RESPONSES OF ECONOMICALLY IMPORTANT CROPS TO CRUDE EXTRACTS OF *CUCUMIS MYRIOCARPUS* FRUIT WHEN USED AS A PRE-EMERGENT BIO-NEMATICIDE

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[redacted]

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TABLE OF CONTENTS

DECLARATION

"I, Tieho Paulus Mafeo, do hereby declare that this thesis submitted to the University of Limpopo, for the degree of Doctor of Philosophy in Agriculture (Horticulture) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been dully acknowledged".

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DEDICATION

I would like to dedicate this thesis to my late parents (Mojalefa and Nyakallo Mafeo), my sons (Nyakallo and Therisano Mafeo) and my wife (Mmakoma Mafeo).

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

High yield losses in various crops due to plant-parasitic nematodes are associated with high initial nematode population densities (Pi). Uses of synthetic nematicides to reduce Pi were dependent on the physiological effect of materials on the protected crops, resulting into the coining of pre-emergent and post-emergent nematicides. Crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit consistently reduced nematode population densities of the southern root-knot nematode (*Meloidogyne incognita*) when used as a post-emergent bio-nematicide. The purpose of this study was to investigate the compatibility of crude extracts of *C. myriocarpus* fruit when used as a pre-emergent bio-nematicide on germination and emergence of commercially important dicotyledonous and monocotyledonous crops using empirical tests and computer-generated models. Studies were conducted over a period of three years to assess the effects of this material on growth of various seedlings. Seven treatments comprising crude extracts of *C. myriocarpus* fruit (0, 2.5, 5, 7.5, 10, 12.5 and 15 g/pot) and test solutions (0, 25, 50, 75, 100, 125 and 150 g/ℓ distilled water) were used for emergence and germination in initial studies. Generally, 18 days after the treatments, variables measured and levels of crude extracts of *C. myriocarpus* fruit had negative quadratic relationships, which suggested that they had density-dependent growth responses. Subsequent studies were conducted using three selected crops each from the families Alliaceae, Gramineae and Solanaceae under greenhouse conditions, each with reduced concentration of 10 treatments (0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.25 g material/pot). Using variables of various organs and crops, significant means were subjected to the Curve-fitting Allelochemical Dosage Response (CARD) computer model, which was characterised by six biological indices, *viz*. threshold stimulation (D_m),

saturation level (R_h), 0% inhibition (D_0), 50% inhibition (D_{50}), 100% inhibition (D_{100}) and transformation level (k). The model demonstrated that the responses of the three crops from each family when regressed to dosages of crude extracts of *C. myriocarpus* fruit exhibited the density-dependent growth patterns, characterised by responses that included stimulation, saturation and inhibition. The integrated sensitivities ($\sum k$) of the tested crops to crude extracts of *C. myriocarpus* fruit ranged from $\sum k = 9$ to $\sum k = 51$, with eggplant (*Solanum melongena*) and sorghum (*Sorghum bicolor*) being the most sensitive, while tomato (*Solanum lycopersicum*) was the least sensitive. Using the data depicting the stimulation range from CARD model, *viz*. (D_m), which is a threshold stimulation dosage and (R_h), which is a saturation dosage, mean dosage stimulation response (MDSR) was determined for chive (*Allium schoenoprasum*), leek (*Allium ampeloprasum*), onion (*Allium cepa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum, eggplant, pepper (*Capsicum annum*) and tomato as being 1.19, 0.68, 0.45, 1.13, 0.86, 1.12, 0.74, 1.11, and 0.53 g, respectively. These MDSR values are dosages which when applied for respective crops at direct seeding would not affect germination or emergence. MDSR values were validated for onion, millet and tomato, resulting in approximately 100% suppression of nematodes in all three test crops. In contrast, 100% emergence occurred in millet and tomato, while the validated MDSR reduced emergence on onion by 15%, which confirmed the sensitivity of this crop to crude extracts of *C. myriocarpus* fruit. In conclusion, crude extracts of *C. myriocarpus* fruit have the potential for use as pre-emergent bio-nematicide in suppression of plant-parasitic nematodes in various crops.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Governments with signatory status to the 1987 Montreal Protocol and the 1997 Kyoto Protocol have been suspending the use of ozone-depleting and greenhouse-inducing compounds from 2005 through 2010 (Ledley *et al.*, 1999; UNEP, 2000; UNEP, 2005). In the management of plant-parasitic nematodes, the suspension of these materials had since resulted in refocusing research into alternative interventions. The Land Bank Chair of Agriculture - University of Limpopo, established and developed the Ground Leaching Technology (GLT), which uses small quantities of crude extracts of wild cucumber (*Cucumis myriocarpus* Naud.) fruit to suppress nematode numbers when applied at transplanting (Mashela, 2002). However, most of the nematode damage on crops occurs at planting, but attempts to apply the material at planting resulted in failure of seedling emergence.

This General Introduction focuses on the (1) background, which includes the description of the problem, its impact, causes of the research problem and the proposed solutions, (2) problem statement, (3) motivation of the study, (4) the objectives, (5) the hypotheses and (6) the format of the thesis.

1.1.1 Description of the research problem

Root-knot nematodes *(Meloidogyne* spp.) cause considerable yield losses in various crops in different parts of the world (Eisenback and Trianataphyllou, 1991; Sikora and

Fernandez, 2005). Over ninety species and two subspecies of *Meloidogyne* species have been reported to infect thousands of different plant species within the broad classification of monocotyledonous and dicotyledonous plants (Eisenback and Trianataphyllou, 1991; Hussey and Janssen, 2002). Crop yield losses are influenced mainly by the aggressiveness of the nematode species involved, the initial population density of nematodes (Pi) at planting, the degree of resistance in the plant, the age of the plant and the presence of abiotic and biotic factors (Mashela *et al*., 1992a,b; Maqbool and Kerry, 1997). Crop yield losses are inversely proportional to the Pi (Seinhorst, 1965). Thus, it is imperative to ensure that at planting, Pi in the soil had been reduced to the lowest level possible.

