THE RELATIVE EFFECTIVENESS OF HOMEOPATHIC PREPARATIONS OF *Pythium spp.* COMPARED TO PREVICUR® (PROPAMOCARB), IN THE CONTROL OF *Pythium* ROOT ROT (DAMPING-OFF) IN CABBAGE AND CUCUMBER SEEDLINGS

by

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I, Jo-Anne Basson, declare that this dissertation represents my own work.

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Dedicated to Len and Val, my earthly parents,

and to my Father in Heaven.

Everything I am and ever hope to be is in Him alone, through Jesus Christ of Nazareth.

"Though the fig tree does not bud and there are no grapes on the vines; though the olive crop fails and the fields produce no food; though there are no sheep in the pen and no cattle in the stalls; yet will I rejoice in the LORD, I will be joyful in God my Saviour.

The Sovereign LORD is my strength; He makes my feet like the feet of a deer,

He enables me to go on the heights."

Habbakuk 3:17-19

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ABSTRACT

The purpose of this study was to expand on the foundations laid by previous homeopathic research, using controlled agricultural experiments as an objective disease system. More specifically, these trials were to investigate and evaluate the efficacy of a fungicide (Previcur®) against homeopathic preparations of a plant pathogenic fungus, *Pythium*, on seedlings infected with this fungus.

Pythium is a worldwide problem and continues to cause substantial crop losses. Few chemical methods are available to control this disease and alternatives are being sought which are more cost-effective, safer for the environment and do not lead to pathogenic resistance. Relatively few trials using Homeopathy in plant pathology have been done to date. Furthermore, many of the earlier trials proved to have little scientific credibility, due to poor methods and insufficient data. More recent trials have shown interesting results, which have opened the doors for further investigation.

This study compared the effects of Previcur® against Homeopathic preparations of *Pythium*, on infected cabbage and cucumber seedlings. There were 28 trays for each crop and seven treatments per crop: four Homeopathic treatments (9CH, 15CH, 30CH and 200CH), two controls (one inoculated and the other un-inoculated) and Previcur®. The seedlings were treated twice weekly, using a 2.5ml drench per seedling, and were harvested three weeks after planting. The trial was run twice, in succession.

Based on the nature of *Pythium* and the disease it causes, four parameters were measured in this trial. To assess the germination rate, the seedlings were counted three days after planting, to establish how many had broken the surface soil. To assess survival rate, the seedlings were counted at the time of harvesting. To assess growth, the seedlings were initially weighed wet (immediately after harvesting) and then dry (after 24 hours in an oven at 50°C).

The data was analysed by two methods: the General Linear Models Procedure (GLMP) produced ANOVA tables, which compared the variation between the four replicates of each of the seven treatments. The data was also analysed with the

Student Newman Keuls (SNK) test, to determine the variation between the seven treatments.

The results showed that Previcur® consistently produced the best or second best results, in terms of mean wet and dry weights, which was evident in the statistical analysis. However, a noticeable trend emerged with the 30CH treatment: it provided the lowest mean weights in seven of the eight parameters measured, while showing evidence of consistent statistical significance from the other treatments throughout both trials. The implications were that this treatment had either enhanced the disease process or had suppressed plant growth.

This trial raised issues regarding the effects of homeopathy on disease, as well as the internal mechanisms of disease control within plant structures. Further research into this area is encouraged, to further the platform already laid by statistically valid trials done thus far.

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DEFINITION OF TERMS

Homeopathy

Homeopathy is a therapeutic method which clinically applies the "Law Of Similars" and which uses medicinal substances in weak or infinitesimal doses (Jouanny 1991).

Isopathy

Treatment of disease by means of the presumed exopathic or endopathic causal agent, or by a product of the manifestation of the same disease (Gaier 1991).

Isotherapeutic agents

These are preparations also called "Homeopathized Allopathica" (Tautopathics) which are defined as homeopathic remedies made from toxic medicines to be used to treat an iatrogenic condition or in that standard method according to similarity of the disease and drug pictures (Gaier 1991).

Law of Similars

The parallel action between the toxicological action of a substance and its therapeutic action (Jouanny 1991).

Nosode

Named from the Greek "nosos" which means disease. Defined as a potency prepared from pathological material – sputum, blood, faeces, pus, neoplastic tissue, etc. – obtained from a patient suffering a particular disease, and usually containing the organism characteristic of the disease (Harling 1974).

Potency

A state of altered remedial activity to which a drug is taken by means of a measured process of deconcentration and the introduction of kinetic energy through succussion or trituration (Gaier 1991).

Potency - Centesimal

A homeopathic potency scale, introduced by Hahnemann, in which one part of mother tincture is added to 99 parts of diluent which is subjected to vigorous shaking or grinding, known as succussion or trituration, respectively. Each successive

decimal potency refers to the number of successive one in ten dilutions (Gaier 1991).

Pharmacopoeia

An authentic reference work containing monographs of medicines and other therapeutic agents. It also contains specifications of the sources and standards for the strengths and purity of the base substances and mother tinctures, formulae and methods of preparation of these substances as well as descriptions of processes for the testing of starting materials (Frazer 1992).

Succussion

The action of shaking up vigorously a liquid dilution of homeopathic medicine in its vial or bottle, where each stroke ends with a jolt, usually by pounding the hand engaged in the shaking action against the other palm (Gaier 1991).

CHAPTER ONE:

Introduction

1.1 THE AIM OF THE STUDY

The aim of this study was to investigate the relative effectiveness of homeopathic preparations of *Pythium* as opposed to the conventionally used fungicide Previcur® (propamocarb), in the control of damping-off in cabbage and cucumber seedlings, in terms of growth (germination, survival/wire-stem, wet weight and dry weight).

1.2 THE STATEMENT OF THE OBJECTIVES

1.2.1. The first objective

The first objective of this study was to investigate the relative effectiveness of homeopathic preparations of *Pythium* 9CH as opposed to Previcur®, in the control of damping-off in cabbage and cucumber seedlings, in terms of growth (germination, survival/wire-stem, wet weight and dry weight).

1.2.2 The second objective

The second objective of this study was to investigate the relative effectiveness of homeopathic preparations of *Pythium* 15CH as opposed to Previcur®, in the control of damping-off in cabbage and cucumber seedlings, in terms of growth (germination, survival/wire-stem, wet weight and dry weight).

1.2.3 The third objective

The third objective of this study was to investigate the relative effectiveness of homeopathic preparations of *Pythium* 30CH as opposed to Previcur®, in the control of damping-off in cabbage and cucumber seedlings, in terms of growth (germination, survival/wire-stem, wet weight and dry weight).

1.2.4 The fourth objective

The fourth objective of this study was to investigate the relative effectiveness of homeopathic preparations of *Pythium* 200CH as opposed to Previcur®, in the control

of damping-off in cabbage and cucumber seedlings, in terms of growth (germination, survival/wire-stem, wet weight and dry weight).

1.3 THE STATEMENT OF THE HYPOTHESES

1.3.1 The first hypothesis

It was hypothesised that in terms of germination, the homeopathic treatments would yield significantly different results compared to the fungicide (Previcur®), the inoculated control and the un-inoculated control.

1.3.2 The second hypothesis

It was hypothesised that in terms of survival, the homeopathic treatments would yield significantly different results compared to the fungicide (Previcur®), the inoculated control and the un-inoculated control.

1.3.3 The third hypothesis

It was hypothesised that in terms of dry weight, the homeopathic treatments would yield significantly different results compared to the fungicide (Previcur®), the inoculated control and the un-inoculated control.

1.3.4 The fourth hypothesis

It was hypothesised that in terms of wet weight, the homeopathic treatments would yield significantly different results compared to the fungicide (Previcur®), the inoculated control and the un-inoculated control.

CHAPTER TWO:

Review of the Related Literature

2.1 FUNGAL PLANT DISEASE

Fungi are predominantly microscopic organisms and of the more than 100 000 known species, approximately 10 000 cause disease in plants. Most fungi have a filamentous body called the mycelium, which branches out in various directions, with the individual branches known as hyphae. The primary means of fungal reproduction is through the dissemination of spores, which are reproductive bodies which can be formed asexually (budding off) or as a product of sexual fertilization. Spore dissemination is generally passive dispersal either through wind, water, soil, animals and humans, thus making pathogen control difficult (Agrios 1997).

Plant pathogenic fungi invade plants in various ways:

- *Obligates* live off the host plant without killing it, and then live in one debris phase. They do not invade the soil.
- *Semi-saprophytes* (e.g. *Pythium sp.)* live off the host plant until it dies, and then live in the soil and plant debris.
- *Hemi-biotrophs* live off the host plant and once it dies, they then live off debris only i.e. not in the soil.
- *Necrotrophs* live in the soil, attacking and killing their hosts, or portions of host tissues on which they live.
- *Saprophytes* are mostly found in the soil, living off debris, but they will invade a host plant should one become available i.e. opportunistic pathogens (Laing 2001).

Pathogenic fungi can cause local or general symptoms when invading the host plant. In general, the most common pattern is general necrosis (killing of plant tissue) which results in stunting (either of the entire plant or certain organs). The effects range from leaf spots and scabs to root rot and damping-off (rapid death and collapse of very young seedlings). Other pathogenic fungi can cause excessive growth of some plant organs, some of the effects include club root to leaf curls, which inadvertently cause stunting of the plant as a whole (Agrios 1997).

2.1.1 The fungus, *Pythium sp.* (see Figure 1)

This fungus is classified as a fungus-like Organism or a Pseudofungus. It falls into the Kingdom Chromista → Phylum Oomycota → Class Oomycetes → Order Peronosporales → Family Pythiaceae → Genus *Pythium* (Agrios 1997).

Pythium is a widely distributed plant pathogen, occurring worldwide from forests to greenhouses. It has a wide host range and the decrease it causes in crop yields is of considerable importance, and has an effect on the economy worldwide (Lifshitz et al. 1984).

Although the greatest damage occurs to the seed and seedling roots, the fungus is capable of infecting plants at all stages of growth. Pre-emergence root rot (damping-off) affects seeds just before or after germination and post-emergence damping-off occurs once the seedling has broken the surface soil, causing seedling death or retarded growth (Laing 1997).

Pythium grows rapidly in response to seed or root exudates (one to two hours after exposure), thus causing infection soon after germination, often within 24 hours. When seeds have been invaded with *Pythium*, they most often fail to germinate and they become soft and "mushy", after which they turn brown, shrivel and disintegrate. The addition of manure can increase fungal growth, as this fungus is also saprophytic (Whipps and Lumsden 1991).

Plants infected with *Pythium* also produce very few lateral roots, thus becoming poorly anchored and easily lifted (Favrin et al. 1988). *Pythium* infection generally results in retarded growth, wilting, discolouration and premature death, caused by inadequate water uptake (van Os et al. 1999). Post-emergence damping-off manifests as wire-stem appearance, commonly resulting in plant death as the plant is unable to support itself (Laing 1997). This is a result of the basal stem thinning out and it is unable to hold the plant upright. If older plants are invaded, they develop root or stem rot, or lesions on their stems or roots which stunt the growth considerably and cause massive retardation and a subsequent decrease in crop yields (Agrios 1997).

