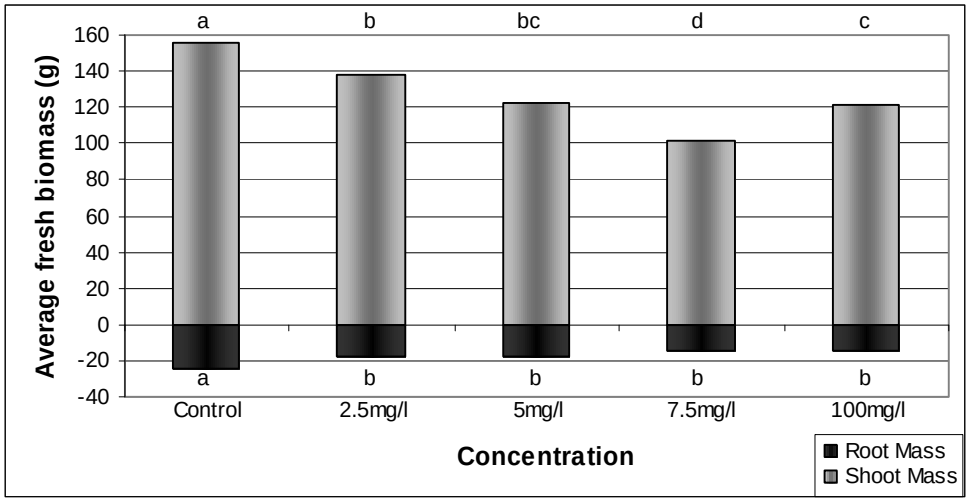


Figure 9: Phytotoxic effects of Prasin® on lettuce plants grown in a gravel bed hydroponic system for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

4.4.2.3 Purogene®

Exposure of lettuce plants to increasing concentrations of Purogene® resulted in an inverted dose response where an increase in fresh plant root and shoot mass was observed up to 10mg.l^{-1} (Fig. 10). A dosage of 50mg.l^{-1} resulted in stunting of plant development and a reduction in fresh plant root and shoot mass in comparison to the untreated control.

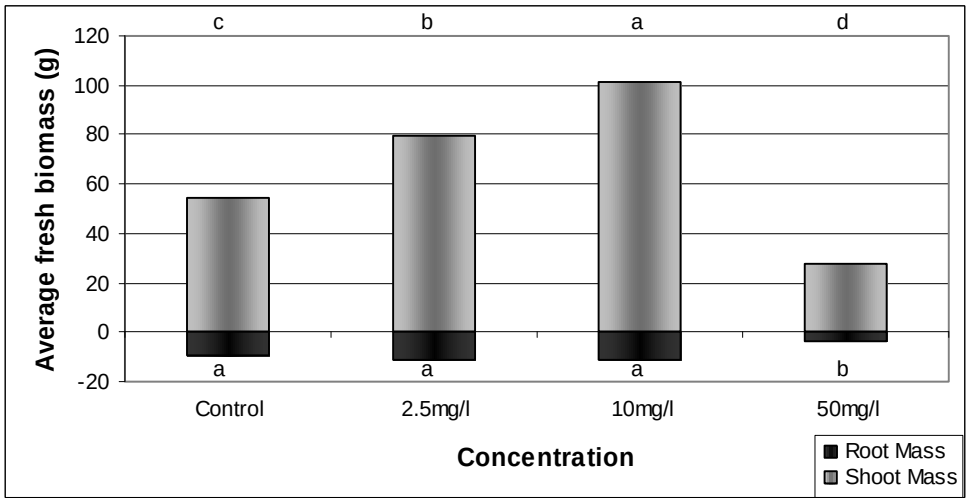


Figure 10: Phytotoxic effects of Purogene® on lettuce plants grown in a gravel bed hydroponic system for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

4.4.2.4 TecsaClor®

Treatment of the nutrient solution with TecsaClor® did not result in any visible or measurable phytotoxic effects on lettuce plants after a 28d exposure time at concentrations up to 100mg.l^{-1} (Fig. 11). A minor increase in fresh plant root and shoot mass compared to the untreated control was observed for each treatment, although not statistically significantly in terms of fresh plant mass (*P*=0.05).

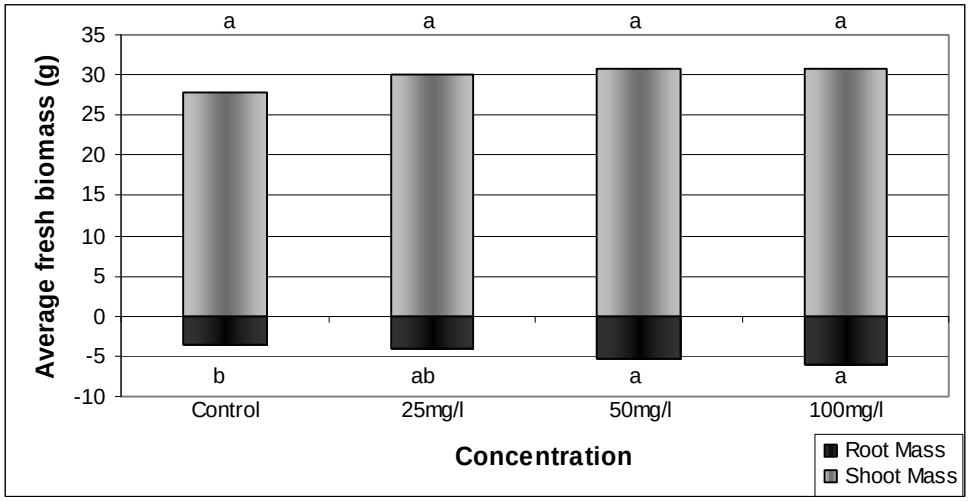


Figure 11: Effects of TecsaClor® on lettuce plants grown in a gravel bed hydroponic system for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

4.5 Discussion

The results obtained from the cucumber model indicated that the selection and design of the model was appropriate for the initial testing since, where applicable, visible phytotoxic effects were seen within the first week of exposure to the sanitisers. This model is therefore suitable for the purpose of the study in accordance with the report of Hund-Rinke and Kordel (2003).

The cucumber model also showed that copper and quaternary ammonium compound-containing sanitisers (Sporekill®, Fitosan® and Prasin®) had definite phytotoxic effects as measured in terms of fresh biomass reduction (Migliore *et al.*, 2003), confirming previous results where similar phytotoxic effects were demonstrated (Wang *et al.*, 2001; Vinit-Dunand *et al.*, 2002; Nalecz-Jawecki *et al.*, 2003). At these low concentrations, phytotoxicity of the above products manifested only as stunting of growth, as opposed to other visible symptoms, which implies that these treatments should not result in any negative consumer impact. Therefore, at the lowest concentrations these sanitisers could still be considered as viable water treatment options if the increase in yield due to disease control outweighs the cost of treatment and crop yield losses due to phytotoxicity.

The most interesting aspect observed in the cucumber model was that the chlorine-dioxide based sanitisers (Purogene® and TecsaClor®) had a slight growth stimulating effect on the cucumber seedlings and no observable phytotoxic effects at concentrations lower than 50mg.l^{-1}. These findings are confirmed by Carrillo *et al.* (1996) where a single dose of chlorine-dioxide was also shown to have growth enhancing effects, while high dosage levels resulted in phytotoxic effects.

The trend seen in the cucumber model was again observed in the small scale gravel bed hydroponic system (lettuce model), where all the sanitiser treatments resulted in similar effects to those seen in the cucumber model. Phytotoxic effects in the lettuce model were limited to growth (leaf and root development) stunting or a total death of the plants within two weeks. No visible signs of wilting, yellowing or other foliar symptoms were observed. As with the cucumber model, the chlorine-dioxide based sanitisers resulted in a growth enhancement, while the quaternary ammonium compound (QAC) based sanitisers resulted in a reduction in fresh biomass, indicating a growth stunting effect.

The growth enhancement seen in the chlorine-dioxide based sanitisers could be attributed to the fact that the active ingredient is volatile, having a lower vapour pressure than water, (detailed in the material safety and data sheet (MSDS)). Thus there would be a rapid initial effect and interaction with the plant roots, after which the active ingredient would volatilise, resulting in the sanitiser returning to a benign state without further action on the roots. This is in direct contrast to the QAC-based sanitisers, which do not volatilise and remain in solution for the duration of the trial (vapour pressure equal to water as described in the MSDS). This constant interaction could be either a direct result on the plant roots due to the minimally toxic nature of the active ingredient, or an additive effect over time as the plant roots take up the QAC.

Actsol®, a unique product, which acts as an oxidising biocide, showed severe phytotoxic effects in both the cucumber and lettuce models, resulting in rapid plant death at the highest concentrations. These observations are in contrast to results obtained by Pernezy *et al.* (2005) where a foliar application resulted in minimal phytotoxic effects. However, one of the active ingredients of Actsol® has been shown to have phytotoxic effects (Monarca *et al.*, 2004). This is possibly due to the Actsol® affecting the regular functioning of the roots due to the combination of a chemical and electro-chemical effect of the Actsol®, likely preventing normal moisture and nutrient uptake by the roots, which then results in plant death observed. At lower concentrations of 1:50 and 1:100 the phytotoxic effects were greatly reduced.

The final result and conclusion from the current study is that the sanitisers could further be tested at the following concentrations for disease control and possible crop yield enhancements in pathogen infested hydroponic systems:
Actsol® at a dilution of 1:50 (one part Actsol® in 50 parts water); Prasin® at a concentration of 5mg.l^{-1}; Purogene® and TecsaClor® at a concentration of 10mg.l^{-1} and Fitosan® at a concentration of 5mg.l^{-1}.

It could further also be concluded that copper sulphate and Sporekill® at a concentration of 1mg.l^{-1} could be viable options for sanitation of hydroponic nutrient solutions, yet the severe phytotoxicity of these products make it unlikely that any benefits would be observed as possible yield reduction due to phytotoxic stunting may outweigh the benefit gained from lowered levels of *Pythium* infestation.

4.6 References

Carrillo, A., Puente, M.E. and Bashan, Y. 1996. Application of diluted chlorine dioxide to radish and lettuce nurseries insignificantly reduced plant development. Ecotoxicology and Environmental Safety 35: 57-66.

Ecological Effects Test Guidelines: OPPTS 850.4150, Terrestrial Plant Toxicity, Tier I (Vegetative Vigour). United States Environmental Protection Agency. Prevention, Pesticides and Toxic Substances (7101) EPA 712–C–96–163 April 1996.

Fravela, D.R. and Larkin, R.P. 2002. Reduction of *Fusarium* wilt of hydroponically grown basil by *Fusarium oxysporum* strain CS-20. Crop Protection 21: 539-543.

Hund-Rinke, K. and Kordel, W. 2003. Underlying issues in bioaccessibility and bioavailability: Experimental methods. Ecotoxicology and Environmental Safety 56: 52-62.

Koponen, H., Avikainen, H. and Tahvonen, R. 1992. The effect of disinfectants on fungi in pure culture and on different surface materials. Agricultural Science Finland 1: 587-595.