Fumigant synthetic nematicides, which have been used as pre-plant nematicides due to their phytotoxicity, had been highly effective in reducing Pi. However, the materials had since been withdrawn due to their depletion of ozone layer, their general biocidal effect, broad spectrum and extended residual effect in the soil (Mashela, 2007). The non-fumigant nematicides, which were ozone-layer friendly, were primarily nemastatic and non-phytotoxic (Mashela, 2007). Consequently, they were used as both pre-emergent and post-emergent nematicides. However, due to their high toxicity levels to mammals including humans, with high residual concentrations in plant produce because of their systematic nature, there is a worldwide advocacy to have them withdrawn from the markets (Barker, 2004; Roberts *et al*., 2005a,b). Options for reducing Pi at planting are increasingly limited and there is therefore, an urgent need to develop alternative nematode control interventions for use at either pre-emergent or pre-planting.

1.1.2 Impact of the research problem

Plant-parasitic nematodes are a severe constraint in crop production, with worldwide surveys suggesting that every crop-producing farmer is being affected. Worldwide, the damage caused by plant-parasitic nematodes had been estimated at approximately US$100 billion per annum (Koenning *et al*., 1999; Berenbaum, 2000; Ferraz and Brown, 2002; Barker, 2004). Yield losses due to plant-parasitic nematodes range from 8% to 20% in various commercial crops (Ferraz and Brown, 2002; Koenning *et al*., 2003; Adegbite and Adesiyan, 2005), with reports of total crop failure in both commercial and subsistence farming systems (Mashela, 2007). In the United States alone, nematode damage in major crops had been estimated at more than US$5 billion per annum in the USA (Koenning *et al*., 2003), whereas in developing nations the estimates, although statistics are scant, are believed to be much higher. Generally, in the Sub-Saharan region, excluding South Africa, crop yield losses due to nematodes are as high as 50% (Oerke, 2006). In South Africa, the estimated annual yield losses caused by plant-parasitic nematodes in cereal, vegetable and fruit crops amounted to approximately 14%, with monetary value estimated at over R200 million (Cadet and Spaull, 2003).

1.1.3 Possible causes of the research problem

The root-knot nematode - a cosmopolitan plant-parasitic nematode with over 90 species which include *M. incognita (*[Kofoid and White] Chitwoodi), *M. javanica* (Treub) Chitwood, *M. arenarea (*Neal and Chitwoodi) and *M. hapla* (Chitwoodi) (Taylor and Sasser, 1978; Eisenback and Trianataphyllou, 1991; Nickle, 1991), have multiple races,

wide geographical distribution and infecting over 3 000 plant species (De Waele and Elsen, 2007). Consequently, *Meloidogyne* species are difficult to manage using plant resistance (Rizvi and Rizvi, 1992). Due to the existence of different biological races, resistant cultivars bred and developed in developed countries like the USA, cannot be used in other countries like South Africa, unless the biological races had been determined. Also, suspension of synthetic nematicides, which were effective in nematode suppression regardless of the country, exacerbated the research problem.

1.1.4 Proposed solution(s)

Currently, “learning to live with nematodes” is receiving greater attention as one of the possible sustainable alternatives for the management of plant-parasitic nematodes (Mashela, 2007). Uses of bio-nematicides and plant resistance are environment-friendly management interventions, which may be sustainable in the long-term (Akhtar and Malik, 2000; Chitwood, 2002). A variety of plant products have been evaluated to lower Pi in various crops, with contradictory results (Alam, 1989; Dash and Padhi, 1990; Rizvi and Rizvi, 1992; Akhtar, 1993; Abid *et al.*, 1995; McSorley and Gallaher, 1995a,b; Chitwood, 2002).

The Ground Leaching Technology (GLT) system, where crude extracts of wild cucumber (*Cucumis myriocarpus* Naud.) fruit (Figure 1.1), castor bean (*Ricinus communis* L.) fruit and fever tea (*Lippia javanica* Burm.f.) leaves are used at transplanting, had consistently suppressed plant-parasitic nematodes (Mashela, 2002; Mashela and Nthangeni, 2002; Ngobeni *et al*., 2004; Mashela *et al*., 2010). The

uniqueness of GLT system is that it uses much smaller quantities (0.20 – 0.70 mt/ha) of plant materials in crude form when compared to excessively large quantities (10-250 t/ha) used in conventional organic amendment methods. In GLT system, selected plant organs are dried at 52°C, ground and applied at transplanting (Figure 1.1), with active ingredients being leached-out of crude extracts through irrigation water (Mashela, 2002). The technology had been successfully used as a post-emergent intervention in suppressing nematodes, with the added advantage of a fertiliser effect on crops. Recently, Mashela *et al*. (2008) demonstrated that the efficacy of crude extracts of C. *myriocarpus* fruit was comparable to those of aldicarb and fenamiphos in the suppression of plant-parasitic nematodes in tomato (*Solanum lycopersicum* L.) production.

1.1.5 General focus of the study

Generally, crop losses due to nematodes are highest when Pi is high (Seinhorst, 1965). Attempts to use crude extracts of *C. myriocarpus* fruit at planting using 2 g material/plant resulted in complete failure of seed germination (unpublished data). Consequently, detailed investigation was necessary to determine responses of various seeds to various levels of crude extracts of *C. myriocarpus* fruit in order to develop an appropriate dosage for pre-emergent bio-nematicide using this potent material.

Figure 1.1 **A**. Fresh fruit of C*ucumis myriocarpus* attached to the vines. **B**. Harvesting of ripe fruit of C. *myriocarpus.* **C.** Ripe fruit of C. *myriocarpus*. **D**. Post-emergent application of crude extracts of C. *myriocarpus* fruit as bio-nematicide*.*

1.2 Problem statement

At 2 g crude extracts of *C. myriocarpus* fruit per plant at transplanting, the material consistently reduced Pi numbers, with fertiliser effect on the test crops. However, using 2 g crude extracts of *C. myriocarpus* fruit per plant at planting in order to reduce Pi resulted in complete failure of seedling emergence. The researcher proposed to investigate the responses of dicotyledonous and monocotyledonous seedlings using various dosages of crude extracts of *C. myriocarpus* fruit in order to modify GLT system or propose an alternative that could reduce Pi at planting in various economic crops.