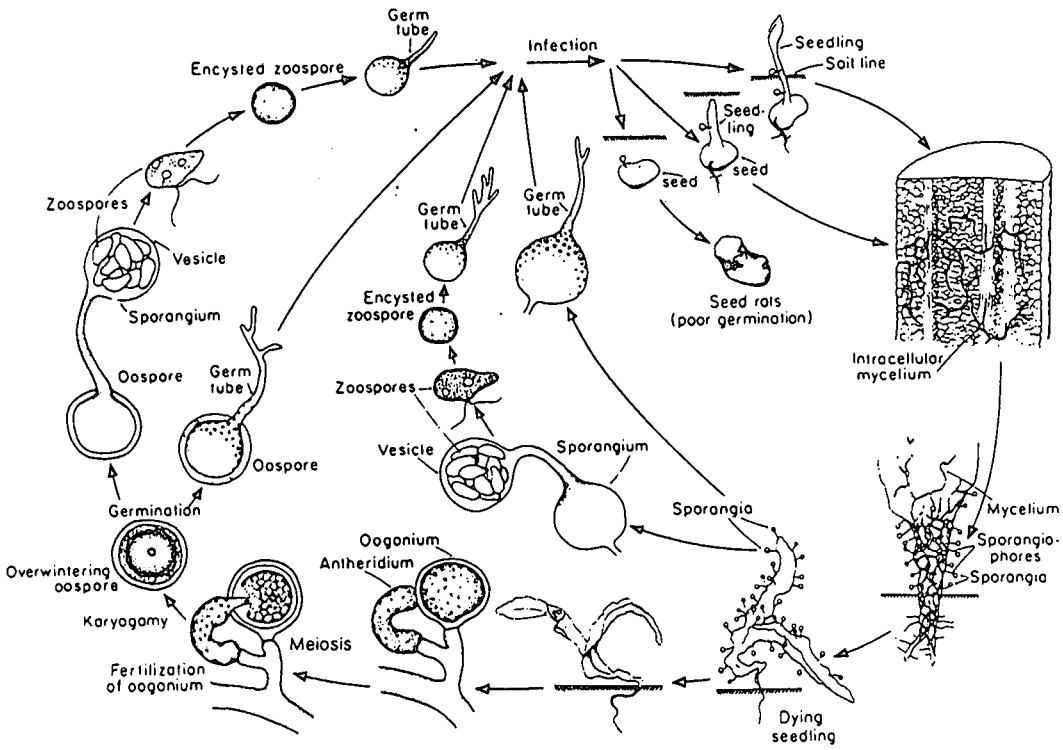

Figure 1 Disease cycle of damping-off and seed decay caused by *Pythium* sp. (Agrios 1997)

2.1.2 Control methods

Damping-off caused by *Pythium* is one of the most destructive and devastating diseases, which affect seedlings and crops. The most prevalent control strategies include pesticides and practices such as crop rotation and irrigation, but the efficacy of these as well as the environmental concerns, have encouraged research into alternative methods of protection against crop diseases (Mao et al. 1998).

The most effective fungicide used for treating *Pythium* infection in South African vegetable nurseries is Previcur® (propamocarb) (Askew & Laing 1994). Fungicide drenches are the preferred method of *Pythium* control. However, since crop losses and decreased yields are becoming an economic problem worldwide, there is a demand for alternative methods of pathogen control, since losses due to *Pythium* crown rot can be as much as 25-30% (McCullagh et al. 1996).

Secondly, since there is an increasing awareness of pathogen resistance to fungicides, there remains a concern for pathogen control as the existing methods appear to become inadequate (Whipps & Lumsden 1991). Because of the ability of pathogens to become resistant, there is a need to find methods of disease control that offer complete resistance, such as plant immunization. On recognition of a potential pathogen, a highly complex and co-ordinated response is activated in a plant. Disease manifests when this mechanism fails to recognize the pathogen or the pathogen is able to avoid or overcome this response. Resistance is due to both physical and chemical barriers, which are activated shortly after infection (Lyon et al. 1995).

As *Pythium* is a soil-borne disease, soil fumigation is a method of disease control. This is dependent on the occurrence of the pathogen in the soil and the effects afterwards have to be considered. The chemical and microbial structure of the soil is altered and further damage has been noted after flooding, as *Pythium* is a very resilient pathogen and can withstand many treatments. The elimination of non-target organisms is a consideration and since *Pythium* is an opportunistic pathogen, the disease infiltration can rapidly increase. It is also important to note that *Pythium* can survive fumigation and flooding (van Os et al. 1999).

Thirdly, fungicides have also become unfavourable due to increasing awareness of environmental pollution, health hazards and possible phytotoxicity (Lifshitz et al. 1984). Furthermore, when any fungicide is applied to an edible crop, the level of chemical residues must be acceptable (Spencer 1981). These considerable limitations have stimulated investigation into alternative means of disease control, or at least suppression.

Biological control has also been utilized to protect crops from fungal infection, although this method has been associated with numerous problems: they are extremely variable, inconsistent, expensive and less effective than fungicides. The biological agents may be impractical to use, as they have a limited shelf life or require specific storage facilities (Whipps & Lumsden 1991) and they may also depend greatly on specific soil or other growth conditions. The result is that field trials often do not coincide with those obtained in the laboratory (Laing 1997) and the variant conditions of the soil have proved to be too unstable for sensitive biocontrol agents (Dunne et al. 1998).

The current trend towards protecting plants from pathogens is to offer complete resistance to infection – it appears to be the most effective and economically viable method to date and it may reduce the occurrence of pathogen resistance or mutation. *Pythium* has been noted as being a very resilient pathogen with extraordinary survival structures and because of its widespread distribution, there is a need for further investigation of this fungus in South Africa (Denman & Knox-Davies 1992).

2.2 HOMEOPATHIC AGRICULTURAL RESEARCH

Numerous studies have been conducted using Homeopathic remedies to prove their efficacy in agriculture. An advantage for using plants to demonstrate the effects of homeopathic remedies is that there is no possibility of suggestion (Pelikan and Unger 1971) and the placebo effect can thus be discounted in plants (Coulter 1980 cited by Kayne 1991).

Trials have been conducted to prove the effect of homeopathic preparations on healthy plants. Koffler (1965) and Wannamaker (1966) carried out trials to test the

effects of potentised Sulphur and Boron on onion plants and the results showed that both remedies affected the weight and length of the plants, as well as their mineral content.

Pelikan and Unger (1971) tested the effects of decimal dilutions of silver nitrate on the growth of wheat seedlings, which showed evidence that potentised substances have an effect on plant growth. Following these trials, Jones and Jenkins (1981) were prompted to undertake a similar study using centesimal potencies of other substances as well as silver nitrate. Certain potencies showed minor - but significant - changes in growth curves with increasing potencies. Jones and Jenkins (1983) conducted further trials, assessing the effect of certain remedies on wheat and yeast growth. Using Pulsatilla, they established that certain potencies inhibited growth, while others stimulated it and the pattern of change was similar for both organisms.

Trials were also conducted to assess the effect of homeopathy on plants that were already diseased. Netien et al. (1966) conducted trials using pea seedlings taken from plants previously poisoned with copper sulphate. They claimed that these seedlings treated with copper sulphate in a 15CH dilution showed better growth than those seedlings grown in distilled water, although these trials could not be reproduced at a later date due to inadequate statistical analysis and methodologies (Scofield 1984).

The response of host plants to three papaya viruses and cucumber mosaic viruses to homeopathic remedies was observed by Khurana (1971). The conclusions drawn from these trials was that homeopathy is more effective when applied prophylactically (preventatively), rather than as a treatment for infected plants and that it is more effective on systemic hosts, rather than local lesions.

Studies done by McIvor (1980) cited by Kayne (1991) claimed to have been successful in treating fruit trees isopathically, using potencies of the fungus responsible for leaf curl. A small hole was drilled into the tree trunk about six inches above ground level and a 6CH dilution was injected into the trees under pressure.

The effects of isopathically prepared remedies of the tobacco mosaic virus were tested by Webb (1997) on tomato seedlings and she found that the potencies 6CH,

12CH and 30CH applied curatively caused an increase in disease, but 200CH caused a significant decrease in disease manifestation.

More specifically for this study, trials using homeopathy to control fungal disease are of particular interest. Khanna and Chandra (1976b, 1977a, 1978) conducted trials to assess the effect of various remedies on fruit rots caused by fungi. The remedies chosen were based on those commonly used to treat human fungal infection against a control (Scofield 1984a) and these were first tested in a large range of potencies to establish whether they had an inhibitory effect on fungal spore germination. Those remedies which showed complete inhibition of spore germination *in vitro* were then tested on infected fruits - either prior to or after homeopathic treatment.

Khanna and Chandra (1976a, 1977b) also studied the effect of homeopathy on leaf blight of wheat. Certain potencies of remedies were used to assess their effect on the spore germination of *Alternaria alternaria*. Some potencies caused inhibition of spore growth and these were then tested *in vivo* by spraying wheat plants 24 hours prior to fungal infection. After 20 days, Arsenicum album 199CH showed a 41% control of the disease and Kalium iodide 200CH showed a 59% control.

Brammer (1994) conducted trials to assess the effect of homeopathic preparations (5CH, 9CH, 15CH and 30CH) of *Peronospora parasitica* (downy mildew) on cabbage seedlings infected with this fungal disease, against three fungicides (S151, Optimo® and Phyton 27®). The results showed that potencies of *P.parasitica* did have a noticeable effect on the control of downy mildew. The lower potencies (5CH and 9CH) resulted in an increase in disease levels, while the medium potencies (15CH and 30CH) provided some control over a short period of time, by lowering the disease incidence. However, the range of potencies used in this trial was very limited.

Brammer's study stimulated a further study by Curnow (1998), in which a higher potency (1M) was introduced. She tested the efficacy of four potencies of *P. parasitica* (9CH, 30CH, 200CH and 1M) against two fungicides (Acrobat® and Bravo®) on cabbage seedlings infected with the same disease. She noted that preventative treatments were more effective for all treatments used, although Acrobat® was the most effective treatment overall. Despite none of the Homeopathic

treatments providing statistically significant results, a trend showed that 30CH was the most effective Homeopathic treatment used in the trial. The 200CH caused a higher level of disease, relative to the other treatments. However, these trials did not have an inoculated control (water only) and comparisons could not be made to show the true effects of the homeopathic preparations.

Dawson (2000) furthered Curnow's trials and sought to demonstrate the Arndt-Schultz Law of Hormesis (inhibition of growth at high concentrations and growth stimulation at low concentrations). She prepared Homeopathic potencies of the fungicide Acrobat® (7XH, 9XH, 12XH, 7CH, 9CH, 12CH, 15CH, 30CH and 200CH) and tested the efficacy on cabbage seedlings infected with *Peronospora parastica* (downy mildew). Trial One did not yield statistically significant results. However, while the second trial showed that Acrobat® was the most successful control overall, 30CH followed by 200CH were the most effective treatments in controlling the disease, although neither were statistically different from the control. It was noted that the 7XH, 9XH, 12XH and 9CH treatments tended to stimulate fungal growth and the 30CH and 200CH treatments tended to inhibit the disease process, which was contrary to the Arndt-Shultz law.