Lemay, A., Redlin, S., Fowler, G. and Dirani, M. 2003. Pest Data Sheet: *Ralstonia solanacearum* race 3 biovar 2. USDA/APHIS/PPQ, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory, Raleigh, NC. http://www.aphis.usda.gov/ppq/ep/ralstonia/ralstoniadatasheet_CPHST.pdf.

Mebalds, M., Bankier, M. and Beardsell, D. 1997. Water disinfections control pathogens. Australian Horticulture. April 1997: 66-68.

Migliore, L., Cozzolino, S. and Fiori, M. 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52: 1233-1244.

Monarca, M., Zani, C., Richardson, S.D., Thruston Jr, A.D., Moretti, M., Feretti, D. and Villarini, M. 2004. A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water. Water Research 38: 3809-3819.

Nalecz-Jawecki, G., Grabinska-Sota, E. and Narkiewicz, P. 2003. The toxicity of cationic surfactants in four bioassays. Ecotoxicology and Environmental Safety 54: 87-91.

Paulitz, T.C., Zhou, T. and Rankin, L. 1992. Selection of rhizosphere bacteria for biological control of *Pythium aphanidermatum* on hydroponically grown cucumber. Biological Control 2: 226-237.

Pernezny, K., Raid, R.N., Havranek, N. and Sanchez, J. 2005. Toxicity of mixed-oxidant electrolyzed oxidizing water to *in vitro* and leaf surface populations of vegetable bacterial pathogens and control of bacterial diseases in the greenhouse. Crop Protection 24: 748-755.

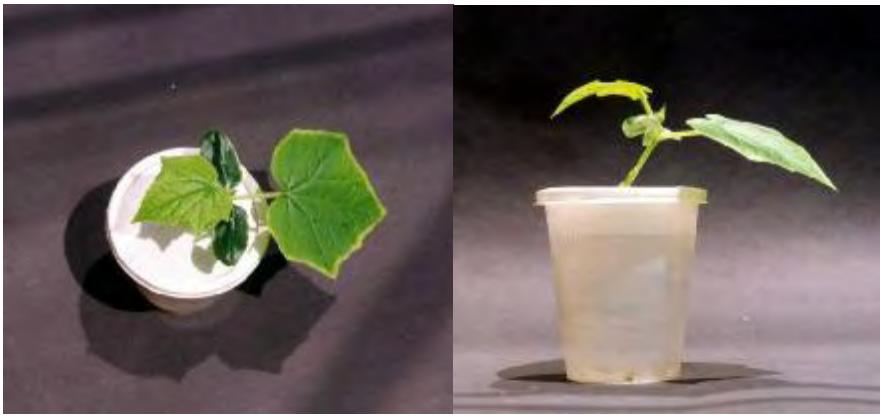
Roux, C. and Botha, W.J. 1997. An introduction to the Pythiaceae in South Africa. Agricultural Research Council. Plant Protection Research Institute, Roodeplaat.

Vinit-Dunand, F., Epron, D., Alaoui-Sosse, B. and Badot, P. 2002. Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. Plant Science 163: 53-58.

Wang, X., Sun, C., Gao, S., Wang, L. and Shuokui, H. 2001. Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*. Chemosphere 44: 1711-1721.

Wang, X., Sun, C., Wang, Y., and Wang, L. 2002. Quantitative structure-activity relationships for the inhibition toxicity to root elongation of *Cucumis sativus* of selected phenols and interspecies correlation with *Tetrahymena pyriformis*. Chemosphere 46: 153-161.

4.7 Plate I



A: Cucumber in static hydroculture, top and side views.

B: Gravel Flow Technique hydroponic system in the greenhouse planted with Butter lettuce at 42d.

C: Visible lightening of cucumber true leaf after a 14d exposure to 2mg.l^{-1} copper sulphate solution.

CHAPTER 5

CONTROL OF *PYTHIUM* WILT AND ROOT ROT OF LETTUCE BY MEANS OF CHEMICAL TREATMENT OF THE NUTRIENT SOLUTION IN RE-CIRCULATING HYDROPONIC SYSTEMS IN THE GREENHOUSE AND FIELD

5.1 Abstract

Results from previous chapters showed that the tested sanitisers were able to control *Pythium* infestation in a water volume while also reducing the levels of *Fusarium* and *Ralstonia.* Secondly, the phytotoxic effects of the sanitisers were determined using two plant models (cucumber and Butter lettuce) grown in hydroponic systems. The aim of the current study was to further test these sanitisers for the control of *Pythium in vivo* using greenhouse and semi-commercial scale hydroponic systems. The hydroponic systems were artificially infested by introducing *Pythium* infected seedlings. The hydroponic nutrient solution was subsequently treated with the sanitisers. Phytex®, Prasin® and Fitosan® significantly reduced the *Pythium* zoospore levels in the nutrient solution assessed at the end of the final week of growth. Purogene® achieved a total eradication (no significant difference from the untreated, uninfected control) of the zoospores. In the semi-commercial field system, Phytex® and Purogene® treatments were able to improve lettuce yield compared to the untreated, *Pythium*-infested control. Agral 90®, Sporekill® and Actsol® resulted in yield decreases when compared to the untreated, *Pythium* infested control. In general, Phytex® and Purogene® rendered the most consistent and positive yield improvements in both the greenhouse and field models. Purogene® also appeared to have a two-fold benefit in that growth was enhanced, while pathogen levels were simultaneously decreased. Prasin® and Fitosan® resulted in some degree of phytotoxicity, while also achieving some measure of *Pythium* control. Although no major yield improvement was obtained, there were no additional negative effects to applying these sanitisers to the nutrient solution. Comparisons between the sanitisers under greenhouse field conditions are discussed.

5.2 Introduction

Pythium has been shown to cause severe disease outbreaks and crop losses over a broad range of hydroponically cultivated vegetable crops, with lettuce and tomato crops being most affected (Stanghellini and Kronland 1986; Paulitz *et al*., 1992; Schwarz and Grosch, 2003; Song *et al*., 2004). Thus *Pythium* is considered one of the most serious pathogens of hydroponic systems (Song *et al*., 2004), with infection and yield losses often going unnoticed due to the ability of this pathogen to cause subclinical infections (Stanghellini and Kronland, 1986). In the recent past, control of this pathogen has been successful with systemic fungicides (Vanachter, 1995; Song *et al*., 2004).

Changes in worldwide regulations have resulted in many hydroponic growth systems being of a recirculating nature to reduce both environmental contamination and water utilisation (Runia 1994). Recirculating hydroponic nutrient solution is an ideal transport medium for pathogen inoculum to rapidly spread throughout and entire hydroponic system (Zinnen, 1988; Stanghellini and Rasmussen 1994; Vanachter 1995).
Current methods of sterilisation of the recirculated nutrient solution are costly or labour intensive while not constantly effective (Schwartzkopf *et al*., 1987; Fravela and Larkin, 2002). Other methods rely on the use of toxic chemicals or substances which have been shown to produce toxic by-products (Date *et al*., 2005). While good pathogen control has also been achieved with fungicides and pesticides (Zinnen, 1988; Song *et al*., 2004), current consumer demand has tended towards preference for products on which pesticide use has been reduced or eliminated (Saba and Messina, 2003).

To satisfy this consumer demand for minimised use of pesticides, while also obtaining consistent sterilisation of the hydroponic nutrient solution, “safer” alternative chemicals such as surfactants and sanitisers (Carillo *et al*., 1996; Allende *et al*., 2006) have been investigated with positive results (Carillo *et al*., 1996; Stanghellini *et al*., 1996)

In Chapter 3 it was determined that certain water sanitisers applied to an aqueous suspension of plant pathogens would result in a lowered contamination level. It was then demonstrated in Chapter 4 that certain of these sanitisers, when applied at low concentrations to a hydroponic nutrient solution, should not result in severe phytotoxic effects or impact negatively on consumer demands.

The aim of this current study was to determine whether the sanitisers are able to reduce crop losses due to *Pythium* infestation, when they are applied in a semi-commercial hydroponic system.

5.3 Method and Materials

Two hydroponic systems were designed namely both an experimental scale greenhouse system as well as semi-commercial scale field system.
To achieve infection of plants by *Pythium*, inoculum was artificially introduced into the hydroponic systems to ensure a high level of infestation.

5.3.1 Small scale gravel bed hydroponic system (greenhouse model)

5.3.1.1 Lettuce variety and germination

Disease-free Butter lettuce (*Lactuca sativa* L. var *capitata* L. cv Nadine) seeds were germinated at a commercial hydroponics grower (Hydrotec, South Africa)) under conditions preventing pathogen infestation. Seedling trays were cleaned with chlorinated water and filled with steam pasteurised vermiculite and peat mixture (80:20) that was used as the germination medium. The seeds were watered every 20min, during daylight hours, by overhead emitters, supplied with pathogen-free borehole water. Seedlings were germinated at optimum environmental conditions under a shade net structure.

5.3.1.2 Design of small scale gravel bed hydroponic system

A small scale gravel bed hydroponic system (based on the gravel flow technique) was assembled in an environmentally controlled greenhouse. This system consisted of ten 100l reservoirs, each containing 100l heat pasteurized tap water and hydroponic nutrient mixture (Appendix I: Solution 2). Each reservoir supplied nutrient solution to three plastic troughs of equal lengths (2.5m) and widths (0.15m) by means of a submersible pump within each reservoir. Each trough was filled to a level of 8cm with washed gravel (crushed dolerite / granite chips of approximately 15mm). Nutrient solution flow was limited to 400mg.min^{-1}.trough^{-1}. Outflow solution was collected at the lower end of each trough due to a gradient and recirculated back into the 100l reservoir by means of gravity flow (Plate II: A).

The entire volume of nutrient solution in each reservoir was replaced on a weekly basis. Sanitisers were added to each reservoir during this preparation of the nutrient solution at the necessary dosages required to achieve the test concentration.
Each trough was planted with 15 28d old Butter lettuce seedlings placed equidistant from each other, resulting in a total of 45 plants per treatment (Plate I: B)
Plants were allowed to grow naturally for a total of 28d and were inspected every 2d for any visible symptoms of phytotoxicity or growth problems.

To achieve and ensure *Pythium* infestation in the hydroponic system, 12 seedlings per treatment were exposed to *Pythium* Group F (PPRI #7079) zoospores for two days prior to planting. This exposure was done by immersing the seedling roots into a water volume containing *Pythium* zoospores at an approximate concentration of 10^3 zoospores.ml^{-1}, obtained by macerating two 7d old cultures of *Pythium* on V8-juice agar in 400ml of sterile deionised water. Four of these infested seedlings were then planted at the head of each trough, to serve as a continuous source of zoospore inoculum into the nutrient solution, which would ensure infection along the entire length of the trough.

5.3.1.3 Growth conditions

Environmental conditions were maintained within the greenhouse at an average RH of 65%, an average maximum daytime temperature of 28°C and average minimum nightly temperature of 18°C.

Initial experiments exposed lettuce plants to a range of concentrations of each sanitiser, while two final experiments compared the optimal concentrations of all the sanitisers against each other. For each experiment the following controls were included: an untreated, uninfested control; a *Pythium* infested, untreated control and a *Pythium* infested control treated with the fungicide Phytex® at the manufacturers recommended dosage rate of 1ml.l^{-1} water.