1.3 Motivation

Modelling physiological responses of various crops to different levels of crude extracts of *C. myriocarpus* fruit would enhance the possibilities of developing a pre-emergent bio-nematicide that can be used at planting to reduce Pi of plant-parasitic nematodes with a fertiliser effect on crop growth.

1.4 Aim and objectives

1.4.1 Aim

The aim of the study was to investigate the compatibility of crude extracts of *C. myriocarpus* fruit when used as a pre-emergent bio-nematicide on germination and emergence of commercially important dicotyledonous and monocotyledonous crops using empirical tests and computer-generated models.

1.4.2 Objectives

1. To determine whether the crude extracts of *C. myriocarpus* fruit could influence seedling emergence of selected dicotyledonous and monocotyledonous crops when used within the recommended range of GLT systems.

2. To investigate whether germination of dicotyledonous and monocotyledonous crops could have density-dependent responses to various levels of crude extracts of *C. myriocarpus* fruit.

3. To determine whether selected crops in the Alliaceae, Gramineae and Solanaceae families respond to crude extracts of *C. myriocarpus* fruit in a density-dependent way that is characterised by stimulation, saturation and inhibition growth patterns, and the appropriate dosage of crude extracts of *C. myriocarpus* fruit when used as a pre-emergent bio-nematicide in selected crops.

1.5 Hypotheses

1. The crude extracts of *C. myriocarpus* fruit could not influence seedling emergence of selected dicotyledonous and monocotyledonous crops when used within the recommended range of GLT systems.

2. The germination of dicotyledonous and monocotyledonous crops could not have density-dependent responses to various levels of crude extracts of *C. myriocarpus* fruit.

3. The selected crops in the Alliaceae, Gramineae and Solanaceae families do not respond to crude extracts of *C. myriocarpus* fruit in a density-dependent way that is characterised by stimulatory, saturation and inhibition growth patterns, and the crude extracts of *C. myriocarpus* fruit do not have the appropriate dosage when used as a pre-plant bio-nematicide in selected crops.

1.6 Format of the thesis

Subsequent to this General Introduction, literature on the research problem was reviewed (Chapter 2). Then, the subsequent chapters each addressed the above

hypotheses in sequence (Chapters 3 – 5), followed by Chapter 6, which provided a summary of the study, the significance of the findings, the recommended future research and conclusions.

CHAPTER 2
LITERATURE REVIEW

The review on the research problem focused on three themes: (i) what has already been written on the research problem, including the findings and/or contradictions, (ii) existing gaps on the research problem and (iii) the explanation on how the existing gaps would be addressed.

2.1 Work done on the research problem

Interventions for lowering the initial population (Pi) in plant-parasitic nematodes and the materials used are important in understanding the work done on the research problem. The main interventions in reducing Pi in plant-parasitic nematodes depend on the time that the control material is applied, namely, before planting (pre-planting), at planting (pre-emergent) or after planting (post-emergent). The focus of the materials used had been in respect to phytotoxicity, which determined the choice of placement time for suppressing Pi.

2.1.1 Interventions for suppressing nematodes

Generally, the materials applied before planting are highly phytotoxic, whereas those applied at planting or after emergence are less phytotoxic. The following are three recognised interventions of reducing Pi in the management of plant-parasitic nematodes.

2.1.1.1 Pre-planting interventions

In these interventions, nematode-suppressive materials are applied before planting due to their phytotoxicity to the cultivated crops. The previously suspended pre-plant fumigant nematicides were liquids that volatilise to a gaseous state soon after entering the soil (Lucas and Talavera, 2009). Pre-plant interventions protect the root system of young sensitive seedlings against nematodes that are usually present in the soil before planting (Starr *et al*., 2002).

During the last 60 years, Pi of *Meloidogyne* species and other economic phyto-parasitic nematodes had been effectively reduced through fumigant nematicides, which were then an integral part of soil preparation (Roberts, 1993; Maqbool and Kerry, 1997; Starr *et al*., 2002; Roberts *et al*., 2005a). Although there were difficulties in delivery to the target pest, fumigant nematicides had been to date rated as the most effective in the control of plant-parasitic nematodes (Maqbool and Kerry, 1997), since they had extended biocidal residues in the soil, in certain instance with residual effects lasting for up to 10 years (Mashela, 2007).

Following President John Kennedy's decree against the unscrupulous use of fumigant pesticides as articulated in Silent Spring (Carson, 1962), the dangers associated with the use of fumigant nematicides increasingly became public-knowledge. Widespread protests resulted in boycotts of treated agricultural produce. Association of these halogenated pesticides with ozone-depletion persuaded worldwide collective action to curb the pending catastrophes from the use of the materials.

The reluctant adoption and implementation of the 1987 Montreal Protocol and the subsequent introduction of the 1997 Kyoto Protocol persuaded signatory governments to enforce, through legislation, the suspension of ozone-depleting and greenhouse-inducing materials, respectively. Among the listed chemicals under the Montreal Protocol were methyl bromide (MB), dichloro-diphenyl-trichloroethane (DDT) and 1, 2-dibromoethane (EDB), all renowned for their high efficacy in reducing Pi of *Meloidogyne* species prior to planting (Maqbool and Kerry, 1997). The cited protocols mandated the elimination of fumigant nematicides in developed countries by 2005, whereas in developing countries the cut-off date was 2010 (UNEP, 2000). Owing to the cost associated with phytotoxicity tests, environmental and health hazard tests, new pre-plant synthetic nematicides had not been forthcoming to substitute fumigant nematicides (Rizvi and Rizvi, 1992; Maqbool and Kerry, 1997).

2.1.1.2 Pre-emergent interventions

In these interventions, the materials were applied at planting and had been mainly the systemic non-volatile nematicides classified either as carbamates, oxy-carbamates or organophosphates (Keetch, 1982; Maqbool and Kerry, 1997). Materials used in these interventions, were either mechanically mixed throughout the soil profile, applied in aqueous solutions or mixed with seeds or with fertilisers in granular form. Most non-fumigant nematicides were applied at planting or even at post-planting due to their limited phytotoxicities.