In summary of the latter three trials, although the fungicides proved to be the best method of control overall, some interesting trends did emerge. In Brammer's (1994) trial, the 5CH and 9CH caused an increase in fungal growth, and while the 200CH in Curnow's (1998) trial caused even higher levels of disease, the 30CH and 200CH inhibited the disease progress in Dawson's (2000) trial. While the results of these trials appear to be inconsistent, it is important to note that Brammer and Curnow did not use a distilled water control, thus it is difficult to assess whether the increased disease levels were because of varying effects of the potencies or a result of the natural progress of the disease. In using a control, it enables comparison between results of disease control and nature of the disease without treatment.

CHAPTER THREE:

Methods and Materials

3.1 INTRODUCTION

The trials were conducted at the University of Natal, Pietermaritzburg in the greenhouse belonging to the Department of Plant Pathology. The study group consisted of two crops: cabbages (Batch A) and cucumbers (Batch B). The trial was repeated twice, in succession. Each batch contained seven treatments, which consisted of:

- Inoculated control
- Un-inoculated control (water only)
- Previcur® (fungicide)
- And the four homeopathic potencies used: 9CH, 15CH, 30CH and 200CH.

Each treatment had four replicates, therefore a total of 28 trays per crop per trial and each tray had the capacity for 24 seedlings. The trays were individually numbered and computerized randomization of the trays was used, to maintain objectivity throughout the trial.

3.2 EXPERIMENTAL DESIGN (See Appendix A)

The experimental design consisted of randomized complete blocks design, whereby a computer randomly allocated the position of the seedling trays for each treatment group. This method is significant in its design and for its contribution towards analysis for the following reasons:

- It ensures that the results are accurate and easy to analyze
- Each block is completely randomized
- Each treatment appears in each block and
- Each set of replicates is separated from the next set when the trays are laid out.

This blocks effect eliminates variation in the results, due to environmental conditions determined by plant positioning. This design is by far the most widely used in agricultural research (Laing 1997).

3.3 TREATMENTS

3.3.1 Selection of treatments

Fungicide: Previcur® (propamocarb) was used for these trials

Homeopathic treatments: these were preparations of the fungus *Pythium spp.* which were applied in the following potencies: 9CH, 15CH, 30CH and 200CH. These potencies were chosen based on results of previous Homeopathic research on plants and because they encompass the range of potencies commonly used in clinical practice - thus covering a broad spectrum of dilutions. Brammer's (1994) study on downy mildew showed that the 9CH potency of *Peronspora parasitica* caused an increase in disease incidence when compared to the control. The medium potencies (15CH and 30CH) provided noticeable disease control over a short period of time, thus these two potencies were chosen for this trial. Curnow (1998) - whose study was to test the efficacy of Homeopathy in the control of downy mildew in cabbage seedlings - found that the 200CH produced the best results prophylactically, when compared to other potencies used.

Water: single-distilled Streem Calypso water was applied to the control group as a treatment.

3.3.2. Preparation of the treatments

Inoculum: *Pythium spp.* was cultivated on agar plates, which was then cut into 2mm x 2mm squares. These squares were placed upside down on each cell, thus causing infection of the seedling.

Fungicide: the concentration of Previcur® was 120ml Previcur/100 litres water.

Homeopathic remedies: *Pythium spp.* was cultivated on agar plates, according to the standard procedures conducted at the University. The fungus was then transported to a recognised Homeopharmaceutical laboratory (Pharma Natura), where it was partially prepared according to the stipulated procedure, as found in the German Homeopathic Pharmacopoeia (Methods 43 and 44) to one potency below the required potencies necessary for the trial (i.e. 8CH, 14CH, 29CH and 199CH). This is due to the fact that potencies are stored in alcohol and are preserved and most stable in this form. Alcohol applied directly to the plants would result in their death, therefore the exact potencies to be used in these trials were prepared in water approximately 30 minutes before use and succussed 100 times before spraying, using the prepared potencies, thus ensuring optimum activity of the remedies. This method of succussion followed that used by Webb (1997) and Curnow (1998). The resulting concentration of alcohol present in these final potencies was insufficient to cause damage to the seedlings (Laing 2001).

The concentration of remedies used was as follows:
1CH = 1/100 ⇒ Therefore one part substance + 99 parts solvent = 100 parts ⇒ Thus 5ml substance (potency) + 495ml solvent (water) = 500ml

Irrigation: the seedlings were automatically irrigated daily at 07h30, 11h00 and 13h00.

Application: the seven treatments were applied twice weekly after the last irrigation of the day.

3.3.3 Dosages of the treatments

Fungicide: Previcur® was applied as a drench using syringes - 62.5ml per seedling tray (2.5ml per seedling)

Homeopathic treatments: the four potencies were also applied as a drench (i.e. 2.5ml per seedling)

3.4 MATERIALS USED

Seeds: one seed per cell was planted (24 per tray with a 95% germination rate), after the soil had been dibbled with a pencil.

Seedling trays: each plastic speedling 24 tray contained 24 seedlings. Per trial there was a subtotal of 64 trays per crop and a total of 128 trays.

Growth medium: composted pine bark (CPB) obtained from Gromed, Crammond, KwaZulu Natal, was available at the Department of Plant Pathology, University of Natal, Pietermartizburg. This medium was thoroughly moistened before use, to prevent water running off a dry surface without penetrating.

Insecticide: Malathion® was applied weekly in a concentration of 2.5ml Malathion/1 litre water, to control aphids.

Fertiliser: 3.1.3. (38) Complete from Ocean Agriculture was used. It contains all the required macro- and micro- nutrients and it was applied daily through the irrigation system. The application levels are 1g/litre of water, which contains nitrogen (100ppm), potassium (100ppm) and phosphorous (33ppm).

Sterilising agent: Plasdip (copper oxychloride in PVA paint) was applied to each cell before planting to sterilise the trays. This was left to dry completely for approximately 24 hours to prevent the copper content from burning the seeds.

MISCELLANEOUS ITEMS

Scissors: these were used to cut off the upper parts of the plants at the end of the trial.

Brown paper bags: these were used to collect the harvested plants.

A "B" pencil: this was used to number the seedling trays and to "dibble" the soil.

3.5 METHOD OF CONDUCTING THE TRIALS

3.5.1 Planting

The 128 seedling trays were sterilised using Plasdip and left to dry completely. The trays were then filled with composted pine bark (CPB) which had been previously moistened and a pencil was used as a dibbler to ensure uniformity of planting depth - approximately 8mm. One seed per cell was planted and covered with CPB.

3.5.2 Treatment and inoculation

After planting, each cell was drenched using a syringe with the treatment randomly allocated to it. Each cell was then individually inoculated with *Pythium*, using the infected agar chips.

3.5.3 Germination

The trays remained in the germination room for approximately one to two days, to maintain optimal and controlled environmental conditions (constant humidity and temperature of 20°C). Once the plumules began to emerge, the seedlings were then moved to the tunnel (greenhouse) and positioned near the wet wall in order to reduce the effects of environmental factors within the testing site. The trays were arranged parallel to each other in two groups: batch A (cucumbers) and batch B (cabbages). They were placed in groups according to the computerized random blocks design, thus ensuring that each block contained each treatment, and objectivity from the researcher.

3.5.4 Drenching

Each seedling was drenched with Previcur®, 9CH, 15CH and 30CH treatments twice weekly (2.5ml per seedling using a syringe), after the final irrigation of the day. In these trials, Previcur® was dosed in error – the correct dosage is once every 30 days.

3.5.5 Harvesting and evaluation

- The first evaluation of the seedlings (germination) was done after three days of germination.
- The seedlings were then evaluated twice weekly to assess survival (wire stem/damping off)
- After 14-21 days (cucumbers) and 30 days (cabbages), the seedlings were individually counted and harvested, by cutting off the upper parts of the plants with scissors (each tray of seedlings was placed into a separate brown paper bag for weighing).
- The harvested seedlings were then weighed and the wet weights were recorded.
- The dry weights of the plants were obtained by placing the brown paper bags into an oven at a constant temperature of 50°C for 24 hours.
- The dried plants were then weighed on an accurate balance to get the total weight and then divided by the number of surviving plants to get the mean weight.

3.6 RECORDING THE DATA/MEASUREMENTS

The seedlings that had broken the surface soil three to five days after planting were counted, to assess the germination rate. After 21 days (cucumbers) and 30 days (cabbages), the seedlings were counted and recorded to assess the survival rate. These seedlings were weighed straight after harvesting to assess the wet weight and then placed in an oven at 50°C for 24 hours and then re-weighed, to assess the dry weight.

Where past studies have used the mean weights of surviving seedlings, there is a problem because of the phenomenon of between-plant compensation growth. This implies that should 24 seedlings grow in the Speedling 24 tray, they all compete for light and space and although are all healthy, their individual weights will be decreased by competition between individual plants. In another tray, where four plants survive damping-off and are widely distributed, there will be no competition

between the surviving plants. The remaining four plants may then be extra large because they will be growing without extra competition.

If the mean weights of the surviving plants is used for the statistical analyses, the latter case of the four survivors will have a larger mean weight per plot, than the 24 healthy plants in the un-inoculated tray. To avoid this problem, the mean weights per plot were each divided by 24, on the basis that there should've been 24 surviving seedlings per plot. This is also appropriate because plants that died before harvesting, are effectively recording a weight of zero grams (Laing 2001).

3.7 METHOD OF DATA ANALYSIS

To determine whether there was a statistical significant difference in these trials, the data was processed using the General Linear Models Procedure (GLMP) which produced ANOVA (Analysis Of Variance) tables – the method most used with the randomised complete blocks design. This analysis provided the mean weights for each treatment and it showed the level of significance and variation between the four replicates of each treatment.

The data was also processed using the Student Newman Keuls (SNK) test to determine levels of significance between the seven treatments.

CHAPTER FOUR:

The Results

For each trial (two cabbage and two cucumber batches) and each measurement parameter (percentage survival, wet weight and dry weight), the results were analysed by the General Linear Models Procedure, which produced ANOVA (Analysis of Variance) tables. These assess the variation between the four replicates of each treatment, compared to the differences between treatments: are the differences *within* each treatment greater or less than the differences *between* treatments?

If the ANOVA F test was significant, then the data was further analysed, using the Student Newman Keuls (SNK) test, to assess the levels of significance between the seven treatments

Ranks are provided to make accessible comparisons of the relative positions of the various treatments.

4.1 THE CABBAGE SEEDLINGS

4.1.1 Trial One (Refer to Appendices B and F)

A summary of the results of the statistical analysis (SNK test) showing the survival rates, wet and dry weights of the cabbage seedlings for Trial One is represented in Table 1.

SURVIVAL RATES

The survival rates showed no significance between treatments in the ANOVA analysis, where P = 0.6682. Previcur® produced the highest survival rate. The results for both Trial One and Trial Two are represented graphically in Figure 1.

WET WEIGHTS

The wet weights showed significance in the ANOVA analysis, where P = 0.0218. The following treatments were significantly different: the un-inoculated control and Previcur®, from the 30CH and 200CH treatments.

DRY WEIGHTS

The dry weights also showed significance in the ANOVA analysis, where P = 0.0072. Previcur® was significantly different from the other treatments.

The results for the wet and dry weights are represented graphically in Figure 2.