5.3.1.4 Yield and infestation assessments

After 28d the lettuce plants were observed for any visible signs of phytotoxic effects after which they were harvested. The shoots and roots were separated from each other and their fresh mass determined separately (Migliore *et al.*, 2003). Harvesting and weight determination were completed before 10am for each experiment to minimize possible growth-cycle differences.

Recirculated nutrient solution was tested for *Pythium* incidence using the citrus leaf disc baiting procedure described by Grimm and Alexander (1973) and plating on a *Pythium*-selective medium (BNPRA) (Roux and Botha, 1997).

5.3.1.5 Analysis

Root and shoot mass data was statistically analysed using Duncan's Multiple Range test at $P = 0.05$, utilising the SAS for Windows version 8.0e software package.

5.3.2 Semi-commercial scale gravel bed hydroponic system in the field

A semi-commercial scale gravel bed hydroponic system (based on the gravel film technique) was constructed under a 20% grey shade net structure on the University of Pretoria experimental farm (Plate II: B), modelled on a commercial farming system (Plate II: C).

Eighteen troughs of 20m lengths were constructed and filled to a depth of 6cm with clean gravel (crushed dolerite / granite chips of approximately 15mm).
Each trough was fed by a 500l reservoir containing a submersible pump supplying a constant flow of $2l.hr^{-1}$ at the head of each trough. Runoff was collected at the lower end of each bed and channelled back into the reservoir by means of gravity.

A commercially available hydroponic nutrient solution pre-mix was used (Appendix I: Solution 2) as the fertigant solution, and the pH was maintained at 6.4 by the addition of

nitric acid. The fertigant solution was replaced weekly with a fresh mixture to which the sanitisers were added at the established dosages (detailed in Table 1).
Each bed was planted with an average of 350 lettuce seedlings equally spaced along the length of the bed in sets of three spaced in a triangular shape with a single seedling at each point of the triangle. Seedlings were allowed to grow naturally for 42d.

To ensure even infestation of *Pythium* across the entire length of each bed, as well as across separate beds, a 96cm Petri dish containing a 7d old *Pythium* culture on V8 medium was cut into four equal sections (3.6cm^2 pieces). Four of these culture pieces were then placed underneath the gravel in contact with plant roots at distances of 0m, 5m, 10m and 15m along each bed.

5.3.2.1 Growth conditions

Environmental conditions fluctuated due to natural climatic conditions. Average daytime temperatures ranged from 27-33 °C and average night time temperatures from 9-14°C
For each experiment an untreated, uninfested control and a *Pythium* infested, untreated control were included.

5.3.2.2 Yield and infestation assessments

After 28d the lettuce plants were observed for any visible signs of phytotoxic effects after which they were harvested. The shoots and roots were separated from each other and their fresh mass determined separately (Migliore *et al*., 2003).

Recirculated nutrient solution was tested for *Pythium* incidence using a citrus leaf disc baiting procedure as described previously (Grimm and Alexander, 1973; Roux and Botha, 1997).

5.3.2.3 Analysis

Shoot mass data was statistically compared using Duncan's Multiple Range test at P=0.05, utilising the SAS for Windows version 8.0e software package. Root mass was not statistically analysed as root mass does not contribute to the marketable yield.

5.3.3 Sanitiser preparation

For both hydroponic systems sanitisers were prepared as follows, with the concentrations tested in each instance detailed in Table 1.

Prasin® (SIDL, South Africa), Fitosan® (Health & Hygiene, South Africa), TecsaClor® (BTC Products, South Africa), Agral 90® (Kynoch Chemicals, South Africa), Sporekill® (Hygrotech Seeds, South Africa) and Phytex® (Horticura, South Africa) were used directly from the solution provided by the manufacturer.

Fresh Purogene® (BTC Products, South Africa) was generated for each experiment by following label instructions (addition of one part supplied activator to ten parts Purogene®). This was allowed to react for 5min before use.

Actsol® was freshly prepared and delivered weekly by Radical Waters. Actsol® solution had an average pH of 7.2 and ORP of 800mV. This solution was used directly in the experiments.

Copper (II) sulphate crystals (Merck, South Africa) were dissolved in de-ionised water and diluted to give the final concentration required. Contact details of all suppliers are provided in Appendix I: C. Details of each sanitiser are provided in Appendix 1: B)

Table 1: Concentrations of sanitisers tested in the greenhouse system (both individually and in comparison) and in the field-scale system.

Product	**Greenhouse system**	**Greenhouse comparison experiment**	**Field system**
Actsol®	1:10; 1:20 and 1:50	1:20 and 1:50	1:20
Prasin®	2,5; 5 and 7.5mg.l^{-1}	7.5mg.l^{-1}	7.5mg.l^{-1}
Purogene®	10; 25 and 50mg.l^{-1}	10mg.l^{-1}	10mg.l^{-1}
TecsaClor®	25; 50 and 75mg.l^{-1}	25mg.l^{-1}	Not tested
Fitosan®	Not tested	7.5mg.l^{-1}	7.5mg.l^{-1}
Phytex®	Not tested	1ml.l^{-1}	1ml.l^{-1}
Sporekill®	Not tested	5mg.l^{-1}	5mg.l^{-1}
Agral 90®	Not tested	5mg.l^{-1}	5mg.l^{-1}
Copper	Not tested	5mg.l^{-1}	Not tested

Concentrations referred to are product concentrations, i.e. concentrations made directly from the stock solutions. Active ingredient concentrations for each product can be found in Appendix I: B.

5.4 Results

5.4.1 Small scale gravel bed hydroponic system (greenhouse model) – evaluation of sanitisers individually at a range of dosage rates.

5.4.1.1 Actsol®

Actsol® at a dilution of 1:10 and 1:20 into a *Pythium* infested hydroponic lettuce system resulted in severe phytotoxic effects, with plant death occurring at the 1:10 dilution after a period of 14d and severely reduced growth and development of plants exposed to a 1:20 dilution after 28d (Fig. 1). A 1:50 dilution resulted in lettuce plants having a significant (P=0.05) higher fresh shoot mass than an infested and untreated control, while also being significantly reduced in fresh shoot mass when compared to an uninfested and untreated control after 28d (Fig. 1).

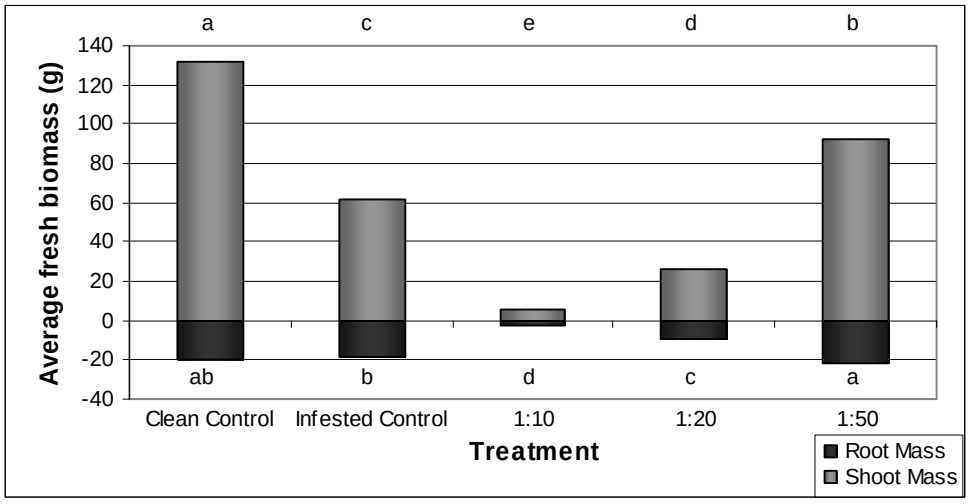


Figure 1: Effect of treatment of the nutrient solution with Actsol® on lettuce yield in the presence of *Pythium* infestation in a small scale gravel bed hydroponic system in the greenhouse. Plants were grown for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

5.4.1.2 Prasin®

Prasin® dosed at a concentration of 7.5mg.l^{-1} into the nutrient supply resulted in the fresh shoot mass being significantly (*P*=0.05) lower than the untreated, uninfested control yet higher than the untreated, infested control (Fig. 2).

Prasin® treatments of 2.5mg.l^{-1} and 5mg.l^{-1} did not result in any significant fresh shoot mass differences from an untreated, *Pythium* infested control, but were significantly lower than the untreated, uninfested control.

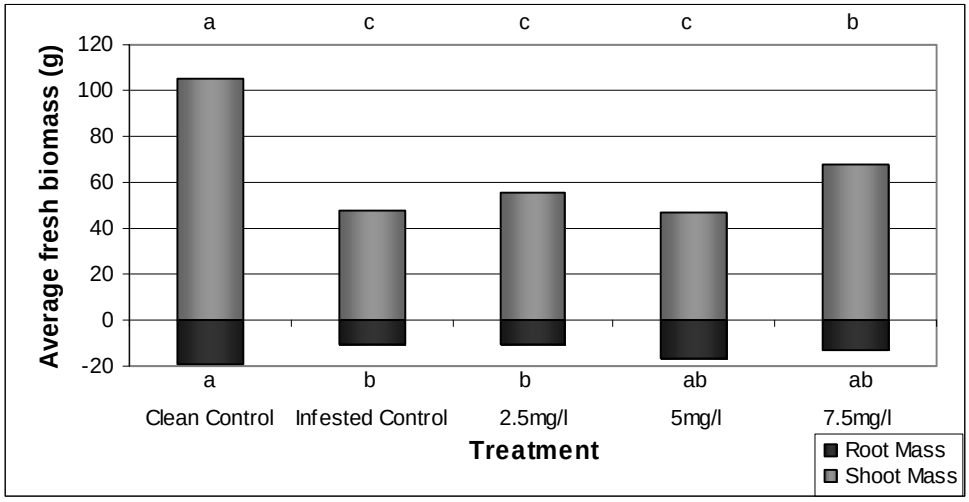


Figure 2: Effect of treatment of the nutrient solution with Prasin® on lettuce yield in the presence of *Pythium* infestation in a small scale gravel bed hydroponic system in the greenhouse. Plants were grown for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

5.4.1.3 Purogene®

Treatment of the nutrient solution with Purogene® at concentrations of 10mg.l^{-1} and 25mg.l^{-1} resulted in no significant (*P*=0.05) differences when compared to an untreated, uninfested control (Fig. 3). None of the Purogene® treatments demonstrated a significant difference to the untreated, *Pythium* infested control. Only the 50mg.l^{-1} treatment resulted in a significant difference in root mass when compared to the uninfested, untreated control although a significant increase in root mass was also observed at a 25mg.l^{-1} concentration.