Bio-nematicides that inhibit germination would obviously not be suitable for use at planting. Inhibition of seed germination in response to phyto-chemicals released by plant species, including sorghum, wheat and rye, have been consistently reported in literature (Inderjit and Duke, 2003; Kupidlowska *et al*., 2006). Most plants in the Cucurbitaceae family, such as the bitter mutant hawkesbury watermelon (*Citrullus vulgaris* Schad.); contain cucurbitacin, which previously inhibited seed germination of watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai], squash *(Cucurbita maxima* L.*)* and tomato (Martin and Blackburn, 2003). However, cucurbitacin from hawkesbury watermelon did not inhibit germination of maize (*Zea mays* L.) seeds. Wild cucumber (*C. myriocarpus*) fruit are known to contain high levels of water-soluble cucurbitacin A (Chen *et al*., 2005). Consequently, it is important to investigate if its crude extracts could not serve as pre-emergent bio-nematicide.

2.1.1.3 Post-planting interventions

In these interventions, the materials are applied after planting either as aqueous solutions, granular or in powder form. However, management interventions in this category include the use of resistant rootstocks, which are currently widely used in vegetable production (Pofu, 2011) and organic amendments, which had proved beyond reasonable doubt that their effectiveness as nematode suppressants warranted further investigation (Stirling, 1991). For instance, inconsistent nematode suppression results, large quantities required to effect nematode suppression (10-250 t/ha), extended waiting periods required to ameliorate negative periods and lowering of soil pH, are

some of the frequently cited drawbacks of organic amendments (Thomason, 1987; Stirling, 1989; Mashela, 2002; Kokalis-Burelle and Rodriquez-Kabana, 2006).

Mashela (2002) introduced the ground leaching technology (GLT) system as one of the post-planting interventions to manage nematodes in vegetable production, with the view of ameliorating drawbacks associated with application of conventional organic amendments. Briefly, the technology involves using small quantities (0.20-0.72 t/ha) of powdered organs from selected plants to suppress plant-parasitic nematodes. The bio-nematicidal potential of crude extracts of *C. myriocarpus* fruit had been demonstrated in various studies (Mashela and Mphosi, 2001; Mashela, 2002; Mphosi *et al*., 2004; Mashela *et al*., 2008). Crude extracts of *C. myriocarpus* fruit suppressed nematode egg-hatch in *vitro* from 97 to 99%, whereas in *vivo M*. *incognita* juvenile numbers were reduced from 92 to 93% in soil (Mashela, 2002). Under both conditions, crude extracts increased electrical conductivity (EC), but had no effect on soil pH. Release of toxic compounds from crude extracts of *C*. *myriocarpus* fruit was believed to be independent of soil microorganisms, suggesting that the toxic compounds were water-soluble (Mashela, 2002). Under field studies, crude extracts of *C. myriocarpus* fruit were independent of the activities of *Bacillus* species (Mabitsela *et al*., 2004; Mphosi *et al*., 2004), confirming the hypothesis which suggested that microbial decomposition was not a prerequisite for the nematicidal activity of the material (Mashela, 2007, 2002).

2.1.2 Phytotoxic concepts in nematode management

The term allelopathy was first introduced by Molisch (1937) and described as biochemical interactions between plants of different species, which included both inhibitory and stimulative responses. Rice (1984) re-defined allelopathy as the effect, both positive and negative, of one plant on the growth of another plant, through the release of chemicals into the rhizosphere. Chemical compounds involved in the interactions were referred to as “allelochemicals”. Generally, allelopathy is dependent upon an allelochemical compound being added to the rhizosphere from another living or dead plant part.

Extracts of most plants through allelopathic pathways are able to kill or suppress nematodes, disrupt their life cycles or discourage them from feeding (Abbasi-Alikamar *et al*., 2005). Allelochemicals exist in virtually all plant tissues and plants generally store them in plant cells in inactive forms, such as water-soluble glycosides and polymers, which include tannins, lignins and salts (Einhellig and Leather, 1988). Allelochemicals are biosynthesised from the metabolism of carbohydrates, fats and amino acids, which arise from mevalonic or the shikimic acid pathways (Akhtar, 1993). Plant phenolics and alkaloids originate from the shikimate pathways, whereas the terpenoids originate from the mevalonic pathways (Inderjit, 1996; Inderjit and Malik, 2002). According to Putnam and Tang (1986), plant phenolics, alkaloids and terpenoids are three major allelochemicals that are implicated in defence mechanisms of plants.

Numerous plants have been investigated for allelopathic activity towards nematodes (Kokalis-Burelle and Rodríguez-Kabana, 2006). A suppressive effect on nematode, possibly mediated by releases of allelochemical compounds have been reported in a wide range of temperate and tropical plants, such as neem (*Azadirachta indica* A. Juss), cabbage (*Brassica* spp.), marigold (*Tagetes* spp.), vetch (*Vicia* spp.), castor bean (*Ricinus communis*), fever tea (*Lippia javanica*), wild watermelon (*Cucumis africanus* L.f.) and *C. myriocarpus* (Ferris and Zheng, 1999; Akhtar and Malik, 2000; Fahey *et al*., 2001; Mashela, 2002; Kokalis-Burelle and Rodríguez-Kabana, 2006; Mashela *et al*., 2010). Experiments which evaluated plant species documented in Chinese traditional medicines identified 153 aqueous plant extracts to possess activity against plant-parasitic nematodes (Ferris and Zheng, 1999).

An allelochemical has a potential to inhibit the growth of a plant species at a certain concentration and to stimulate the growth of the same species or another at a lower concentration (Rice, 1984; Putnam and Tang, 1986). Most allelochemicals are phytotoxic, but have the potential of being used as pesticides or as templates for new pesticides, since they are responsible for defence in plants. Allelochemicals are released through volatilisation, root exudation and decomposition of plant residues or extracts (Putman and Tang, 1986, Einhellig and Leather, 1988; Inderjit, 1996).