TABLE 1: Cabbage seedlings - Trial One

	SURVIVAL *			WET WEIGHT *			DRY WEIGHT *		
Treatments	**%**	**SNK**	**Rank**	**Mean (g)**	**SNK**	**Rank**	**Mean (g)**	**SNK**	**Rank**
Uninoculated Control	81.25	A	6	1.49	A	4	0.52	B	5
Inoculated Control	94.79	A	2	1.78	AB	2	0.54	B	2
Previcur®	96.88	A	1	1.96	A	1	0.74	A	1
9CH	87.50	A	3	1.51	AB	3	0.53	B	4
15CH	83.33	A	4	1.47	AB	5	0.50	B	7
30CH	82.29	A	5	1.29	B	7	0.50	B	6
200CH	73.96	A	7	1.37	B	6	0.53	B	3

* Means with the same SNK letter are not significantly different at the 95% confidence limit (P = 0.05)

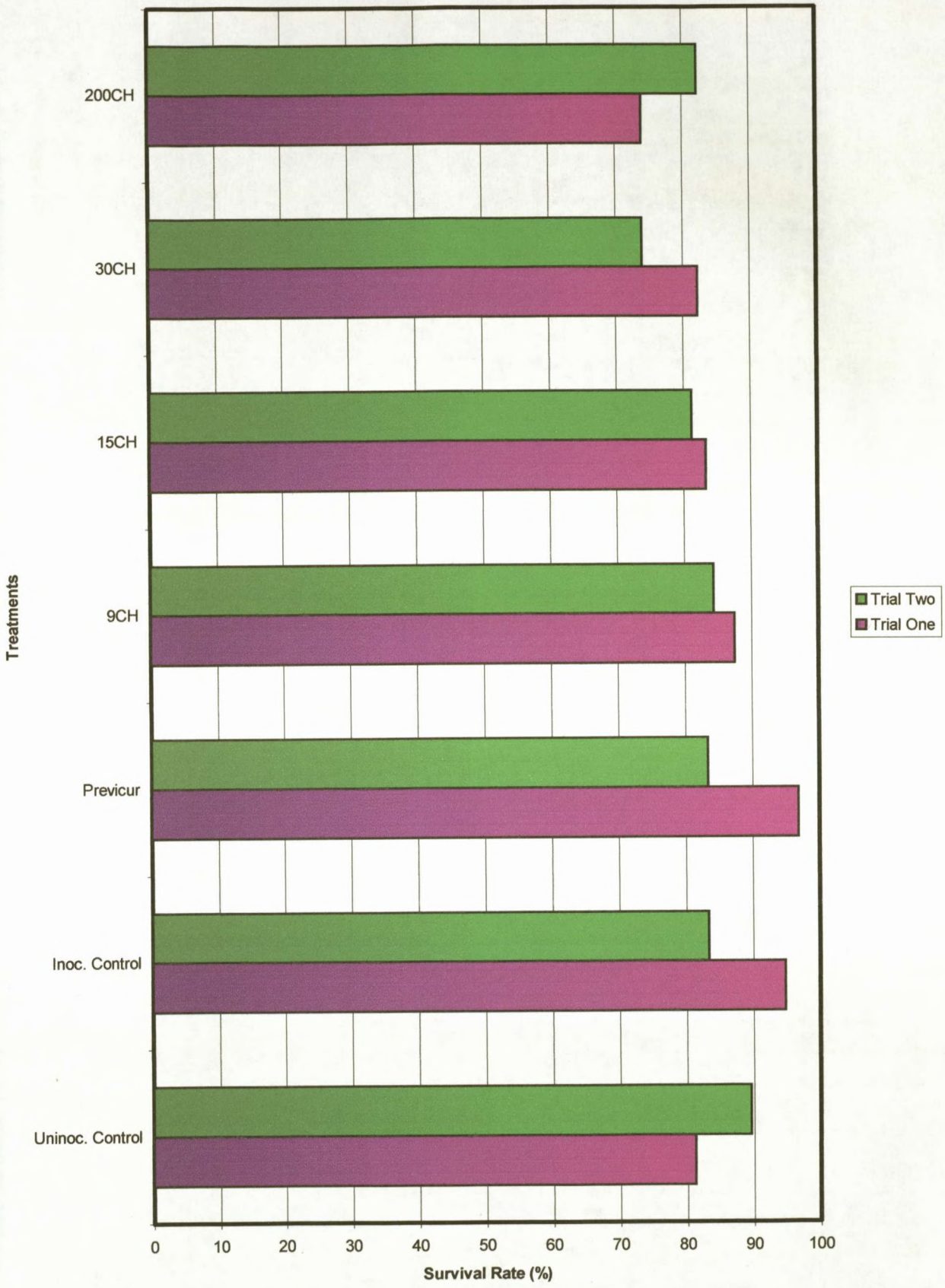

FIGURE 1: - TRIAL ONE AND TRIAL TWO

Histogram to represent the percentage survival rates of the cabbage seedlings

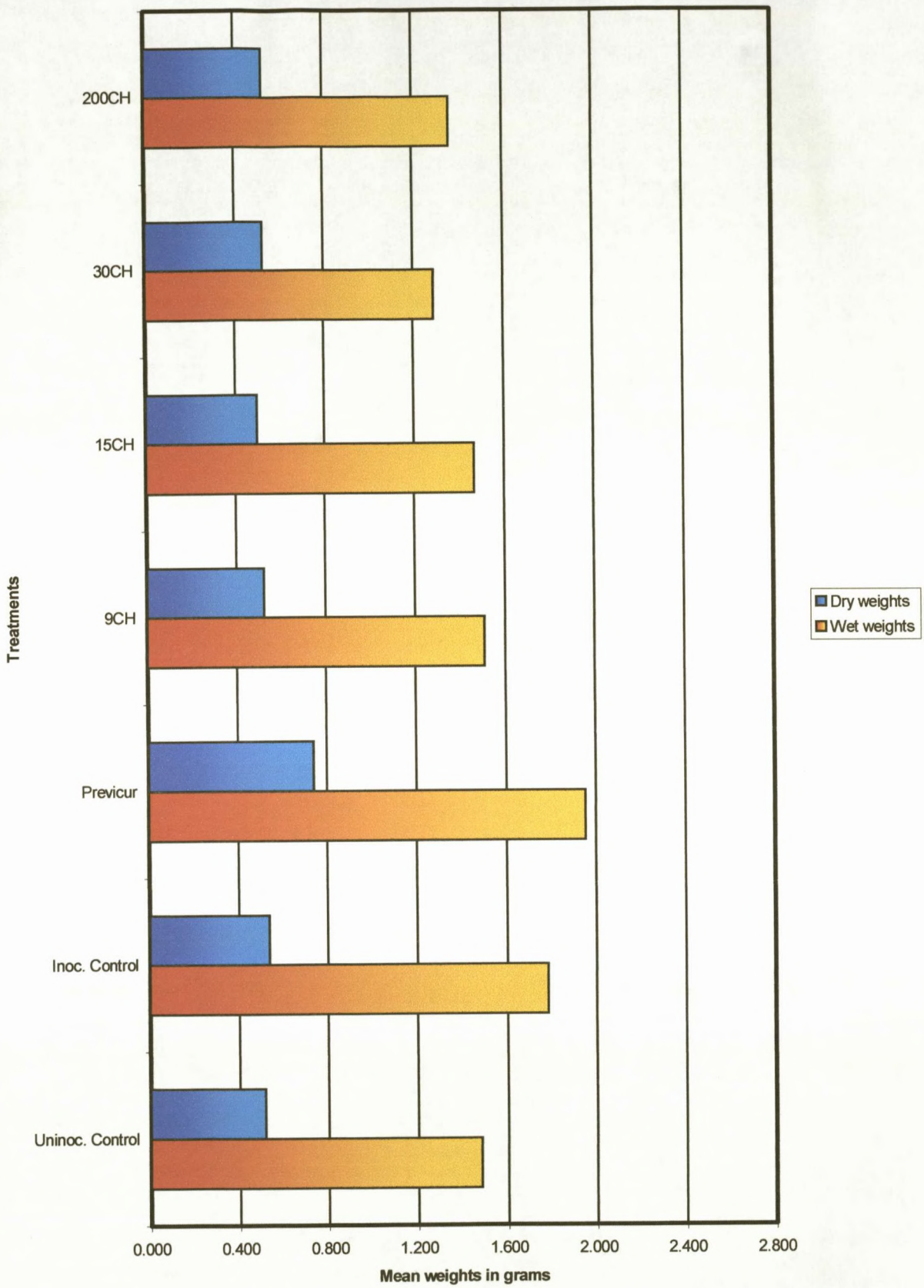

FIGURE 2: - TRIAL ONE

Histogram to represent the wet and dry weights of the cabbage seedlings

4.1.2 TRIAL TWO (Refer to Appendices C and G)

A summary of the results of the statistical analysis (SNK test) showing the survival rates, wet and dry weights of the cabbage seedlings for Trial Two is represented in Table 2.

SURVIVAL RATES

The survival rates showed no significance between treatments in the ANOVA analysis, where P = 0.1623. The un-inoculated control produced the highest survival rate. The results for both Trial One and Trial Two are represented graphically in Figure 1.

WET WEIGHTS

The wet weights were nearly significant at the 5% level, where the ANOVA analysis showed that P = 0.0672. Previcur® and the 30CH were significantly different from each other.

DRY WEIGHTS

The dry weights showed no significance in the ANOVA analysis, where P = 0.2169.

The results for the wet and dry weights are represented graphically in Figure 3.

TABLE 2: Cabbage seedlings - Trial Two

	SURVIVAL *			WET WEIGHT *			DRY WEIGHT *		
Treatments	**%**	**SNK**	**Rank**	**Mean (g)**	**SNK**	**Rank**	**Mean (g)**	**SNK**	**Rank**
Uninoculated Control	89.58	A	1	1.81	AB	6	0.49	A	6
Inoculated Control	83.33	A	3	1.85	AB	2	0.50	A	2
Previcur®	83.33	A	4	2.13	A	1	0.52	A	1
9CH	84.38	A	2	1.82	AB	5	0.49	A	5
15CH	81.25	A	6	1.83	AB	4	0.50	A	3
30CH	73.96	A	7	1.71	B	7	0.49	A	7
200CH	82.29	A	5	1.85	AB	3	0.50	A	4

* Means with the same SNK letter are not significantly different at the 95% confidence limit (P = 0.05)