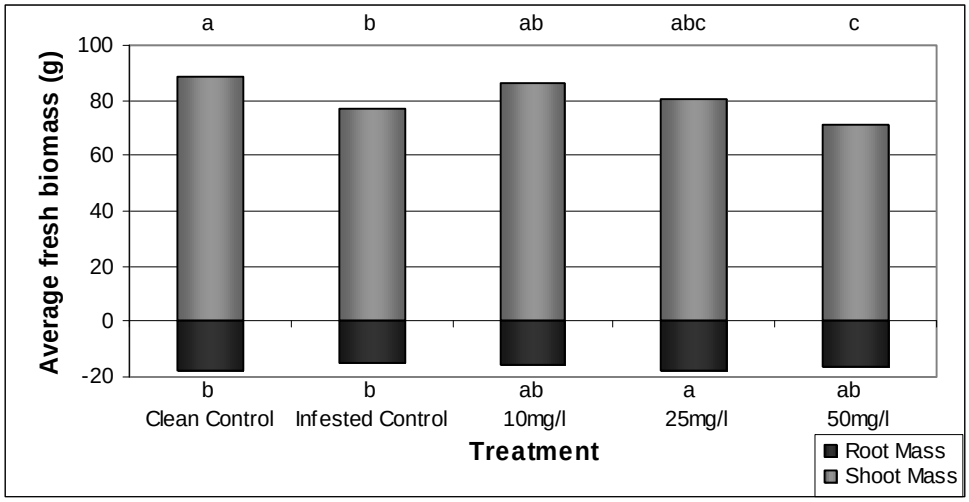


Figure 3: Effect of treatment of the nutrient solution with Purogene® on lettuce yield in the presence of *Pythium* infestation in a small scale gravel bed hydroponic system in the greenhouse. Plants were grown for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (*P*=0.05).

5.4.1.4 TecsaClor®

TecsaClor® at all treatment concentrations did not result in any significant (*P*=0.05) differences in fresh shoot mass when compared to an untreated, *Pythium* infested control, nor between treatment concentrations (Fig. 4). All treatments did result in a significant reduction in fresh shoot mass when compared to an untreated, uninfested control. Root mass was also significantly decreased by all treatments when compared to the uninfested, untreated control, while a 25mg.l^{-1} concentration resulted in a significant increase in root mass when compared to an untreated, *Pythium* infested control.

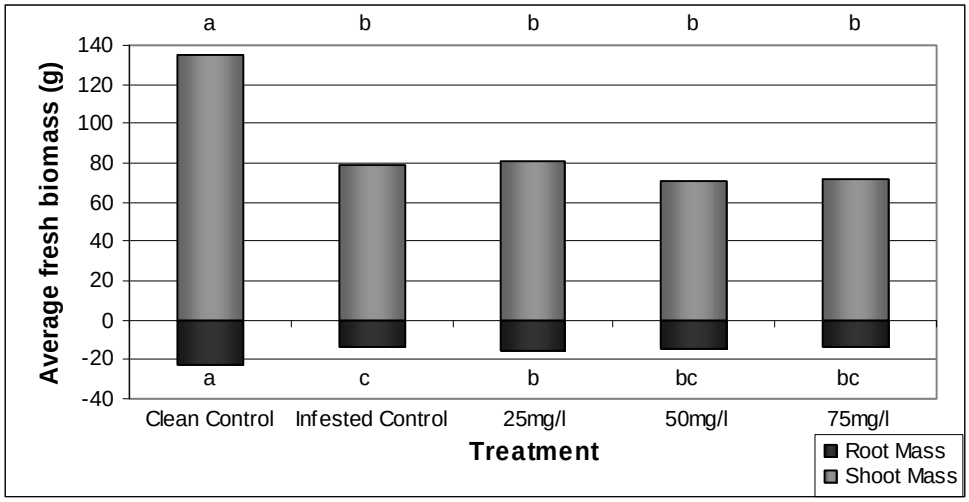


Figure 4: Effect of treatment of the nutrient solution with TecsaClor® on lettuce yield in the presence of *Pythium* infestation in a small scale gravel bed hydroponic system in the greenhouse. Plants were grown for 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

5.4.2 Comparison of different sanitisers at optimum dosage rates in the greenhouse

5.4.2.1 Preliminary experiment

The preliminary comparison experiment showed that the untreated, uninfested control exhibited significantly (P=0.05) reduced growth as measured by fresh shoot mass (Fig. 5). Root mass was also reduced. This biomass was less than an untreated, *Pythium* infested control, indicating that *Pythium* contamination had likely occurred in the uninfested control, which was later confirmed by root platings on *Pythium* selective media. This data was therefore considered unreliable.

The data presented here shows that Actsol® at a 1:20 dilution resulted in total plant death, while other treatments indicated that Prasin® at 7.5mg.l^{-1}, TecsaClor® at 25mg.l^{-1}, Phytex® at 1ml.l^{-1}, Fitosan® at 7.5mg.l^{-1} and Purogene® at 10mg.l^{-1} could be most effective in decreasing order.

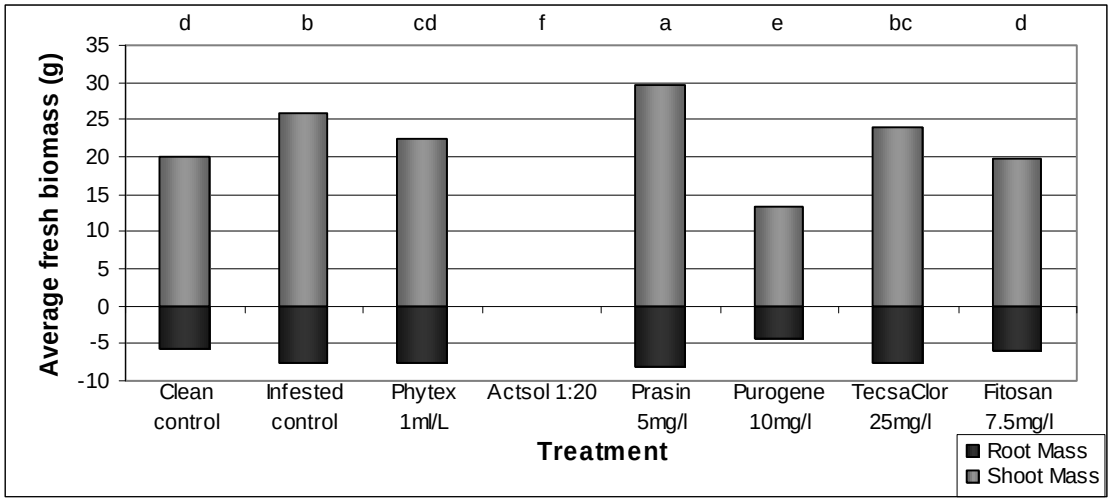


Figure 5: Effect of sanitisers at optimum dosages on yield of *Pythium* infested lettuce in a small scale gravel bed hydroponic system in a greenhouse, over a period of 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

5.4.2.2 Comparison of primary sanitisers in a small scale gravel bed hydroponic system (greenhouse model)

Due to the results obtained in the preliminary experiment, the procedure was repeated, with Actsol® reduced to a 1:50 dilution. The untreated, uninfested and untreated, *Pythium* infested controls showed a significant (P=0.05) difference in shoot mass indicating that *Pythium* infection resulted in a 29% reduction in yield (Fig. 6). Phytex® at 1ml.l^{-1} resulted in a significant increase in fresh shoot mass when compared to the untreated, uninfested control whilst Purogene® at 10mg.l^{-1} showed no significant difference in fresh shoot mass. Both these treatments resulted in a significant increase in fresh shoot mass when compared to the untreated, *Pythium* infested control achieving a 69% and 39% increase respectively. Prasin® at 7.5mg.l^{-1} and Fitosan® at 7.5mg.l^{-1} did not differ significantly from each other, nor from the untreated, *Pythium* infested control. Actsol® at a 1:50 dilution and TecsaClor® at 25mg.l^{-1} showed a significant decrease in fresh shoot mass in comparison to the untreated, *Pythium* infested control (Fig. 6).

Phytex®, Prasin® and Fitosan® significantly reduced the *Pythium* zoospore levels in the nutrient solution at the end of the final week of growth, while only Purogene® achieved a total elimination (same as the untreated, uninfested control) (Fig. 7). Plants treated with Actsol® and TecsaClor® showed no significant difference in *Pythium* levels when compared to the untreated, *Pythium* infested control.

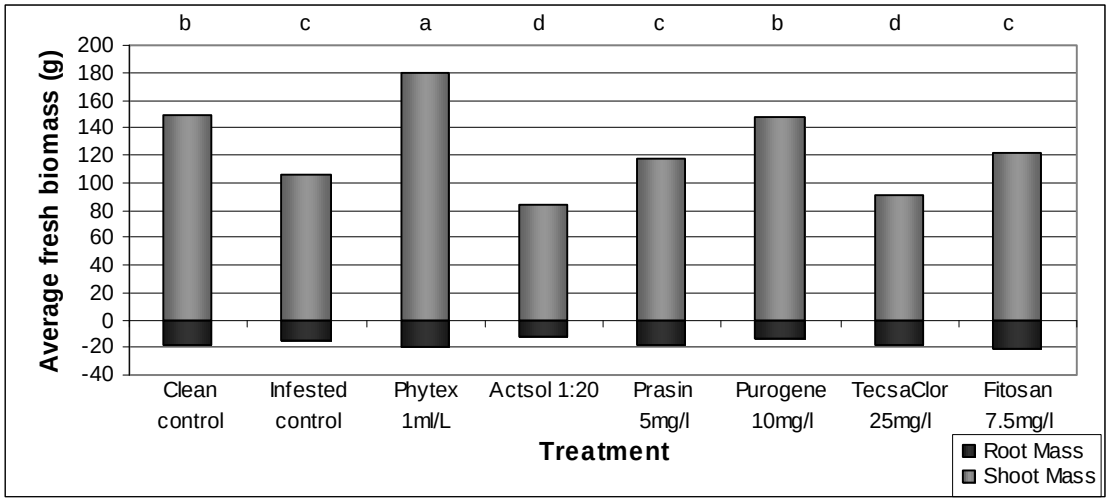


Figure 6: Effect of sanitisers at optimum dosage on yield of *Pythium* infested lettuce in a small scale gravel bed hydroponic system in a greenhouse, over a period of 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

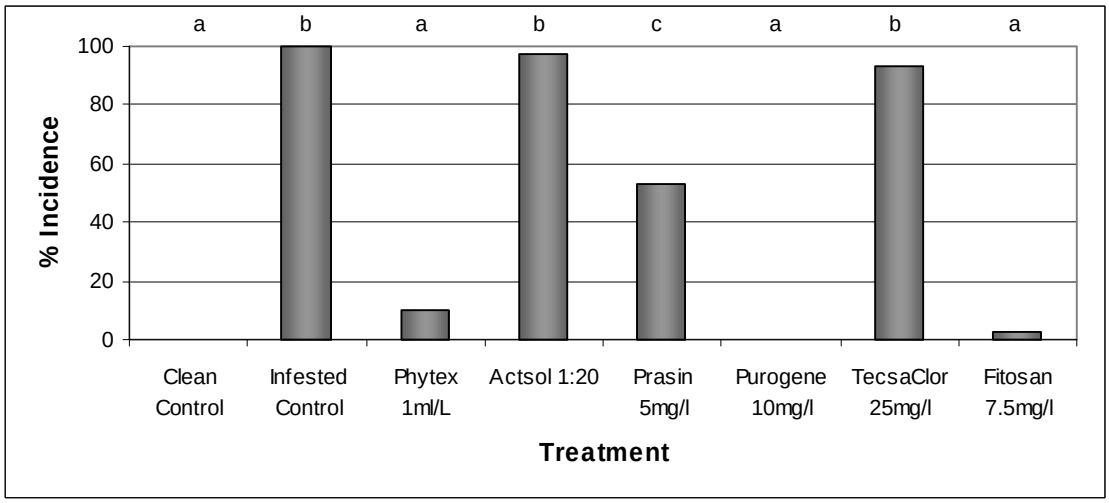


Figure 7: Effect of chemical treatments on *Pythium* infestation in recirculated nutrient solution at the end of the 28d of lettuce growth in a small scale gravel bed hydroponic system in the greenhouse. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

5.4.2.3 Comparison of additional sanitisers in a small scale gravel bed hydroponic system in the greenhouse

All sanitiser treatments, and the untreated, *Pythium* infested control showed a significant (P=0.05) reduction in fresh shoot mass in comparison to the untreated, uninfested control (Fig. 8). Phytex® dosed at 1ml.l^{-1} and Agral 90® dosed at 5mg.l^{-1} resulted in a significant

increase in fresh shoot mass when compared to the untreated, uninfested control, while copper sulphate dosed at 5mg.l^{-1} and Sporekill® dosed at 5mg.l^{-1} resulted in a significant decrease in shoot mass in comparison to the untreated, *Pythium* infested control.