Toxic components in crude extracts of *C*. *myriocarpus* fruit are cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$), which are collectively referred to as cucurbitacins (Van Wyk *et al*., 1997). Cucurbitacin A accumulates in fruit and roots of *C*. *myriocarpus,* whereas

cucurbitacin B accumulates in all organs of *C. africanus*. The cucurbitacins are amongst the bitterest substances known to man (Rimington, 1938; Jeffery, 1978), with more than 12 isolated and identified molecular structures (Chen *et al*., 2005; Cry *et al*., 2006). Mashela *et al*. (2008) demonstrated that the efficacy of crude extracts of *C. myriocarpus* fruit on suppression of *M. incognita* race 2 in tomato was similar to those of aldicarb and fenamiphos.

2.2 Work not yet done on the research problem

Much work using crude extracts of *C. myriocarpus* fruit, have been done in the management of the southern root-knot nematode at transplanting of various crops (Mashela, 2002; Mphosi *et al*., 2004; Mashela *et al*., 2008). However, the compatibility of crude extracts of *C. myriocarpus* fruit with seed germination and seedling emergence, for the material to be used to suppress Pi in various crops, has not been investigated.

2.3 Addressing the identified gaps

Generally, biological systems respond to extrinsic or intrinsic factors in accordance to the density-dependent growth pattern, which is characterised by specific concentration-dependent dosages for stimulation, inhibition and saturation of growth (Mamphiswana *et al*., 2010). In order to successfully investigate whether crude extracts of *C. myriocarpus* fruit could be used as pre-emergent bio-nematicide, a series of experiments needed to be conducted, to determine the appropriate concentration (dosage) of crude extracts of *C. myriocarpus* fruit for various crops in relation to density-dependent growth pattern responses. In order to determine the dosages, one had first to establish the position

where the normal dosage used in GLT for suppression of *M. incognita* stood in relation to density-dependent growth responses (Salisbury and Ross, 1992), which required computer modelling.

The Curve-fitting Allelochemical Response Data (CARD) model, developed to quantify allelopathic responses in various biological systems (Liu *et al*., 2003), is suitable for use to identify dosages of crude extracts of *C. myriocarpus* fruit responsible for stimulation, saturation and inhibition growth responses. The CARD model was briefly reviewed as originally described (Liu *et al*., 2003): When R was the response of a testing organism, D a dosage of an allelochemical, and R_c the response of untreated control in the bioassay, the model was written as follows:

$$R = R_C + E(D) \quad (1)$$

where $E(D)$ was the effect of the allelochemical. Stimulation corresponded to $E(D) > 0$, whereas inhibition occurred when $E(D) < 0$. First, consider the case where $E(D)$ was a simple quadratic equation, so that:

$$E(D) = \alpha D - \beta D_2 \quad (2)$$

where α, β (> 0) were constants. When stimulation corresponded to $D < \alpha/\beta$, and inhibition to $D > \alpha/\beta$, then Equation 1 became:

$$R = R_C + \alpha D - \beta D_2 \quad (3)$$

When *D* was large, *R* would be negative, which was physiologically unacceptable. Consequently, the model would only apply over the range where $R > 0$. When $\alpha > 0$ and $\beta > 0$, the response curve had stimulation at low dosages; otherwise there would be no stimulation. Equation 3 was basically a quadratic function. The choice of the quadratic equation roots emanated from the consideration of inverted U-shaped biological responses with the mathematical curve shape. In practice, however, a quadratic equation would hardly possess a feature of flexibility in describing biological responses. In order to overcome this, the *D* term in Equation 3 was replaced by a function of the dosage, *g(D)*, so that:

$$R = R_c + \alpha g(D) - \beta [g(D)]^2. \quad (4)$$

To analyse the similarities in plant and animal responses to allelochemical stress, Lovett *et al*. (1989) used $g(D) = ln(D+1)$, which gave a good fit to several sets of data. In the present model, this approach was generalized as:

$$g(D) = \overbrace{\ln(\ln(\cdots(\ln}^{k}(D + \overbrace{1)\cdots+1)+1)}^{k} \quad (5)$$

where *k* is the number of $ln(D + 1)$ transformations. Equation 4 then became:

$$R=R_c+\alpha\ln(\ln(\ldots\ln(D+1)\ldots+1)+1)-\beta[\ln(\ln(\ldots\ln(D+1)\ldots+1)+1]^2. \quad \textbf{(6)}$$

The case of k = 0 was denoted as no transformation. Thus, when k = 0, Equation 3 was referred. Features of Equation 6 were that the value of the untreated control remained at zero [*i.e*., ln(ln(.ln(0 + 1).+1) +1) = 0], and the stimulation peak changed from a standard quadratic curve (when k = 0). Thus, Equation 6 could account for a wide range of stimulation-inhibition responses. The k might biologically be a sensitive indicator of stimulation. The equation was symmetrical quadratic when R was plotted against $g(D)$.

To look at the properties of the equation, Equation (4) could be written as:

$$R=R_c+\frac{\alpha^2}{4\beta}-\beta\left[g(D)-\frac{\alpha}{2\beta}\right]^2. \quad \textbf{(7)}$$

The maximum value of R, when defined as R_m converted equation 7 to:

$$R_m=R_c+\frac{\alpha^2}{4\beta}. \quad \textbf{(8)}$$

Thus, the highest stimulation value (R_h), would convert equation 8 to:

$$R_h=R_m-R_c=\frac{\alpha^2}{4\beta}. \quad \textbf{(9)}$$

By defining D_m as the dosage that gave the highest stimulation, from Equations 5 and 7, D_m could be as follows:

$$D_m=\exp\left(\ldots\left(\exp\left(\exp\left(\frac{\alpha}{2\beta}\right)-1\right)-1\right)\ldots\right)-1. \quad \textbf{(10)}$$

When defining D_p as the dosage that resulted in a p% reduction in the process, due to the allelochemical, from Equation 4, the following resulted:

$$g(D_p)=\frac{\alpha+\sqrt{\alpha^2+0.04p\beta R_c}}{2\beta} \quad \textbf{(11)}$$

and hence

$$D_p=Exp\left(\ldots\left(Exp\left(Exp\left(\frac{\alpha+\sqrt{\alpha^2+0.04p\beta R_c}}{2\beta}\right)-1\right)-1\right)\ldots\right)-1. \quad \textbf{(12)}$$

In particular, the dosages corresponding to 0 and 50% reduction, D_0 and D_{50} respectively, were calculated by the computer using Equation 12. D_0 was the threshold dosage below which stimulations occurred, and above which inhibitions appeared. D_{50} could be used as a measure of the inhibition potency of an allelochemical or the sensitivity of the testing organism to the allelochemical.