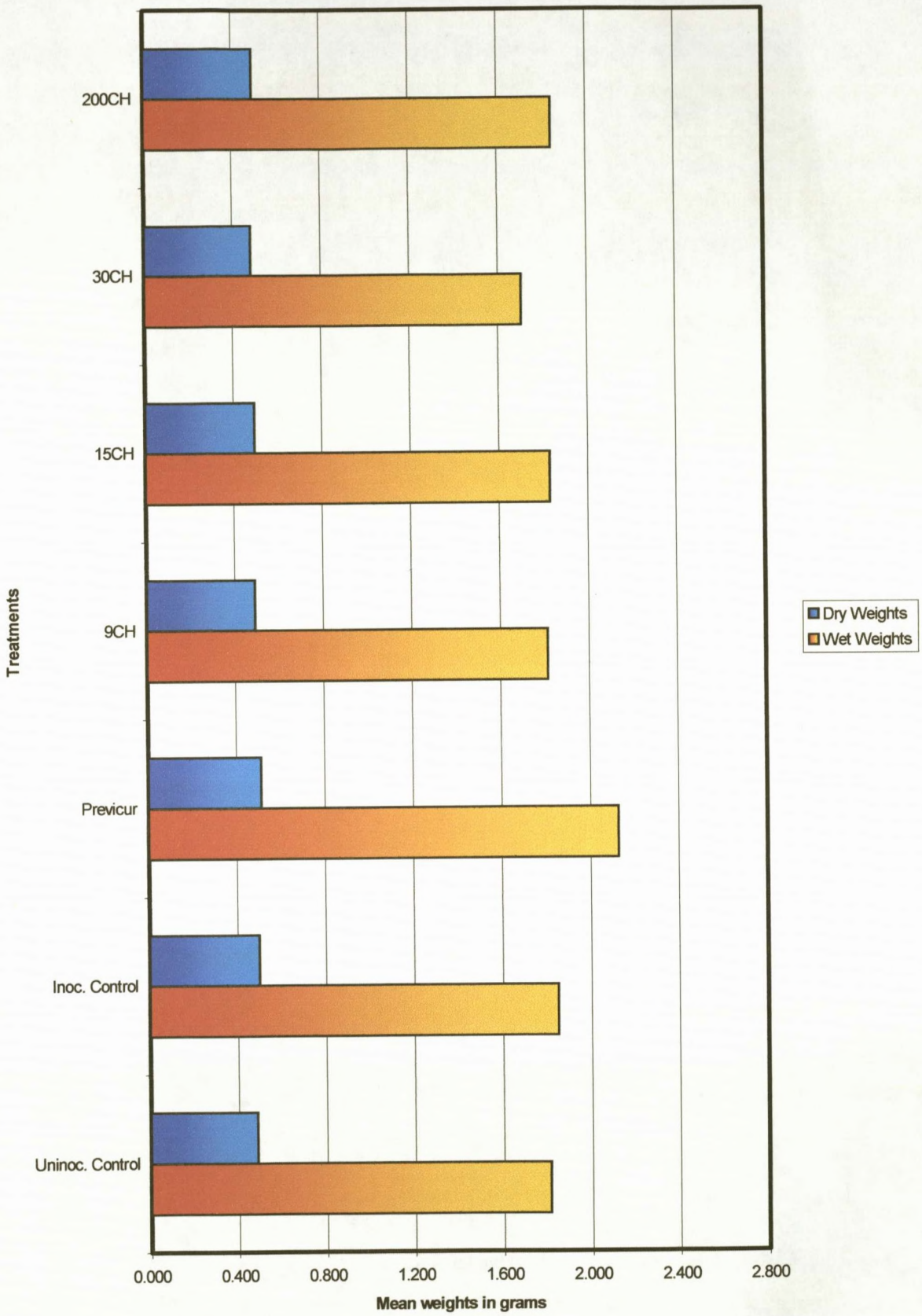

FIGURE 3: - TRIAL TWO

Histogram to represent the wet and dry weights of the cabbage seedlings

4.2 THE CUCUMBER SEEDLINGS

4.2.1 TRIAL ONE (Refer to Appendices D and H)

A summary of the results of the statistical analysis (SNK test) showing the survival rates, wet and dry weights of the cabbage seedlings for Trial One is represented in Table 3.

SURVIVAL RATES

The 15CH treatment produced the highest survival rate and none of the treatments showed significance. The results for both Trial One and Trial Two are represented graphically in Figure 4.

WET WEIGHTS

The ANOVA analysis revealed showed no significant differences in the wet weights, where P = 0.1870.

DRY WEIGHTS

The ANOVA analysis also revealed no significant differences in the dry weights, where P = 0.8934.

The results for the wet and dry weights are represented graphically in Figure 5.

TABLE 3: Cucumber seedlings - Trial One

	*			*			*		
	SURVIVAL			WET WEIGHT			DRY WEIGHT		
Treatments	%	SNK	Rank	Mean (g)	SNK	Rank	Mean (g)	SNK	Rank
Uninoculated Control	66.67	A	5	2.20	A	6	1.03	A	7
Inoculated Control	67.71	A	3	2.28	A	4	1.20	A	5
Previcur®	71.88	A	2	2.25	A	5	1.17	A	6
9CH	67.71	A	4	2.57	A	1	1.31	A	3
15CH	75.00	A	1	2.51	A	2	1.32	A	2
30CH	54.17	A	7	1.98	A	7	1.36	A	1
200CH	62.50	A	6	2.33	A	3	1.26	A	4

* Means with the same SNK letter are not significantly different at the 95% confidence limit (P = 0.05)

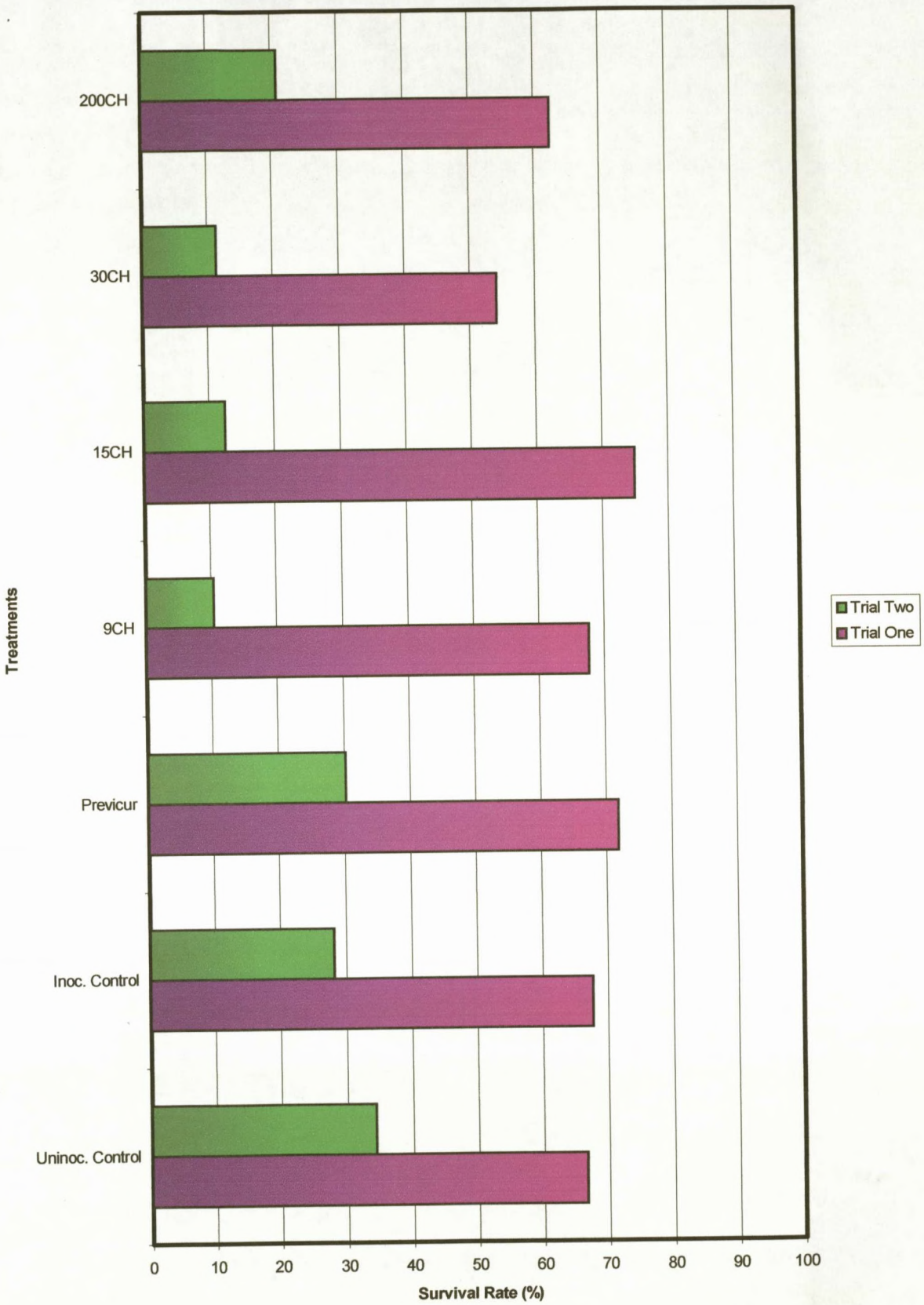

FIGURE 4:- TRIAL ONE AND TRIAL TWO

Histogram to represent the percentage survival rates of the cucumber seedlings

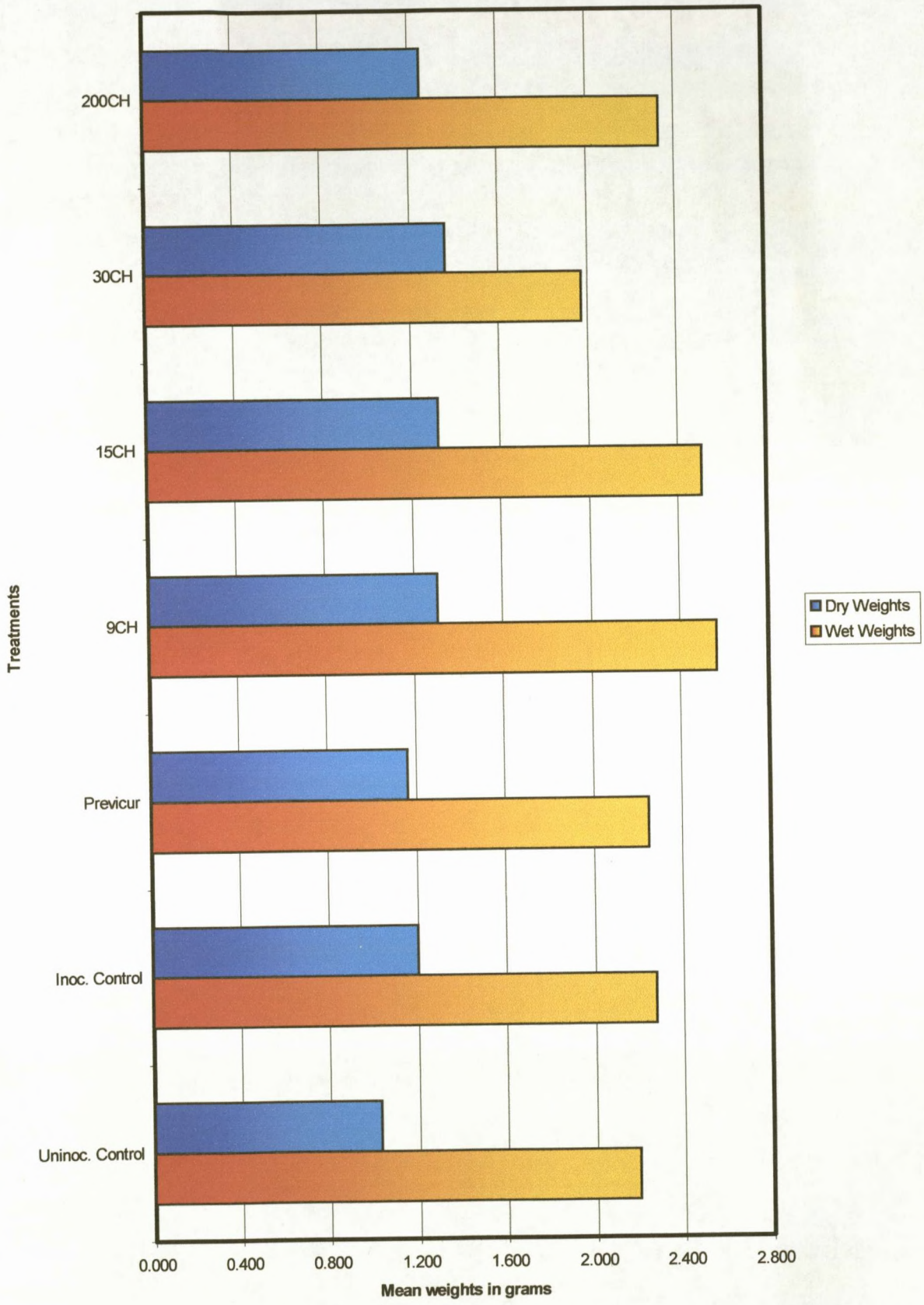

FIGURE 5: - TRIAL ONE

Histogram to represent the wet and dry weights of the cucumber seedlings

4.2.1 TRIAL TWO (Refer to Appendices E and I)

A summary of the results of the statistical analysis (SNK test) showing the survival rates, wet and dry weights of the cabbage seedlings for Trial One is represented in Table 4.