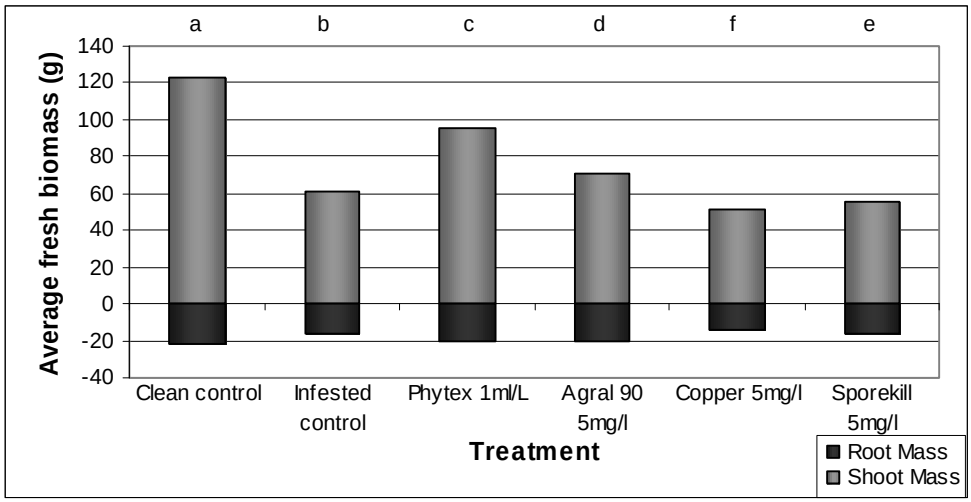


Figure 8: Effect of additional sanitisers at optimal dosages on yield of *Pythium* infested lettuce in a small scale graven bed hydroponic system in the greenhouse, over a period of 28d. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

Treatment with Sporekill® resulted in the highest level of *Pythium* in the nutrient solution at the end of the final week of the 28d growth period, which was significantly greater than the untreated, *Pythium* infested control (Fig. 9). Phytex® resulted in a significant reduction in *Pythium* incidence, while Agral 90® and copper sulphate treatments resulted in a complete eradication of *Pythium* in the nutrient solution, which was the same as the untreated, un-infested control.

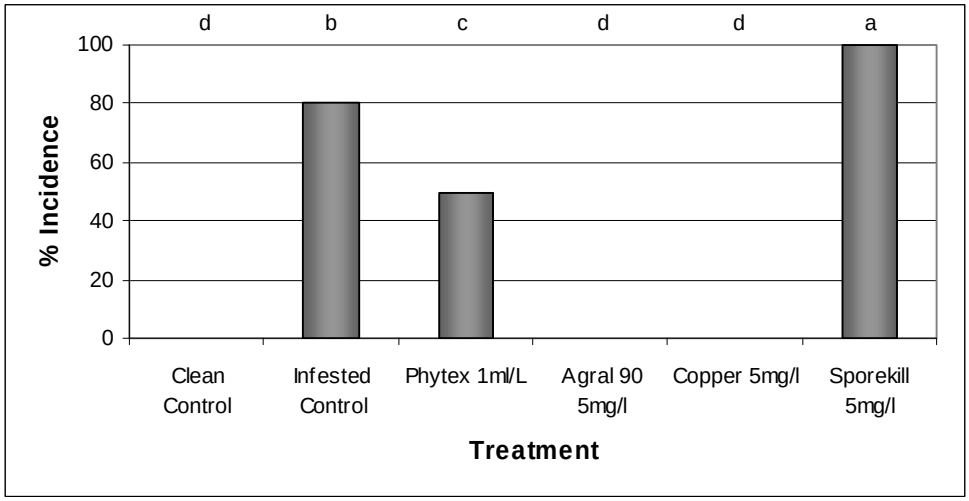


Figure 9: Effect of chemical treatments on *Pythium* incidence in recirculated nutrient solution at the end of the 28d of lettuce growth in a small scale gravel bed hydroponic system in the greenhouse. Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

5.4.3 Treatment comparisons in a semi-commercial gravel bed hydroponic field system – (multi-sanitiser trial)

5.4.3.1 Comparison of sanitisers in a semi-commercial scale gravel bed hydroponic system in the field

In two trials in the semi-commercial gravel bed hydroponic field system, only Phytex® dosed at 1ml.l^{-1} was able to achieve a significant (P=0.05) increase (37%) in fresh shoot mass over the untreated, *Pythium* infested control. Purogene® dosed at 10mg.l^{-1} was able to achieve a 7% improvement in mass compared to the untreated, *Pythium* infested control, yet this was not statistically significant (Figs. 10 and 11).

With the exception of Phytex®, which achieved the maximum lettuce yield, no sanitiser treatment was able to achieve growth levels equivalent to or significantly greater than the untreated, uninfested control.

Treatments with Prasin® and Fitosan®, both at 7.5mg.l^{-1}, yielded fresh shoot biomass significantly equivalent to the untreated, *Pythium* infested control (Fig. 10), while Agral 90® and Sporekill®, each applied at 5mg.l^{-1}, showed severe reductions in shoot mass of

15% and 20% respectively, which were not significantly different from the untreated, *Pythium* infested control (Fig. 11). Actsol® dosed at a 1:20 dilution showed the greatest yield reduction of 61% which was significantly different from both the untreated, *Pythium* infested control, as well as the untreated, uninfested control.

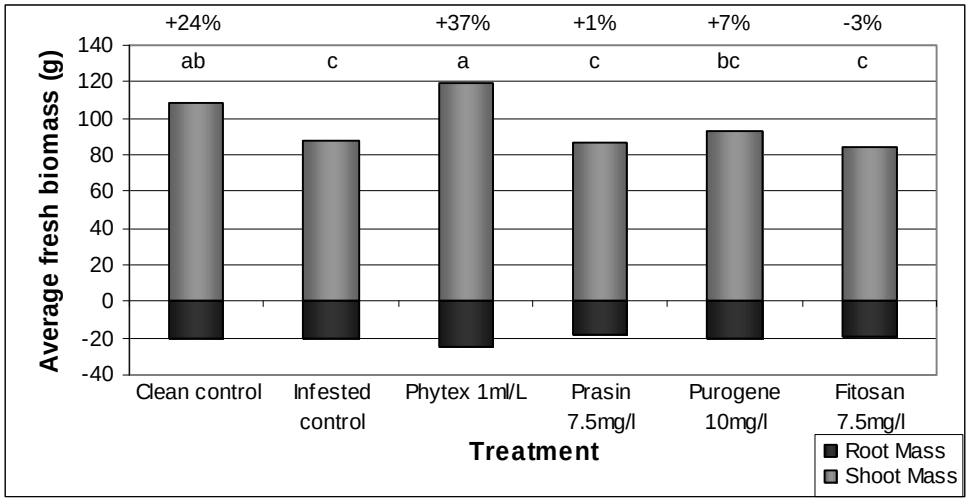


Figure 10: Effect of chemical treatments on yield of *Pythium* infested lettuce in a semi-commercial scale gravel bed hydroponic system in the field, after 42d growth (values at top indicate yield increase over infested control). Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

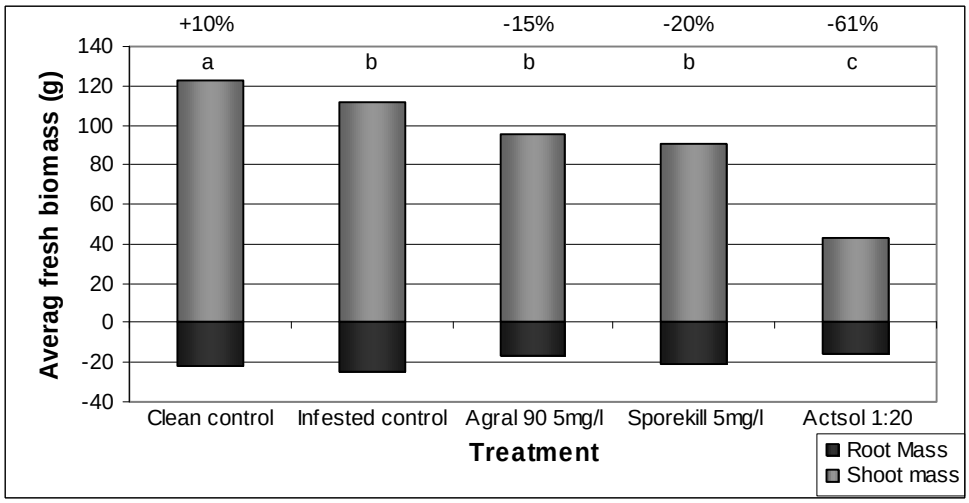


Figure 11: Effect of chemical treatments on yield of *Pythium* infested lettuce in a semi-commercial scale gravel bed hydroponic system in the field, after 42d growth (values at top indicate yield increase over infested control). Bars with the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

5.5 Discussion

In the greenhouse trials, Actsol® exhibited a trend of increasing yield with increasing dilution which is attributed to increased phytotoxicity at the higher concentrations causing phytotoxic stress and root damage with a related increase in susceptibility to disease. This was again confirmed in the comparison trial where a 1:20 dilution resulted in plant death. Only a 1:50 dilution resulted in a significant yield improvement over the *Pythium*-infested, untreated control, yet this was significantly lower than the untreated, uninfested control. This could be attributed to the fact that disease control was not complete and low levels of phytotoxicity being present, both factors preventing optimal growth. The high levels (not significantly different to the untreated, *Pythium* infested control) of *Pythium* inoculum recorded in the comparison trial further indicate that disease control was not maximal and was not affected by a reduction of *Pythium* in the nutrient solution but rather at an infection stage at root level.