The Curve-Fitting Procedure for Equation 6 was illustrated in Figure 2.1. The approach made successive transformations, which were fitted the data to Equation 4 for each transformation. Multilinear regression analysis was then used to determine the parameters, $R_{c,i}$, α_i, β_i, where *i* equalled 0, 1, 2, for nil, 1, 2, logarithmic transformations, respectively. The predicted values, $\tilde{R}_i = R(R_{c,i}, \alpha_i, \beta_i)$, were calculated for each transformation. Then, linear regression was used to fit predicted values, $\tilde{R}_i$, to the observed values, R_0, resulting into the following hypothetical dosage-response curve:

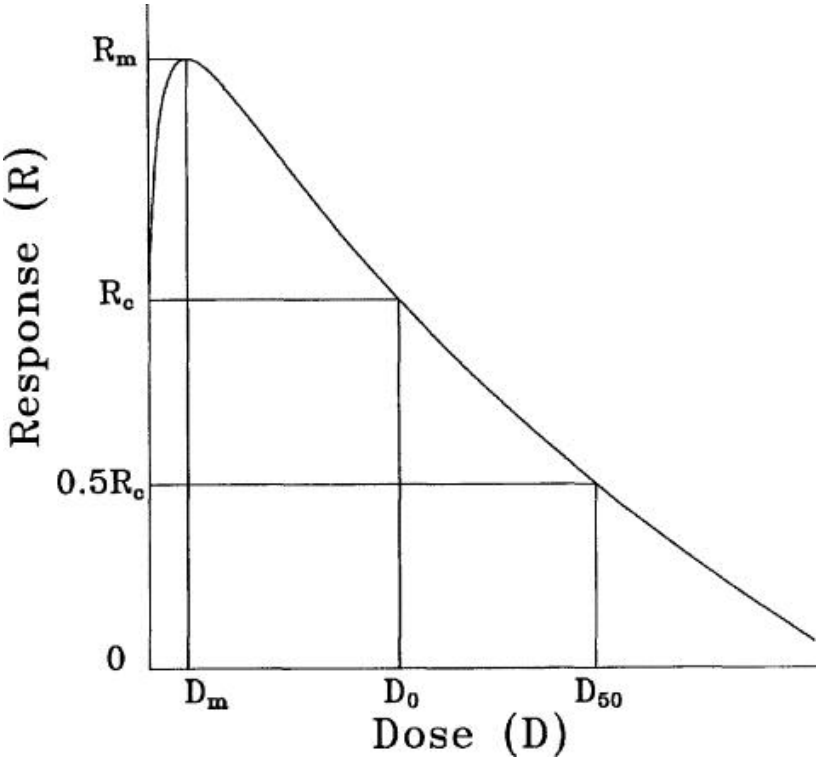

Figure 2.1 A hypothetical allelochemical dosage-response curve. R_m was the maximum stimulating peak, D_m was the dosage that gave the stimulating peak, D_0 was the dosage that gave no effect and D_{50} was the dosage that gave 50% reduction of untreated control yield.

$$\tilde{R}_i = a + bR_o + \varepsilon. \quad \textbf{(13)}$$

The number of transformations was determined when the *k*-transformations gave the highest coefficient of determination (R^2). The criterion for determination of *k* was:

$$r_{k-1}^2 < r_k^2 > r_{k+1}^2 \text{ or } r_{k+1}^2 - r_k^2 \leq 0.01 \quad \textbf{(14)}$$

where the subscription denoted the number of transformations.

2.4 Summary of the gaps to be investigated

Crude and aqueous extracts of *C. myriocarpus* fruit would be used in the present study to determine their compatibility with selected dicotyledonous and monocotyledonous crops. Empirical tools and models would be employed to establish dosages of crude extracts of *C. myriocarpus* fruit required for various crops when applied at planting.

CHAPTER 3
INFLUENCE OF C*UCUMIS* BIO-NEMATICIDE ON SEEDLING EMERGENCE OF SELECTED DICOTYLEDONOUS AND MONOCOTYLEDONOUS CROPS

3.1 Introduction

The damage caused by plant-parasitic nematode is directly proportional to the initial nematode population density (Pi) at planting or transplanting (Seinhorst, 1965). Thus, it is preferable that Pi, at all times be at its lowest at planting. Fumigant nematicides, used as pre-plant intervention tactics, were the most effective since they reduced Pi to the minimum. Also, most non-fumigant synthetic nematicides have been successful in crop production because they could be used as pre-emergent nematicides without inducing any phytotoxicity. However, most of these materials, due to their eco-unfriendliness and mammalian toxicity had been withdrawn from the agro-chemical markets, with increased focus being on alternatives such as organic amendments. Drawbacks of conventional organic amendments in suppression of plant-parasitic nematodes, includes inconsistent results in nematode suppression, large quantities needed to achieve effective control, unavailability of most effective materials, high transport costs, waiting-period required for microbial decomposition and lowering of soil pH (Muller and Gooch, 1982; Rodriquez-Kabana, 1986; Stirling, 1991; Bello, 1998; McSorley and Gallaher, 1995a; Mashela, 2002).