SURVIVAL RATES

The inoculated control produced the highest survival rate, which also showed significance from the following treatments: 9CH, 15CH and 30CH. The results for both Trial One and Trial Two are represented graphically in Figure 4.

WET WEIGHTS

The ANOVA analysis revealed levels of significance, where P = 0.0365 and the un-inoculated control was significantly different from the 30CH treatment.

DRY WEIGHTS

The dry weights also showed levels of significance in the ANOVA analysis, where P = 0.0435. The un-inoculated control and Previcur® were significantly different from the 30CH treatment.

The results for the wet and dry weights are represented graphically in Figure 6.

TABLE 4: Cucumber seedlings - Trial Two

	SURVIVAL *			WET WEIGHT *			DRY WEIGHT *		
Treatments	**%**	**SNK**	**Rank**	**Mean (g)**	**SNK**	**Rank**	**Mean (g)**	**SNK**	**Rank**
Uninoculated Control	34.38	A	1	0.95	A	1	0.71	A	1
Inoculated Control	28.13	AB	3	0.76	AB	3	0.61	AB	3
Previcur®	30.21	AB	2	0.82	AB	2	0.68	A	2
9CH	10.42	B	7	0.56	AB	6	0.49	AB	6
15CH	12.50	B	5	0.60	AB	5	0.53	AB	5
30CH	11.46	B	6	0.46	B	7	0.36	B	7
200CH	20.83	AB	4	0.66	AB	4	0.55	AB	4

* Means with the same SNK letter are not significantly different at the 95% confidence limit (P = 0.05)

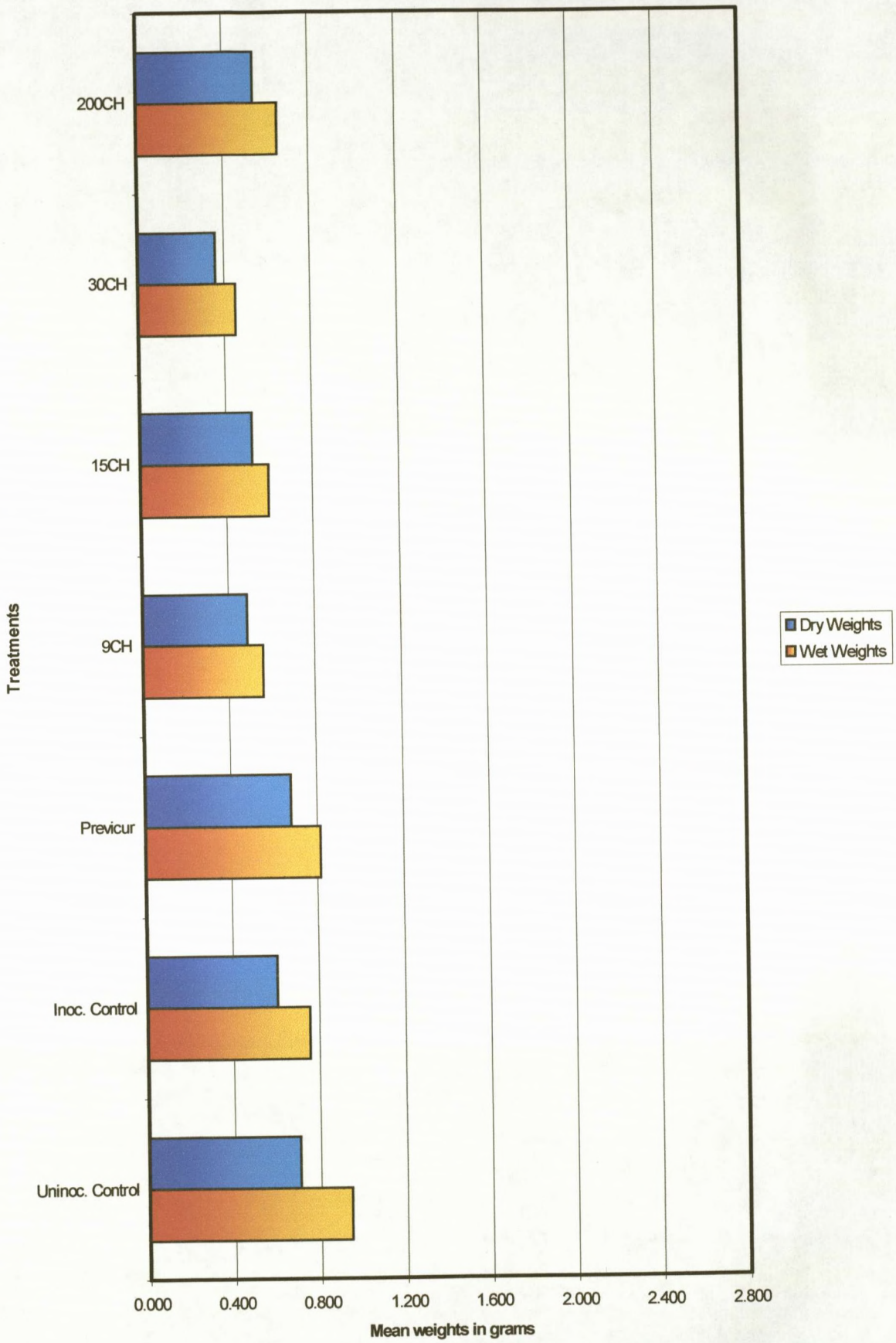

FIGURE 6: - TRIAL TWO

Histogram to represent the wet and dry weights of the cucumber seedlings

Of the four trials conducted (two cabbage and two cucumber), the seven treatments were ranked according to the three parameters measured i.e. survival, wet weights and dry weights.

The results have been tabulated in Table 5.

Conclusions:

Previcur® produced the highest overall ranking, indicating that it was the most successful treatment used in these trials.

The un-inoculated control, the 9CH and 15CH treatments each produced the third highest rankings – each treatment having the same mean rank. This indicates that these two homeopathic treatments produced similar results to the control, which was included in the trials to show the best case of growth.

The 30CH treatment produced the lowest overall ranking, which was consistent with the results obtained throughout both trials. This indicates that this treatment was the least successful treatment used in these trials.

A more detailed explanation and discussion of the results is available in Chapter Five.

Table 5: A Table of Ranking Each Treatment in Four Trials, Measured with Three Parameters

	CABBAGES TRIAL ONE			CABBAGES TRIAL TWO			CUCUMBERS TRIAL ONE			CUCUMBERS TRIAL TWO			Mean Rank	Overall Rank
Treatments	***Surv.***	***Wet***	***Dry***	***Surv.***	***Wet***	***Dry***	***Surv.***	***Wet***	***Dry***	***Surv.***	***Wet***	***Dry***		
Uninoculated Control	6	4	5	1	6	6	5	6	7	1	1	1	4.083	3=
Inoculated Control	2	2	2	3	2	2	3	4	5	3	3	3	2.833	2
Previcur®	1	1	1	4	1	1	2	5	6	2	2	2	2.333	1
9CH	3	3	4	2	5	5	4	1	3	7	6	6	4.083	3=
15CH	4	5	7	6	4	3	1	2	2	5	5	5	4.083	3=
30CH	5	7	6	7	7	7	7	7	1	6	7	7	6.167	7
200CH	7	6	3	5	3	4	6	3	4	4	4	4	4.417	6

Surv. = survival
Wet = Wet weights
Dry = Dry weights

CHAPTER FIVE:

Discussion and Conclusions

The data was statistically analysed using two methods. The General Linear Models Procedure (GLMP) produced ANOVA (Analysis of Variance) tables, showing the F-values for each trial. This test (i.e. ANOVA) is used to detect real differences between treatments. It does so by measuring the variation within each treatment, by comparing the results of each replicate - this provides the error term (value). This is then compared to the values of each treatment, therefore comparing the variation *within* each treatment, versus the difference *between* treatments. It does so by dividing the error value into the treatment value, to give the F-value. Each F-value can then be evaluated for significance against tabulated values, which provide the P-value i.e. a measure of significance.

In simple terms, when an F-value is large, it's P-value will be small and significant. Conversely, when the F-value is small, the P-value approaches 1 and is not significant. In most of science, a P-value of 0.05 or smaller is considered significant. In other words, there is less than 5 chances in 100 that the result is by chance, whereas there is a 95 out of 100 probability that the measured difference is real.

If the F-test is significant, it indicates that at least two treatments in the trial are significantly different from each other. However, it does not explain how each treatment differs from every other treatment. To establish this, the SNK test is run, which compares each treatment mean to the other treatment means. In order to compare the variation and levels of significance between the seven treatments, the data was analysed using the Student Newman Keuls (SNK) test. Mean values with the same letter are not considered significantly different.

The cabbage seedlings

In *Trial One*, the wet weights of the cabbage seedlings showed significance in both the ANOVA analysis (P = 0.0218) and the SNK test, which revealed that the un-inoculated control, Previcur® were significantly different from the 30CH and 200CH treatments. The dry weights of the cabbage seedlings also showed significance in

the ANOVA analysis Trial One (P = 0.0072), although the SNK test did not show any significance between treatments. In terms of survival rate, Previcur® ranked highest (96.88%) although the treatments were not significant from each other.

In *Trial Two*, the ANOVA test revealed that the wet weights were nearly significant at the 5% level, where P = 0.0672. The SNK test revealed that Previcur® and the 30CH treatment were significantly different from the other treatments. The dry weights showed no significance, where P = 0.2169. The SNK test did not reveal any significant differences between treatments. The un-inoculated control produced the highest survival rate (89.58%) and the treatments showed no significance.

Previcur® consistently produced the highest wet and dry weights in both Trial One and Trial Two, ranking first in all four of the parameters measured. It also produced the highest survival rate in Trial One. These trends indicate that Previcur® was the most successful treatment used for the cabbage seedlings – this was confirmed in Trial One, where it showed a significant difference from the other treatments (the un-inoculated control, 30CH and 200CH). However, Previcur® was not significantly different from the other treatments in Trial Two.