Prasin® exhibited an inverse trend to all other sanitisers tested in the greenhouse trial where a significant yield increase over an untreated, *Pythium* infested control was only noted at the highest concentration tested (7.5mg.l^{-1}) with lower concentrations not appearing to have significant beneficial effects. This was as expected, since previous research showed that Prasin® was most effective against *Pythium* zoospores in suspension at a 7.5mg.l^{-1} concentration with a 10min exposure time, while not being as effective at lower concentrations. In the sanitiser comparison experiment, it was shown that *Pythium* infestation was significantly lowered in comparison to the untreated, *Pythium*-infested control, further validating the hypothesis that the yield improvement noted was due to a disease control effect. None of the treatments resulted in optimum growth which may have been due to a combination of inadequate disease control and various phytotoxic effects. This indicated that, as with Actsol®, there was both a disease control benefit as well as a phytotoxic effect. Unlike Actsol® the disease control appears to be as a result of inoculum reduction within the nutrient solution, which also explains the poor performance of the low concentration treatments where failure to improve plant yields may be due to the *Pythium* inoculum not being sufficiently reduced.

With the exception of Purogene® at a 50mg.l^{-1} concentration, both Purogene® and TecsaClor® (which have chlorine dioxide as an active ingredient) exhibited equivalent trends, which were similar to Actsol® treatments. An increase in concentration of these sanitisers resulted in lowered yields, which did not differ significantly. A 50mg.l^{-1} Purogene® treatment resulted in a significantly reduced yield. This is attributed to the higher phytotoxic effects of chlorine-dioxide at high concentrations and is similar to findings by Carillo *et al.* (1996) where chlorine dioxide application did not significantly reduce lettuce plant development under nursery conditions.

In the sanitiser comparison trial under greenhouse conditions it was shown that the 10mg.l^{-1} treatment of Purogene® was able to totally eradicate *Pythium* inoculum from the nutrient solution indicating that the growth improvement may be due to inoculum reduction in the nutrient solution. The increased concentrations would expectedly have the same effect on disease severity, yet the phytotoxic effects would be increased, preventing optimal growth. These results do not follow the expected trend directly since a dual benefit was expected at treatment with lower concentrations where disease incidence would be lowered or eradicated with a simultaneous growth enhancement as described in Chapter 4, with the growth enhancing aspect of low concentrations of chlorine dioxide also being described by Lee *et al.* (2004) and Pernezy *et al.* (2005). The reduced yield at the highest concentration, attributed to phytotoxicity, was expected and the phytotoxic nature of high concentrations of chlorine dioxide has also been previously described (Lee *et al.*, 2004; Pernezy *et al.*, 2005).

TecsaClor® was unable to achieve a significant improvement in yield over an untreated, *Pythium* infested control. As TecsaClor® previously exhibited minimal phytotoxicity (Chapter 4) and low levels of chlorine dioxide have been shown to be minimally phytotoxic (Carillo *et al.*, 1996) with a possible growth enhancing factor (Lee *et al.*, 2004 and Pernezy *et al.*, 2005) the failure of TecsaClor® to effect an improved yield may be due to poor disease control. In the sanitiser comparison experiment it was shown that TecsaClor® at 25mg.l^{-1} was unable to significantly reduce *Pythium* inoculum levels in the nutrient solution in comparison to the untreated, *Pythium* infested control. The lack of improvement in yield may have been due to minimal disease control, combined with minimal phytotoxicity and growth enhancing factors which resulted in the treatments being very similar to an untreated, *Pythium* infested control.

In one of the preliminary experiments comparing all sanitisers under greenhouse conditions the untreated, uninfested control showed poorer growth than an untreated, *Pythium* infested control. This was later determined by random root plating (data not shown) to be due to contamination of the uninfested control by *Pythium*. Actsol® at a 1:20 dilution was again shown to be phytotoxic to a level resulting in plant death. Purogene® and TecsaClor® demonstrated an expected trend, possibly due to phytotoxic effects where the Purogene® treatment resulted in reduced growth in comparison to the TecsaClor® treatment. Prasin® and Fitosan® did not conform to any expected trend since results were expected to be similar between the two treatments.

In the first successful comparison experiment the expected trends were observed for all the treatments with the exception of the Phytex® treatment which resulted in a significantly increased yield when compared to an untreated, uninfested control. Phytex® was also unable to eliminate *Pythium* inoculum from the hydroponic nutrient solution, although this was significantly reduced in comparison to levels noted in the untreated, *Pythium*-infested control. This was understandable because Phytex® is a systemic fungicide based on phosphorous acid, exerting disease control within the plant and plant roots (Fenn and Coffey, 1984) as opposed to directly affecting pathogen inoculum in the nutrient solution. Phosphorous acid has also been observed to have a growth stimulating effect on plants when applied at low concentrations (Chaluvaraju *et al.*, 2004) and this may have resulted in the maximum growth observed with Phytex® treatment.

Purogene®, Fitosan®, Prasin®, TecsaClor® and Actsol® resulted in progressively decreasing lettuce yields in ascending order, along with increasing *Pythium* inoculum presence in the nutrient solution. These results followed an expected trend noted in the previous trials where only Purogene® was able to effect a significantly increased yield over an untreated, *Pythium* infested control along with a total eradication of *Pythium* inoculum from the nutrient solution. Fitosan® and Prasin® (both having similar active ingredients) resulted in similar, insignificantly different, yields, while Fitosan® was unexpectedly shown to have the greater effect on *Pythium* inoculum. This may be attributed to the higher level of phytotoxicity exerted by Fitosan®, resulting in lowered yields even though disease control was more effective than Prasin® treatment. TecsaClor® and Actsol® were not significantly different from each other with neither able to significantly reduce *Pythium* inoculum levels in the nutrient solution, which might have resulted in the poor growth and yield in these

treatments. Furthermore, phytotoxic stresses caused by these treatments may have resulted in the significantly lowered yield when compared to the untreated, *Pythium* infested control as plants may have been more susceptible to disease.

In the second greenhouse comparison experiment Phytex® was again demonstrated to significantly increase lettuce yield (plant mass) over an untreated, *Pythium* infested control while not completely eliminating *Pythium* inoculum in the nutrient solution, although a significant reduction was achieved. Unlike the previous experiment no additional increase over an untreated, uninfested control was noted. Neither Agral 90®, Sporekill® or copper sulphate at 5mg.l^{-1} were able to improve lettuce yield over an untreated, *Pythium* infested control although both Agral 90® and copper sulphate were able to eliminate *Pythium* inoculum from the nutrient solution. This effect of Agral 90® on zoospores has previously been reported by Stanghellini and Tomlinson (1987) and Stanghellini *et al.* (1996). The low lettuce yield observed even though *Pythium* control was high, is probably due to the high levels of phytotoxicity of copper sulphate, as seen in Chapter 4 of this study, while growth reduction by non-ionic surfactants has been reported by Garland *et al*. (2004).

When tested over two experiments in a semi-commercial scale gravel bed hydroponic system in the field, the trends seen in previous experiments were again observed. Only Phytex® was able to achieve a significantly increased lettuce yield in comparison to the untreated, *Pythium* infested control, with a further insignificant improvement over an untreated, uninfested control.

In the semi-commercial field system, Phytex® and Purogene® treatments were able to improve lettuce yield over that of an untreated, *Pythium*-infested control, while Prasin® and Fitosan® achieved yields similar to this control. Agral 90®, Sporekill® and Actsol® showed yield decreases when compared to the untreated, *Pythium*-infested control. As previously shown, Purogene® was the only other treatment to result in an improved lettuce yield over an untreated, *Pythium*-infested control, although this improvement was not statistically significant. Following previous trends Prasin®, Fitosan®, Agral® and Sporekill® did not achieve a significantly different yield in comparison to the untreated, *Pythium*-infested control, while Actsol® again showed a significantly reduced yield.

Phytex® at 1ml.l^{-1} and Purogene® at 10mg.l^{-1} demonstrated the most consistent and positive yield improvements under both greenhouse and field conditions. This yield improvement may be due to two aspects where *Pythium* inoculum in the nutrient solution is reduced (or eliminated in the case of Purogene® treatment) along with a growth stimulation effect on the lettuce plants. Phytex® may also have a third aspect where disease control is effected by the systemic nature of this product. Both Phytex® and Purogene® are thus indicated as having beneficial effects when dosed into hydroponic nutrient solution.

Prasin®, Fitosan®, Agral® and Sporekill® treatments did not result in improved lettuce yields even though some measure of disease control was exerted. This may be due to a combination effect of benefits due to disease control coupled to yield reduction caused by the phytotoxic nature of these products. Although no direct benefit was seen in the current trial setup using only *Pythium*, addition of these sanitisers in commercial hydroponic systems may result in yield improvement due to general pathogen inoculum reduction in the nutrient solution and the *Pythium* control may be beneficial under stress conditions when plants are more susceptible to infection.

Actsol® consistently showed poor inoculum control from the nutrient solution along with decreased yield mass when compared to an untreated, *Pythium* infested control, indicating that the tested concentrations are not suited for application into hydroponic nutrient solutions and a negative impact is observed.

5.6 References

Allende, A., Tomas-Barberan, F.A. and Gil, M.I. 2006. Minimal processing for healthy traditional foods. Trends in Food Science and Technology 17: 513–519.

Carrillo, A., Puente, M.E. and Bashan, Y. 1996. Application of diluted chlorine dioxide to radish and lettuce nurseries insignificantly reduced plant development. Ecotoxicology and Environmental Safety 35: 57-66.

Chaluvaraju, G., Basavaraju, P., Shetty, N.P., Deepak, S.A., Amruthesh, K.N. and Shetty, H.S. 2004. Effect of some phosphorous-based compounds on control of pearl millet downy mildew disease. Crop Protection 23: 595–600.

Date, S., Terabayashi, S., Kobayashi, Y. and Fujime, Y. 2005. Effects of chloramines concentration in nutrient solution and exposure time on plant growth in hydroponically cultured lettuce. Scientia Horticulturae 103: 257-265.

Fenn, M. and Coffey, M.D. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-A1 and phosphorous acid. Phytopathology 74: 606-611.

Fravela, D.R. and Larkin, R.P. 2002. Reduction of *Fusarium* wilt of hydroponically grown basil by *Fusarium oxysporum* strain CS-20. Crop Protection 21: 539-543.

Garland, J.L., Levine, L.H., Yorio, N.C. and Hummerick, M.E. 2004. Response of graywater recycling systems based on hydroponic plant growth to three classes of surfactants. Water Research 38: 1952-1962.

Grimm, G.R. and Alexander, A.F. 1973. Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. Phytopathology 63: 540-541.

Lee, S., Gray, P.M., Dougherty, R.H. and Kang, D. 2004. The use of chlorine dioxide to control *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples. International Journal of Food Microbiology 92: 121-127.

Migliore, L., Cozzolino, S. and Fiori, M. 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52: 1233-1244.

Paulitz, T.C., Zhou, T. and Rankin, L. 1992. Selection of rhizosphere bacteria for biological control of *Pythium aphanidermatum* on hydroponically grown cucumber. Biological Control 2: 226-237.

Pernezny, K., Raid, R.N., Havranek, N. and Sanchez, J. 2005. Toxicity of mixed-oxidant electrolyzed oxidizing water to *in vitro* and leaf surface populations of vegetable bacterial pathogens and control of bacterial diseases in the greenhouse. Crop Protection 24: 748-755.

Roux, C. and Botha, W.J. 1997. An introduction to the Pythiaceae in South Africa. Agricultural Research Council: Plant Protection Research Institute, Roodeplaat, Pretoria.

Runia, W. 1994. Disinfection of recirculation water from closed cultivation systems with ozone. Acta Horticulturae 361: 388-396.