In an attempt to ameliorate the drawbacks of conventional organic amendments, the Land Bank Chair of Agriculture - University of Limpopo, developed the ground leaching technology (GLT) system, which involves spot application of powdered materials from selected plant organs in a shallow hole around the base of the stem of the transplant at 2 g (Mashela and Mphosi, 2001; Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2010). The GLT system relies on irrigation water to leach potent bio-chemicals into the soil (Mashela and Nthangeni, 2002). In a number of screening trials,

ground wild cucumber (*Cucumis myriocarpus* Naud.) fruit, castor bean (*Ricinus communis* L.) fruit and fever tea (*Lippia javanica* Burm.f.) Mill) leaves consistently reduced densities of the southern root-knot nematode (*Meloidogyne incognita* [Kofoid and White] Chitwood) in root and soil samples (Mashela and Mphosi, 2001; Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2010). Regardless of the organic amendment source, when used as post-emergent bio-nematicide, the material had fertiliser effect, but had no effect on soil pH, with the exception of *L. javanica* leaves, which reduced soil pH (Mashela *et al*., 2010). The efficacy of ground *C. myriocarpus* fruit on nematode suppression was comparable to that of synthetic systemic nematicides, *viz*. aldicarb and fenamiphos (Mashela *et al*., 2008). However, growth responses of emerging seedlings to crude extracts of *C. myriocarpus* fruit are not documented. The objective of the study was to determine the effect of crude extracts of *C. myriocarpus* fruit on seedling emergence in dicotyledonous and monocotyledonous crops when used within the recommended range of GLT systems.

3.2 Materials and methods

Separate experiments, each comprising one of the ten dicotyledonous or eight monocotyledonous crops, were conducted at the Horticultural Unit of the University of Limpopo (23º53’10”S, 29º44’15”E) from May 2008 through December 2010. Fruit of *C. myriocarpus* were locally collected from the wild, cut into pieces and dried at 52°C for 5 days in air-forced ovens to minimise the loss of volatile phytochemicals (Makkar, 1999). Dried materials were ground in a Wiley mill through 1-mm-mesh sieves. Prior to use, the ground material was stored at room temperature in sealed plastic bags. Thirty 15-cm-diameter plastic pots were placed on the greenhouse benches and filled with 5 ℓ

growing mixture, comprising pasteurised sand and Hygromix at 3:1 (v/v). Ambient day/night temperatures averaged 27°C/18°C, with maximum temperatures managed using automatic electrical thermostat.

3.2.1 Experimental design and cultural practices

Seven treatments, *viz*. 0, 2.5, 5, 7.5, 10, 12.5 and 15 g crude extracts of *C. myriocarpus* fruit per pot, were arranged in a randomised complete block design, with five replications. Individual experiments on dicotyledonous crops included bean (*Phaseolus vulgaris* L.) cv*. '*Contendor', chilli (*Capsicum frutescence* L.) cv. 'Long Slim Cayenne', cucumber (*Cucumis sativus* L.) cv. 'Delight Green F1', eggplant (*Solanum melongena* L.) cv. 'Black Beauty', lettuce (*Latuca sativa* L.) cv. 'Great Lakes', pea (*Pisum sativum* L.) cv. 'Hygrotech J12082', pepper (*Capsicum annum* L.) cv. 'Capistrano', sunflower (*Helianthus annaus* L.) cv. 'PAN 7033', tomato (*Solanum lycopersicum* L.) cv. 'Floradade' and watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cv. 'Crimson Giant'. Experiments on monocotyledonous crops included chive (*Allium schoenoprasum* L.) cv. 'Hygrotech J03940', leek (*Allium ampeloprasum* L.) cv. 'Hygrotech G07157), maize (*Zea mays* L.) cv. 'SNK 2147', millet (*Panicum miliaceum* L.*)* cv. 'Babala [OPV]', onion (*Allium cepa* L.) cv. 'Texas Grano', rye (*Secale cereal* L.*)* cv. 'ARC-FRI PBR', sorghum (*Sorghum bicolor* (L.) Moench) cv. 'Pannar 8609' and wheat (*Triticum aestivum* L.*)* cv. 'Caledon. The time of planting for each crop was as proposed in the 2009 Hygrotech Planting Guide (Hygrotech, 2009).

Meloidogyne incognita race 2 inoculum was prepared by extracting eggs and second-stage juveniles (J2s) from roots of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' plants in 1% NaOCl (Hussey and Barker, 1973). The J2s were collected after eggs had been incubated for 5 days on modified Baermann trays (Rodriguez-Kabana and Pope, 1981). Two seeds per pot were planted at commercially prescribed depths (Hygrotech, 2009). At planting, organic amendments were applied in separate holes and covered with growing medium, which was irrigated to field capacity. A day after planting, pots were each infested with nematodes by dispensing approximately 5 000 J2s of *M. incognita* race 2 using a 20-mℓ plastic syringe by placing into 5-cm-deep holes on the cardinal points of the seeded hole. Each pot was irrigated with 250 mℓ tapwater every other day.

3.2.2 Data collection

Successful seedling emergence was recorded as the appearance above the soil surface of the hypocotyl for dicotyledonous crops, whereas for monocotyledonous crops and the garden pea the epicotyl appearances were recorded and marked to ensure that recording was done once. Data were daily recorded for 14 days and expressed as percentage seedling emergence. Nematodes were extracted from 250 mℓ soil subsamples using the sugar-floatation and centrifugation method (Coolen and d'Herde, 1972). Juveniles were further separated from aliquots using the Baermann method (Rodriguez-Kabana and Pope, 1981) in order to exclude dead nematodes, with nematodes counted from a 10-mℓ aliquot under a stereomicroscope.

3.2.3 Data analysis

Prior to analysis, nematode and emergence data were transformed using $Log_2 (x +1)$ in order to homogenise the variances (Gomez and Gomez, 1984), but untransformed data were reported. Nematode and seedling emergence data were subjected to analysis of variance (ANOVA) with SAS program (SAS Institute Inc., 2004). Treatment means ($P \leq 0.05$) were separated using Waller-Duncan multiple-range test, and lines of the best fit between variables measured and dosages of crude extracts of *C. myriocarpus* fruit were generated. Unless stated otherwise, only treatment means that were significant at the probability level of 5% are discussed.