These results indicate that Previcur® had one or more effects:

- it caused an increased growth rate in cabbage seedlings
- it caused a decrease in the disease development caused by *Pythium*
- it increased the internal resistance mechanisms to disease in the seedlings

The results also revealed a trend in the effects of the 30CH treatment on the cabbage seedlings. In three of the four of the parameters measured, this treatment consistently produced the lowest wet and dry mean weights – the exception being Trial One, where it produced the second lowest dry weights. The dry weights were not significantly different from the other treatments, but the wet weights revealed significant differences from the other treatments: Previcur®, the un-inoculated control and the 200CH treatment (Trial One) and Previcur® (Trial Two). In two of the four parameters measured, the 30CH produced the lowest survival rate: 54.17% for the

cucumber seedlings (Trial One) and 73.96% for the cabbage seedlings (Trial Two). In the other two parameters, it showed the second lowest and third lowest survival rates for cucumber seedlings (Trial Two) and the cabbage seedlings (Trial One) respectively.

These results indicate that the 30CH treatment had one or more of the following effects:

- it caused a decreased growth rate of the cabbage seedlings
- it caused an increase in *Pythium* proliferation and disease development
- it suppressed the normal plant resistance mechanisms

They appear to be the reverse of the effects of Previcur® on the cabbage seedlings. The inoculated control did not show any significance, but it consistently produced the second highest mean weights, indicating a possibility that the strain of *Pythium* used for this trial was not as pathogenic as expected i.e. without any treatment, the seedlings still produced high mean weights. The un-inoculated control produced the second lowest mean weights, suggesting a possibility that the seed quality was poor. These two results can be confirmed by the absence of a significant difference between the two controls, in three of the four parameters measured.

The other three homeopathic treatments (9CH, 15CH and 200CH) did not reveal any noticeable or consistent trends throughout both trials, apart from the 200CH treatment, which showed a significant difference from the other treatments in the wet weights (Trial One).

The cucumber seedlings

In *Trial One*, both the wet and dry weights of the cucumber seedlings showed no significant difference in the both the ANOVA analysis, where $P = 0.1870$ and $P = 0.8934$ respectively. The SNK test also revealed no significance between treatments. In terms of survival rate, the 15CH treatment ranked highest (75%), although none of the treatments showed significance in this parameter.

In *Trial Two*, the ANOVA test revealed a significant difference in wet weights, where P = 0.0365) and the SNK test also revealed that the un-inoculated control and the 30CH treatment were significantly different from each other. The dry weights also showed significance in both ANOVA (P = 0.0435) and the SNK test, where the un-inoculated control, Previcur® and the 30CH treatment were significantly different from the other treatments. The un-inoculated control ranked highest in terms of survival rate (34.38%) and the treatments showed significance, where P = 0.0065. The un-inoculated control is significantly different from the 9CH, 15CH and 30CH treatments.

Noticeable trends emerged on comparing the results between Trial One and Trial Two. Trial One did not show evidence of any significant differences in the wet and dry weights, both between treatments and between replicates. The results of Trial One were not consistent with Trial Two, or when compared with the cabbage seedlings.

The following points were noted by the researcher:

- Previcur® produced lower mean weights in Trial One: it ranked fifth (wet weights) and sixth (dry weights), which was not consistent with Trial Two (Previcur® ranked second in both the wet and dry weights) or with the cabbage seedlings, where it ranked first throughout both trials.

- The 30CH treatment produced the highest mean dry weight in Trial One, which was inconsistent with the rest of the results: 30CH ranked seventh, producing the lowest mean weights in seven of the eight parameters measured.

- The other homeopathic treatments (9CH, 15CH and 200CH) ranked higher in the mean weights, which was not consistent with the other results.

Although there was no statistical significance in Trial One, the homeopathic treatments appear to have been the most successful treatments in this trial. They ranked in the first three positions, indicating that they produced the highest mean weights when compared to the other treatments.

However, it can be concluded that Trial One was not successful or noteworthy, because of one or more of the following reasons:

- The seeds used were not hybrid or of a high quality. This is evidenced in the results: the un-inoculated control ranked fifth (wet weights) and seventh (dry weights) and showed no significant difference from the other treatments. A possible reason for this result is that a different packet of seeds was used for Trial Two, which produced results more consistent with the rest of the trials.

- Trial One was grown in October and Trial Two was grown in November, therefore the seedlings may have been affected by the different environmental conditions (e.g. humidity, sunlight etc)

The results of Trial Two were almost identical: the mean weight rankings were the same and the un-inoculated control and the 30CH treatment were significantly different from the other treatments in both the wet and dry weights, with Previcur® showing a significant difference in the latter parameter.

The researcher noted the following trends:

- Previcur® ranked second in both the wet and dry weights, indicating that it had the expected effects on the cucumber seedlings i.e. increased plant growth and/or decreased *Pythium* infection and disease progression.

- The 30CH treatment ranked seventh, by producing the lowest mean wet and dry weights, showing evidence that it had the same effects on both the cabbage and cucumber seedlings i.e. decreased plant growth and/or increased disease progression.

The un-inoculated control produced the highest mean wet and dry weights, indicating that it produced the highest rate of seedling growth. It can be assumed that the seeds used were of a good quality. This was evidenced in the statistical analysis, where it showed a significant difference from the other treatments in both parameters measured. The inoculated control produced the third highest mean weights, indicating that the fungus was not as pathogenic as expected. The 9CH, 15CH and

200CH treatments were not significantly different from the other treatments, but they showed consistent rankings in their mean weights for Trial Two.

The un-inoculated control

The un-inoculated control was included in these trials, to provide a comparison between the inoculated control i.e. the former would produce the best case of growth and the latter, the worst case of growth. The un-inoculate control was also included to show the quality of the seeds used. This control showed significance from the other treatments in three of the eight parameters measured: cabbage wet weights (Trial One) and cucumber wet and dry weights (Trial Two).

It is interesting to note that the yield of the cabbage seedlings was fairly consistent in both trials, but there was a considerable difference in the yield of the cucumber seedlings in both trials. Each treatment had four replicates, therefore each potential yield was 96 seedlings. The un-inoculated cabbage seedlings yielded 78 seedlings in Trial One (81.25%) and 86 in Trial Two (89.58%), producing the second lowest and highest survival rates respectively. In terms of wet and dry weights, the un-inoculated cabbages produced the second lowest weights in Trial Two, confirming that while the yield was substantial, the quality of the seedlings was poor. The un-inoculated cucumber seedlings showed interesting results: the average yield for Trial One was 64 (66.67%) and 33 (34.38%) for Trial Two. In Trial One, this control ranked second among the lowest in wet and dry weights and in survival rates, indicating low yield and poor quality seedlings. In Trial Two, the un-inoculated seedlings produced the highest survival rate and the highest wet and dry weights - however, the discrepancy between Trial One is vast. A survival rate of 34.38% is not remarkable, when compared to higher yields of cucumber seedlings in Trial One, and the cabbage seedlings in both trials.

This trend is supported the statistical analysis that the quality of the seeds used was not consistent, or as high as expected. It is important to note that the same packet was used for the cabbage seedlings and was re-sealed between trials. However, two separate packets were used for the cucumber seedlings – the results show the inconsistency of seed quality.

This indicates a necessity for pre-trial germination tests, to assess the quality of the seeds to be used and that fresh packets are opened before planting i.e. separate packets of seeds for each trial.

The inoculated control

Like the un-inoculated control, this control was included to provide a framework of comparison with the other treatments. The inoculated control was also included to show a measure of the pathogenicity of the *Pythium* strain used. It consistently produced the second highest mean cabbage weights throughout both trials and the third highest weights in the cucumber seedlings (Trial Two). This control also showed no significant difference from the other treatments throughout both trials.

In terms of survival rates, the inoculated control was consistent with its ranking in two of the four parameters measured. In Trial One, the cabbage seedlings showed the second highest survival rate (94.79%), while also ranking second highest in both the wet and dry weights. In Trial Two, the inoculated control ranked second highest in terms of wet and dry weights, while showing the third highest in the survival rate (83.33%). This showed that a high yield of heavy plants were harvested, therefore confirming that the strain of *Pythium* used in these trials was not as pathogenic as anticipated.

Previcur®

It was anticipated that Previcur® and the un-inoculated control would produce the best results and that the inoculated control would produce the worst results.

From these trials, it is evident that Previcur® consistently produced the best or second best results, even where there was no evidence of statistical significance from the other treatments. It produced the highest or second highest mean weights in six of the eight parameters measured.

The following overall results can be deduced from the trends and statistical analysis:

i. The strain of *Pythium* used was not very pathogenic

ii. Previcur® consistently stimulated plant growth and inhibited fungal growth

iii. The Homeopathic remedies did not work as well as expected - in particular, the 30CH produced the worst results.

The homeopathic treatments

The effects of the 30CH treatment on the seedlings has been discussed. The 9CH, 15CH and 200CH treatments did not reveal any noticeable trends or show evidence of statistical significance through both trials, in terms of wet and dry weights and survival rates.

Conclusions

The aim of this study was to investigate the relative effectiveness of homeopathic preparations (9CH, 15CH, 30CH and 200CH) of *Pythium* as opposed to Previcur®, in the control of damping-off in cabbage and cucumber seedlings, in terms of growth.

The four hypotheses stated that each of the homeopathic treatments would yield significantly different results, compared to Previcur®. Based on previous trials done using homeopathy on plants, as discussed in chapter two, it was expected that the homeopathic treatments would have an effect on the disease caused by *Pythium*. At best, they would produce better results than the inoculated control and at worst, produce results worse than the un-inoculated control.

These trials indicate that the homeopathic treatments were not adequate in controlling disease caused by *Pythium,* nor was their action comparable to that of Previcur®. The 9CH, 15CH and 200CH did not reveal any evidence of adequate control. Several possibilities can be considered.

In terms of the homeopathic remedies:

i. They have no effect on plant diseases.

ii. They have no effect on plant pathogenic fungi.

However, these assumptions are not consistent with results in previous research and with the results of the 30CH treatment in these trials, as discussed previously in this thesis.

Other possibilities can be considered:

i. Homeopathic remedies are dose dependent and may show noticeable results when applied for a longer period of time i.e. until the plant reaches maturity.

ii. The drenches were applied too infrequently and further trials should be conducted, comparing results after daily drenching, twice weekly drenching and weekly drenching.

It can be concluded that Previcur® was the most successful treatment used in this research project, in terms of disease control, consistently producing results that were expected i.e. increasing seedling growth and/or decreasing disease progression.

The 30CH treatment was not successful in controlling disease progression caused by *Pythium* infection, but it produced consistent results and trends, which were backed with a scientifically valid methodology and statistically significant results. The mechanism of action of homeopathic dilutions has been highlighted in these trials, proving that they do have an effect on seedling (plant) growth.

The spheres of plant pathology and homeopathy create vast potential for further investigation. Many more aspects can be investigated, through scientific, reproducible and valid trials, using plants as experimental subjects as an objective method of demonstrating the activity of homeopathic preparations. These trials indicate the need for future research to be conducted, using knowledge from previous trials as the platform for further discovery.