Saba, A. and Messina, F. 2003. Attitudes towards organic foods and risk/benefit perception associated with pesticides. Food Quality and Preference 14: 637-645.

Schwartzkopf, S.H., Dudzinski, D. and Minners, R.S. 1987. The effects of nutrient solution sterilisation on the growth and yield of hydroponically grown lettuce. HortScience 22: 873-874.

Schwarz, D. and Grosch, R. 2003. Influence of nutrient solution concentration and a root pathogen (*Pythium aphanidermatum*) on tomato root growth and morphology. Scientia Horticulturae 97: 109-120.

Song, W., Zhou, L., Yang, C., Cao, X., Zhang, L. and Liu, X. 2004. Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. Crop Protection 23: 243-247.

Stanghellini, M.E. and Kronland, W.C. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by *Pythium dissotocum*. Plant Disease 70: 1053-1056.

Stanghellini, M.E. and Rasmussen, S.L. 1994. Hydroponics: A solution for zoosporic pathogens. Plant Disease 78: 1129-1138.

Stanghellini, M.E. and Tomlinson, J.A. 1987. Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. Phytopathology 77: 112-114.

Stanghellini, M.E., Kim, D.H., Rasmussen, S.L. and Rorabaugh, P.A. 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. Plant Disease 80: 1113-1116.

Vanachter, A. 1995. Development of *Olpidium* and *Pythium* in the nutrient solutions of NFT grown lettuce, and possible control methods. Acta Horticulturae 382: 187-196.

Zinnen, T.M. 1988. Assessment of plant diseases in hydroponic culture. Plant Disease 72: 96-99.

5.7 Plate II

A: Semi-commercial gravel bed hydroponic system in the field

B: Semi-commercial gravel bed hydroponic system in the field, planted with butter lettuce seedlings

C: Commercial gravel bed hydroponic system planted with butter lettuce

CHAPTER 6

General Discussion

6.1 Discussion

Pythium zoospores in aqueous suspension were exposed to concentration ranges of Actsol®, Prasin®, Purogene® and TecsaClor®, where the exposure resulted in zoospores being inactivated or destroyed (Chapter 3).

Pythium zoospore survival in aqueous suspension was reduced by 80% or greater within 10min by the following treatments: Actsol® at a 1:10 dilution; Prasin® at a concentration of 5mg.l^{-1}; Purogene® at a concentration of 10mg.l^{-1} and TecsaClor® at a concentration of 25mg.l^{-1}. Additional tests also showed that Fitosan® at a 7.5mg.l^{-1} concentration, Agral 90®, and copper sulphate at concentrations of 1mg.l^{-1} and Sporekill® at a concentration of 5mg.l^{-1} were also able to reduce *Pythium* zoospore levels by 80% or greater within a 10min exposure time. Although Agral 90® proved to be effective against *Pythium* zoospores, increased concentrations of this sanitiser resulted in an unexpected reduction in efficacy. This correlated with results of Stanghellini and Tomlinson (1987), who showed a similar reduced efficacy of Agral 90® with increasing concentrations. This may be due to the higher concentrations of Agral 90® causing a rapid encystment of the zoospores with an associated decreased sensitivity. This rapid encystment has been described by Morris and Ward (1992), although the decreased sensitivity to chemicals has not. From the above results, it can be summarised that all the tested chemicals dosed at low concentrations had good efficacy against *Pythium* zoospores in a water volume. This indicated that these sanitisers could have a beneficial use in *Pythium* infested hydroponic nutrient solutions.

Parallel trials (Chapter 3) showed that among *Pythium* zoospores, *Fusarium* conidia and *Ralstonia* cells, the *Pythium* zoospores proved to be the most sensitive to the effects of the sanitisers, while *Ralstonia* cells were shown to be the least sensitive in all experiments. For *Fusarium* conidia and *Ralstonia* cells, effective reduction in inoculum concentration was only noted at sanitiser concentrations much higher than required for the *Pythium* zoospores. This was expected and proves the hypothesis that increasing complexity of the outer cell barrier will result in decreasing sensitivity to sanitisers.

Ralstonia cells, being of a Gram negative form (have the most complex structure comprising of cell membranes and a cell wall, with the possibility of an outer capsule) (Agrios, 2005; Claessens *et al.*, 2006) was thus least sensitive to the sanitiser treatments due to a multi-barrier protection. As both *Fusarium* and *Ralstonia* required much higher concentrations to achieve similar efficacy, it is also surmised that the presence of a cell wall (Claessens *et al.*, 2006) results in a larger degree of resistance to the effects of the sanitisers, while not necessarily making the pathogen immune.

The phytotoxicity studies (Chapter 4) showed that Actsol® and copper sulphate were most phytotoxic to both cucumber and lettuce plants. Phytotoxic effects of electrochemically activated water (Actsol® solution) have been described previously (Pernezny *et al.*, 2005). The level of phytotoxicity observed in the current study was far greater than expected, possibly due to the fact that in this study the plant roots were directly exposed to Actosl® for an extended time, allowing a cumulative phytotoxic effect to manifest. Copper sulphate phytotoxicity was extreme even at low concentrations, which was to be expected and also similar to the effects observed by Vinit-Dunand *et al.* (2002), who demonstrated that cucumber plants are sensitive to copper at these low concentrations with the resulting phytotoxic effects of growth retardation and leaf discolouration being similar to those observed in this study.

The quaternary ammonium (QAC) based sanitisers (Prasin®, Sporekill® and Fitosan®) all demonstrated phytotoxic effects as retardation of foliar and root growth and development in cucumbers, with Fitosan® being less phytotoxic than Sporekill®. Prasin® demonstrated similar trends under both the cucumber and lettuce model where increasing levels of phytotoxicity were observed at increasing concentrations, with the exception of an anomalous observation where a 100mg.l^{-1} treatment in the lettuce model resulted in a reduced level of growth retardation. The reason for this is unclear and this might be attributed to experimental error. Phytotoxic effects of QAC's have been described on a variety of crops at similar dosage levels (Nalecz-Jawecki *et al.*, 2003) and the above results demonstrated an expected trend.

Prasin® further demonstrated less phytotoxic effects in the lettuce model than in the cucumber model which indicate that the lettuce plants have a higher tolerance to the phytotoxic effects. A previous study has shown that sanitisers of this nature can break

down when introduced into hydroponic systems (Garland *et al.*, 2000; 2004). This phenomenon could have resulted in the lowered phytotoxicity noted on the lettuce grown in the recirculating system, where such breakdown would be more likely.

The lack of severe phytotoxic effects, such as plant death, at the tested concentrations (Chapter 4), combined with the results that these concentrations are also able to reduce the levels of pathogenic inoculum from a water volume (Chapter 3), indicated that the sanitisers could have a beneficial effect if dosed into the nutrient solution of a *Pythium* infested hydroponic system since *Pythium* inoculum should be reduced, thus reducing disease incidence and severity, while minimal negative effects due to phytotoxic interactions would be experienced.

When tested in an experimental gravel bed hydroponic system under greenhouse controlled environmental conditions (Chapter 5), Purogene® at 10mg.l^{-1}, Prasin® at 7.5mg.l^{-1} and Actsol® at a 1:20 dilution, in descending order, were able to improve yield of lettuce plants over an untreated, *Pythium* infested control. TecsaClor® proved to be an exception to this trend where no increase in yield was observed. None of these treatments were able to achieve a similar yield mass as an untreated, uninfested control, indicating that either disease control was insufficient, or the phytotoxic effects described in Chapter 4 were causing a reduction in maximum potential yield, or a combination of these two factors was being experienced.

Comparison studies in a gravel bed hydroponic system under greenhouse conditions (Chapter 5) showed that treatment of the nutrient solution with Purogene® at 7.5mg.l^{-1} resulted in the most beneficial effects. *Pythium* zoospore levels in nutrient solutions treated with Purogene® were completely eradicated while lettuce yield was significantly increased in comparison to the untreated, *Pythium* infested control. This eradication of the *Pythium* inoculum from the nutrient solution, reported in Chapter 2, combined with the growth enhancing effects described in Chapter 4, correlated well with the results obtained in the current trial. Fitosan® at 7.5mg.l^{-1} and Prasin® at 7.5mg.l^{-1} reduced *Pythium* zoospore incidence in the nutrient solution although no significant improvement in yield was observed, indicating that the reduction in disease was probably overshadowed by the negative phytotoxic effects of these products. TecsaClor® at 25mg.l^{-1} did not achieve any growth improvement and was unable to reduce the levels of *Pythium* in the nutrient

solution, indicating that this product is not suited for use in the more complex nature of recirculating hydroponic systems. Although TecsaClor® at 25mg.l^{-1} previously showed a growth improvement of lettuce (Chapter 4) and was able to reduce the levels of *Pythium* zoospores in a water volume (Chapter 3), these were both in basic systems and the results were marginal, thus the current results were not unexpected. Actsol® at a 1:20 dilution was able to eliminate *Pythium* zoospores from the nutrient solution, although the extensive phytotoxic effects of this sanitiser (described in Chapter 4) resulted in a significantly decreased lettuce yield. This indicates that this sanitiser would not be acceptable for use in hydroponic systems of this nature. Agral 90® treated nutrient solution at 5mg.l^{-1} showed no *Pythium* incidence, and lettuce plants showed a significant improvement in yield over an untreated, *Pythium* infested control. This correlates well with previous findings by Stanghellini *et al.* (1996) and De Jonghe *et al.* (2005) who obtained similar levels of *Pythium* zoospore reduction and associated growth improvements.

Results obtained in the field-scale gravel bed hydroponic system (Chapter 5) differed from those obtained in the greenhouse system (Chapter 5) where a far lower level of *Pythium* control was achieved, although the trends exhibited by all the tested sanitisers closely mirrored those observed in the greenhouse system. Only Purogene® at 7.5mg.l^{-1} was able to achieve some control and resulted in an increase in lettuce yield from this system when compared to the untreated, *Pythium* infested control, although not of significant levels. Prasin® at 7.5mg.l^{-1} and Fitosan® at 7.5mg.l^{-1} were unable to effect a change in yield when compared to the untreated, *Pythium* infested control, while Agral 90® at 5mg.l^{-1}, Sporekill® at 5mg.l^{-1} and Actsol® at a 1:20 dilution resulted in a decrease in yield. The data indicates that these latter three treatments have a negative phytotoxic effect outweighing any positive benefits due to disease control. Therefore these treatments are not considered applicable for use in this hydroponic system.

In both the greenhouse system as well as the field system, none of the sanitisers tested were able to achieve the same measure of growth and yield improvement as the commercial fungicide, Phytex®, used at the manufacturer's recommended dosage rate of 1ml.l^{-1}, even though *Pythium* inoculum was not eradicated from the nutrient solution. This was most likely due to the fact that Phytex® is a systemic fungicide having a specific effect on pythiaceous fungi and acting primarily within the plant (Fenn and Coffey, 1984).

Phosphorous acid (the active ingredient of Phytex®) has also been reported to have a growth enhancing effect when applied at low concentrations (Chaluvaraju *et al.*, 2004).