CHAPTER SIX:

Recommendations

From the results obtained, certain homeopathic treatments do seem to have an effect on plant growth and disease management.

However, it does appear that the strain of *Pythium* used for this trial was not as pathogenic as expected. It would be beneficial to conduct a test the pathogenicity of the pathogen before starting the trials, by infecting seedlings and assessing the disease progress.

It is evident that the quality of the seeds used was inconsistent – the cabbage seeds were taken from the same packet for both trials, whereas the cucumber seeds were taken from two separate packets, opened just before planting began. In future, it would be advisable to conduct a germination test beforehand, whereby random seeds are grown in cotton wool for three to five days, to assess their quality. It would also be beneficial to use fresh seeds for each trial, as opposed to resealing old packets.

To assess the exact mechanism of action the 30CH treatment has on both the seedlings and plants, future trials in these areas would be useful. To assess whether it merely has an effect on stunting plant growth, future trials could be done on un-inoculated plants. To assess whether 30CH has an effect on the internal resistance of plants to disease, future trials could be done using several plants infected with various plant pathogens, to determine if 30CH has an effect on other fungal infections or only *Pythium*.

To establish the effect of dose response of homeopathic remedies, it is suggested that trials are conducted, whereby one homeopathic potency (concentration) is used, although the frequency of drenching is varied.

To establish alternatives for disease control, it would be beneficial to treat the infected plants with homeopathic remedies selected on the basis of disease similarity, according to the Law of Similars.

APPENDIX A

LAYOUT OF THE SEEDLING TRAYS AS GENERATED BY THE COMPUTER (RANDOMIZED COMPLETE BLOCKS DESIGN)

POSITION	TREATMENT
1	9CH
2	Inoculated Control
3	30CH
4	200CH
5	Previcur
6	Uninoculated Control
7	15CH
8	Uninoculated Control
9	Previcur
10	15CH
11	200CH
12	9CH
13	30CH
14	Inoculated Control
15	15CH
16	Previcur
17	Inoculated Control
18	Uninoculated Control
19	9CH
20	200CH
21	30CH
22	30CH
23	9CH
24	15CH
25	Inoculated Control
26	200CH
27	Previcur
28	Uninoculated Control

NO.	TREATMENTS	TRAY NUMBERS
1	Uninoculated Control	6, 8, 18, 28
2	Inoculated Control	2, 14, 17, 25
3	Previcur	5, 9, 16, 27
4	9CH	1, 12, 19, 23
5	15CH	7, 10, 15, 24
6	30CH	3, 13, 21, 22
7	200CH	4, 11, 20, 26

APPENDIX B

MEAN WET & DRY WEIGHTS (g) OF THE CABBAGE SEEDLINGS

TRIAL ONE

TRAY	SURV.	WET WEIGHT	DRY WEIGHT
1	21	1.48	0.52
2	23	2.10	0.55
3	21	1.13	0.49
4	24	1.83	0.53
5	24	2.12	0.98
6	16	1.56	0.51
7	23	1.51	0.49
8	16	1.31	0.50
9	22	1.97	0.55
10	20	1.60	0.50
11	23	1.66	0.65
12	20	1.62	0.53
13	20	1.32	0.49
14	24	1.63	0.53
15	17	1.37	0.50
16	24	1.83	0.61
17	23	1.74	0.54
18	22	1.62	0.53
19	22	1.37	0.51
20	1	0.48	0.41
21	18	1.36	0.52
22	20	1.36	0.51
23	21	1.57	0.54
24	20	1.39	0.51
25	21	1.66	0.54
26	23	1.49	0.52
27	23	1.90	0.82
28	24	1.45	0.53

Surv. = number of surviving seedlings harvested (maximum = 24 per tray)

APPENDIX C

MEAN WET & DRY WEIGHTS (g) OF THE CABBAGE SEEDLINGS

TRIAL TWO

TRAY	SURV.	WET WEIGHT	DRY WEIGHT
1	22	1.69	0.48
2	21	1.75	0.49
3	16	1.47	0.46
4	21	1.84	0.49
5	20	2.28	0.52
6	23	2.04	0.50
7	18	1.79	0.49
8	19	1.72	0.48
9	20	2.03	0.51
10	18	1.53	0.47
11	17	1.61	0.48
12	19	1.78	0.49
13	18	1.75	0.49
14	21	1.87	0.49
15	20	1.86	0.50
16	18	2.03	0.51
17	18	1.83	0.51
18	22	1.84	0.49
19	19	1.77	0.49
20	20	1.93	0.50
21	17	1.70	0.49
22	20	1.91	0.51
23	21	2.02	0.51
24	22	2.15	0.53
25	20	1.95	0.51
26	21	2.00	0.51
27	22	2.17	0.52
28	22	1.65	0.48

Surv. = number of surviving seedlings harvested (maximum = 24 per tray)

APPENDIX D

MEAN WET & DRY WEIGHTS (g) OF THE CUCUMBER SEEDLINGS

TRIAL ONE

TRAY	SEEDS	WET WEIGHT	DRY WEIGHT
1	14	2.57	1.08
2	18	2.66	1.43
3	10	2.03	1.34
4	16	2.53	1.34
5	13	2.21	0.94
6	14	2.32	0.57
7	18	2.87	1.44
8	15	2.39	1.23
9	18	2.44	1.77
10	20	2.69	1.50
11	12	2.13	1.21
12	17	2.45	1.13
13	16	2.16	1.23
14	14	2.11	0.85
15	13	1.73	0.64
16	19	2.39	1.00
17	13	1.75	1.15
18	15	1.90	1.20
19	14	2.33	0.82
20	15	2.42	0.94
21	14	1.86	1.39
22	12	1.85	1.49
23	20	2.91	2.21
24	21	2.74	1.71
25	20	2.58	1.37
26	17	2.24	1.54
27	19	1.95	0.95
28	20	2.17	1.12

Surv. = number of surviving seedlings harvested (maximum = 24 per tray)

APPENDIX E

MEAN WET & DRY WEIGHTS (g) OF THE CUCUMBER SEEDLINGS

TRIAL TWO

TRAY	SEEDS	WET WEIGHT	DRY WEIGHT
1	4	0.60	0.48
2	5	0.62	0.48
3	6	0.72	0.49
4	6	0.84	0.69
5	8	0.90	0.77
6	8	0.91	0.72
7	1	0.43	0.45
8	9	1.02	0.78
9	11	1.11	0.93
10	4	0.66	0.59
11	2	0.55	0.49
12	3	0.61	0.51
13	3	0.59	0.49
14	9	0.97	0.76
15	3	0.58	0.47
16	5	0.63	0.50
17	8	0.84	0.71
18	4	0.65	0.58
19	1	0.53	0.50
20	7	0.66	0.53
21	2	0.52	0.47
22	0	0.00	0.00
23	2	0.51	0.47
24	4	0.73	0.59
25	5	0.60	0.48
26	5	0.59	0.47
27	5	0.63	0.53
28	12	1.20	0.74

Surv. = number of surviving seedlings harvested (maximum = 24 per tray)

APPENDIX F

GENERAL LINEAR MODELS PROCEDURE
TRIAL ONE

ANOVA VALUES OF CABBAGE SEEDLINGS - WET WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.32494286	0.22082381	3.19	0.0218
Error	21	1.45212500	0.06914881		
Corrected Total	27	2.77706786			

R_Square	C.V.	Root MSE	Mean
0.477101	16.95355	0.2629161	1.551071

ANOVA VALUES OF CABBAGE SEEDLINGS- DRY WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.17257143	0.02876190	4.08	0.0072
Error	21	0.14812500	0.00705357		
Corrected Total	27	0.32069643			

R_Square	C.V.	Root MSE	Mean
0.538115	15.26019	0.0839855	0.550357

ANOVA VALUES OF THE CABBAGE SEEDLINGS - SURVIVORS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	88.85714286	14.80952381	0.68	0.6682
Error	21	458.00000000	21.80952381		
Corrected Total	27	546.85714286			

R_Square	C.V.	Root MSE	Mean
0.162487	22.70171	4.6700668	20.571429

APPENDIX G

GENERAL LINEAR MODELS PROCEDURE
TRIAL TWO

ANOVA VALUES OF CABBAGE SEEDLINGS - WET WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.40018571	0.06669762	2.36	0.0672
Error	21	0.59390000	0.02828095		
Corrected Total	27	0.99408571			

R_Square	C.V.	Root MSE	Mean
0.402567	9.062247	0.1681694	1.855714

ANOVA VALUES OF CABBAGE SEEDLINGS - DRY WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00214286	0.00035714	1.53	0.2169
Error	21	0.00490000	0.00023333		
Corrected Total	27	0.00704286			

R_Square	C.V.	Root MSE	Mean
0.304260	3.07703	0.0152753	0.496429

ANOVA VALUES OF THE CABBAGE SEEDLINGS - SURVIVORS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	29.85714286	4.97619048	1.73	0.1623
Error	21	60.25000000	2.86904762		
Corrected Total	27	90.10714286			

R_Square	C.V.	Root MSE	Mean
0.331352	8.54543	1.6938263	19.821429

APPENDIX H

GENERAL LINEAR MODELS PROCEDURE
TRIAL ONE

ANOVA VALUES OF CUCUMBER SEEDLINGS - WET WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.93683571	0.15613929	1.63	0.1870
Error	21	2.00575000	0.09551190		
Corrected Total	27	2.94285871			

R_Square	C.V.	Root MSE	Mean
0.318372	13.44113	0.3090500	2.299286

ANOVA VALUES OF CUCUMBER SEEDLINGS - DRY WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.31277143	0.05212857	0.36	0.8934
Error	21	3.00692500	0.14318690		
Corrected Total	27	3.31969643			

R_Square	C.V.	Root MSE	Mean
0.094217	30.63086	0.3784005	1.235357

ANOVA VALUES OF CUCUMBER SEEDLINGS - SURVIVORS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	62.71428571	10.45238095	1.25	0.3238
Error	21	176.25000000	8.39285714		
Corrected Total	27	238.96428571			

R_Square	C.V.	Root MSE	Mean
0.262442	18.14702	2.8970428	15.964286

APPENDIX I

GENERAL LINEAR MODELS PROCEDURE
TRIAL TWO

ANOVA VALUES OF CUCUMBER SEEDLINGS - WET WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.66008571	0.11001429	2.81	0.0365
Error	21	0.82360000	0.03921905		
Corrected Total	27	1.48368571			

R_Square	C.V.	Root MSE	Mean
0.444896	28.88054	0.1980380	0.685714

ANOVA VALUES OF CUCUMBER SEEDLINGS - DRY WEIGHTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.33457143	0.05576190	2.67	0.0435
Error	21	0.43792500	0.02085357		
Corrected Total	27	0.77249643			

R_Square	C.V.	Root MSE	Mean
0.433104	25.80354	0.1444077	0.559643

ANOVA VALUES OF CUCUMBER SEEDLINGS - SURVIVORS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	135.85714286	22.64285714	4.17	0.0065
Error	21	114.00000000	5.42857143		
Corrected Total	27	249.85714286			

R_Square	C.V.	Root MSE	Mean
0.543739	45.94227	2.3299295	5.071429

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