The five sanitisers tested were able to effectively reduce *Pythium*, *Fusarium* and *Ralstonia* from a water volume at reasonably low concentrations. Although some phytotoxicity was observed this was not of an extreme nature at the optimum concentrations selected for testing. When tested for *Pythium* disease control in an experimental system in a greenhouse positive results were obtained by treatments with Purogene® (10mg.l^{-1}), Prasin® (7.5mg.l^{-1}) and Fitosan® (7.5mg.l^{-1}). TecsaClor® dosed at a concentration of 25mg.l^{-1} did not result in any improvement in growth or disease control, while Actsol® at a dilution of 1:20 eliminated *Pythium* but was unable to improve growth, presumably due to phytotoxic effects. Purogene® at 10mg.l^{-1} was the only sanitiser to effectively improve growth and reduce disease in a field-scale system

Although *Pythium* disease control in a field scale recirculating gravel hydroponic system was not achieved as would be expected from results seen in Chapter 3, this does not preclude the use of sanitisers in these systems. Dosage of the nutrient solution with Purogene® at 10mg.l^{-1} and Prasin® at 7.5mg.l^{-1} could prevent rapid and devastating outbreaks of various non-*Pythium* diseases, specifically during times of plant stress and associated increased susceptibility to disease. In a commercial hydroponic system the general reduction of a wide range of disease propagules, not limited only to *Pythium*, may result in a yield improvement above the norm.

Sanitisers such as Actsol® which are touted to be environmentally friendly with no resultant residues, could also be targeted for use in hydroponic systems where the nutrient solution is not re-used directly or is re-directed for alternate uses such as field irrigation, where prolonged direct exposure to roots is avoided.

The sanitisers tested in this study may also result in beneficial effects in bag and ebb-and-flow type hydroponic systems where the sanitiser / root interaction is minimised, thus possibly reducing the level of phytotoxicity while still reducing pathogen levels in the nutrient solution. Other hydroponic crops may also demonstrate lesser phytotoxic effects than the lettuce crop chosen for this study.

None of the sanitisers were able to achieve a level of *Pythium* disease control similar to a commercial fungicide (Phytex®), yet this does not give a complete indication of the benefits of the use of these sanitisers, since a sanitiser will reduce the levels of most pathogens due to the general sanitising effect, while most registered fungicides or pesticides do not have the same broad-spectrum sanitation action.

Further research using multi-pathogen infested hydroponic systems, similar in setup to those described in Chapter 5, would aid to confirm the true benefits of these sanitisers at a scientific level, while application into a commercial hydroponic system cultivating a variety of crops would identify the wider range of benefits which these sanitisers could offer a commercial hydroponic grower. Additionally these sanitisers should also be investigated using a variety of hydroponic systems, cultivating a single crop, to determine whether phytotoxic effects would be lessened and beneficial effects increased depending on the type of hydroponic system.

A further useful study for near term application would be a viability assessment on an agricultural economic basis to determine whether the benefits demonstrated by Phytex® and Purogene® in Chapter 5 would be economically viable and beneficial to the average commercial grower. This would primarily be the case if income due to yield enhancement and disease control exceeded the direct (product cost) and hidden (transport, storage, training, time and application) costs of these products. What may not be addressed in such a study is again the potential cost saving due to prevention of a disease outbreak which could cause severe yield reductions or even total crop loss.

6.2 References

Agrios, G. N. 2005. Plant Pathology. 5th Ed. Elsevier Academic Press. UK. pp 439 & 619.

Chaluvaraju, G., Basavaraju, P., Shetty, N.P., Deepak, S.A., Amruthesh, K.N. and Shetty, H.S. 2004. Effect of some phosphorous-based compounds on control of pearl millet downy mildew disease. Crop Protection 23: 595–600.

Claessens, J., van Lith, Y., Laverman, A.M. and Van Cappellen, P. 2006. Acid–base activity of live bacteria: Implications for quantifying cell wall charge. Geochimica et Cosmochimica Acta 70: 267–276.

De Jonghe, K., De Dobbelaerea, I., Sarrazynb, R. and Höfte, M. 2005. Control of brown root rot caused by *Phytophthora cryptogea* in the hydroponic forcing of witloof chicory (*Cichorium intybus* var. *foliosum*) by means of a nonionic surfactant. Crop Protection 24: 771-778.

Fenn, M. and Coffey, M.D. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-A1 and phosphorous acid. Phytopathology 74: 606-611.

Garland, J.L., Levine, L.H., Yorio, N.C., Adams, J.L. and Cook, K.L. 2000. Graywater processing in recirculating hydroponic systems: Phytotoxicity, surfactant degradation, and bacterial dynamics. Water Research 34: 3075-3086.

Garland, J.L., Levine, L.H., Yorio, N.C. and Hummerick, M.E. 2004. Response of graywater recycling systems based on hydroponic plant growth to three classes of surfactants. Water Research 38: 1952-1962.

Morris, P.F. and Ward, E.W.B. 1992. Chemoattraction of zoospores of the soybean pathogen, *Phytophthora sojae*, by isoflavones. Physiological and Molecular Plant Pathology 40: 17-22.

Nalecz-Jawecki, G., Grabinska-Sota, E. and Narkiewicz, P. 2003. The toxicity of cationic surfactants in four bioassays. Ecotoxicology and Environmental Safety 54: 87-91.

Pernezny, K., Raid, R.N., Havranek, N. and Sanchez, J. 2005. Toxicity of mixed-oxidant electrolyzed oxidizing water to *in vitro* and leaf surface populations of vegetable bacterial pathogens and control of bacterial diseases in the greenhouse. Crop Protection 24: 748-755.

Stanghellini, M.E. and Tomlinson, J.A. 1987. Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. Phytopathology 77: 112-114.

Stanghellini, M.E., Kim, D.H., Rasmussen, S.L. and Rorabaugh, P.A. 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. Plant Disease 80: 1113-1116.

Vinit-Dunand, F., Epron, D., Alaoui-Sosse, B. and Badot, P. 2002. Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. Plant Science 163: 53-58.

APPENDIX I:

A. Solution 1: Static hydroculture nutrient solution

Agrosol' O	at 0.9g.l^{-1}	(Fleuron, South Africa)
Micromax	at 0.3g.100l^{-1}	(Fleuron, South Africa)
$Ca(NO_3)_2$	at 0.6g.l^{-1}	(Ocean Agriculture, South Africa)

Solution 2: Greenhouse and Field scale nutrient solution mix

Hydrogro	at 0.45g.l^{-1}	(Ocean Agriculture, South Africa)
Hortical (Calcium Nitrate)	at 0.60g.l^{-1}	(Ocean Agriculture, South Africa)

B. Information on sanitisers used in the current study.

Name used	Active ingredient	Type of product	Supplier	Notes & Formulation	Referenced in
Agral 90®	90% m.m^{-1} alkaryl polyglycol ether	Agricultural surfactant	Kynoch chemicals	Nonnionic, SL	3.4.6
Actsol®	Mixed oxidant & metastable species e.g hypochlorous acid, hypochlorite, chlorate, perchlorate (180mg.l^{-1} total)	Electro chemically activated water	Radical Waters	Anionic, SL	3.4.1; 4.4.1.1; 4.4.2.1; 5.4.1.1; 5.4.2.1; 5.4.2.2
Copper sulphate	Copper (II) sulphate pentahydrate supplying Cu^{2+}	Chemical	Merck		3.4.7; 4.4.1.6
Fitosan® (F10 Agricultural)	Quaternary ammonium & biguanide (5.8%)	Agricultural sanitiser	Health & Hygiene	Cationic, SL	3.4.5; 4.4.1.5; 5.4.2.1; 5.4.2.2
Phytex® (marketed as Phytex 200SL)	Potassium phosphonate (200g.l^{-1})	Fungicide	Horticura	SL	5.4.2.1; 5.4.2.2
Prasin® (marketed as Prasin Agri®)	Polymetric biguanide hydrochloride & quaternary ammonium (7%)	Agricultural sanitiser	SIDL cc	Cationic, SL	3.4.2; 4.4.1.2; 4.4.2.2; 5.4.1.2; 5.4.2.1; 5.4.2.2
Purogene® (with activator)	Chlorine dioxide (3g.l^{-1} max)	General & agricultural sanitiser	BTC products & services	Nonionic, SL	3.4.3; 4.4.1.3; 4.4.2.3; 5.4.1.3; 5.4.2.1; 5.4.2.2
Sporekill®	N,N-Didecyl N,N-dimethyl ammoniumchloride (12%)	Agricultural sanitiser	Hygrotech Seed	Nonionic, SL	3.4.8; 4.4.1.7
TecsaClor®	Chlorine dioxide (2-3g.l^{-1})	General & agricultural sanitiser	BTC products & services	Nonionic, SL	3.4.4; 4.4.1.4; 4.4.2.4; 5.4.1.4; 5.4.2.1; 5.4.2.2

C. Information on supplier referenced in the current study.

Company name	**Supplier of:**	**Address**	**Telephone #**
BTC Products & Services	Purogene® TecsaClor®	P.O. Box 1611, Randburg, 2125, South Africa	011 794 9239
Fleuron (PTY) Ltd.	Agrasol'O Micromax	Unit 2, Kroft Park, Lower Germiston, Heriotdale, Germiston, P.O.Box 31245, Braamfontein 2017, South Africa	011 626-2928
Health & Hygiene	Fitosan®	Unit 2, Marvil Park, 84 Ratchet Avenue, Stormill, Roodepoort, 1709, South Africa	011 474 1668
Hydrotec	Lettuce Seedlings	Middel Avenue, Uitzicht, Gauteng, South Africa	011 376 2910
Hygrotech SA (PTY) Ltd.	Cucumber seed Sporekill®	P.O.Box 17220, Pretoria North, Gerard Braak Street, Pyramid, 0120, South Africa	012 545 8000
Kynoch Chemicals (PTY) Ltd.	Agral 90®	272 Pretoria Avenue, Ferndale, Randburg, 2125, South Africa	011 787-0419
Lowveld Agrochem*	Prasin Agri®	PO Box 32462, Glenstantia, Pretoria 0010, South Africa	012 998 5909
Merck SA	Copper (II) Sulphate Culture media	1 Friesland Drive, Longmeadow Business Estate South, Modderfontein 1640, South Africa	011 372 5000
Ocean Agriculture (PTY) Ltd.	Hortical ($Ca(NO_3)_2$) Hydrogro	P.O. Box 741, Muldersdrift, 1747, Gauteng, South Africa	011 662 1947
Radical Waters	Actsol®	19 Indianapolis crescent, Kyalami Business Park, Kyalami, 1684, Midrand, South Africa	011 466 0610
SIDL cc*	Prasin®	533 Jonathan St, Waterkloof Glen, Pretoria OR 47 Verwoerd Street, Pierre van Ryneveld, 0045, South Africa	012 9934265

* Note that Prasin Agri® is the current trade name for Prasin® and is distributed by Lowveld Agrochem.

D. Poster presentations.

Bagnall, R.C., and Labuschagne, N. 2003. Control of *Pythium* in hydroponic systems by means of water treatment with sanitisers. 41st Congress of the South African Society for Plant Pathology (SASPP). Bloemfontein.

Bagnall, R.C., and Labuschagne, N. 2004. Control of *Pythium* in hydroponic fertigation water by means of chemical sanitisers. 42nd Congress of the South African Society for Plant Pathology (SASPP). Cathedral Peak,