3.3 Results

In the untreated control, there were averages of 638 juveniles of *Meloidogyne* species, whereas in all treatment levels the nematode counts were from zero to negligible numbers (data not shown). Crude extracts of *C. myriocarpus* fruit inhibited emergence of the test plants regardless of whether they were dicotyledonous or monocotyledonous crops. Partitioning of the sum of squares for dicotyledonous crops indicated that the treatment explained 81%, 81%, 88%, 70% and 52% of the total treatment variation in emergence of bean, chili, cucumber, eggplant and lettuce, respectively (Appendices 3.2 - 3.6). In pea, pepper, sunflower, tomato and watermelon, the total treatment variation in emergence was explained by 81%, 88%, 79%, 54% and 64%, respectively (Appendices 3.7 - 3.11). Similarly, in monocotyledonous crops the treatment explained 81%, 84%, 88%, 63% and 79% of the total treatment variation in emergence of chive, leek, maize and millet, respectively (Appendices 3.12 - 3.15). In emergence of onion, rye, sorghum

and wheat, the total treatment variation was explained by 78%, 78%, 60% and 84%, respectively (Appendices 3.16 - 3.19).

Emergence of all cultivars tested had strong negative quadratic relationships when regressed against the crude extracts of *C. myriocarpus* fruit, regardless of whether seeds were from dicotyledonous or monocotyledonous crops. Treatment levels on dicotyledonous seeds (Figures 3.1-3.10) contributed 99%, 98%, 99%, 98%, 98%, 98%, 98%, 93%, 97% and 94% to the total treatment variation in mean seedling emergence of bean (Figure 3.1), chilli (Figure 3.2), cucumber (Figure 3.3), eggplant (Figure 3.4), lettuce (Figure 3.5), pea (Figure 3.6), pepper (Figure 3.7), sunflower (Figure 3.8), tomato (Figure 3.9) and watermelon (Figure 3.10), respectively. Similarly, treatment levels on monocotyledonous seeds (Figures 3.11-3.18) contributed 98%, 98%, 98%, 98%, 99%, 95%, 99% and 97% to the total treatment variation in mean seedling emergence of chive (Figure 3.11), leek (Figure 3.12), maize (Figure 3.13), millet (Figure 3.14), onion (Figure 3.15), rye (Figure 3.16), sorghum (Figure 3.17) and wheat (Figure 3.18), respectively.

FIGURES 3.1 – 3.10: DICOTYLEDONOUS CROPS (PAGES 30 – 39)

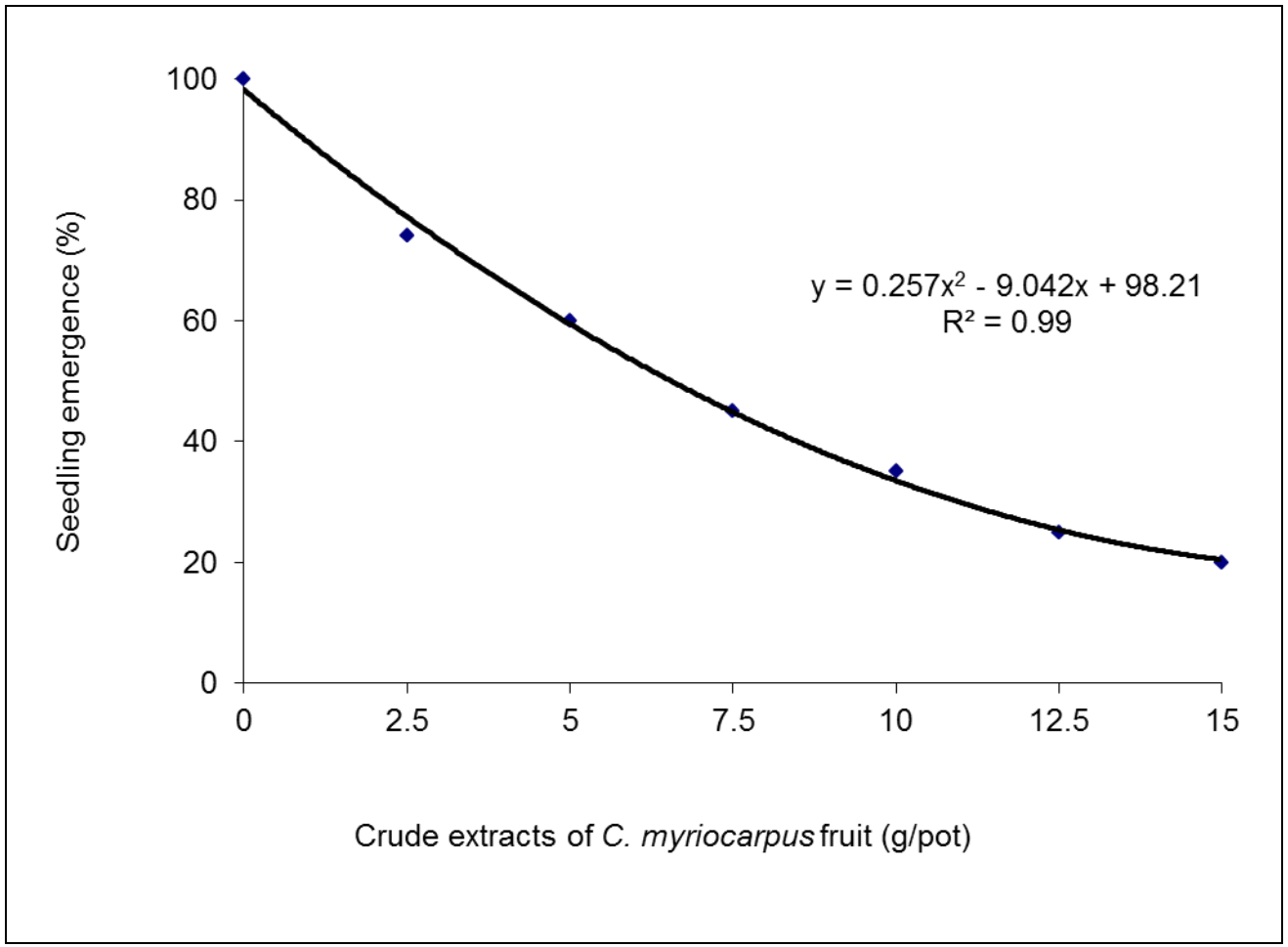

Figure 3.1 Quadratic relationship between bean seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

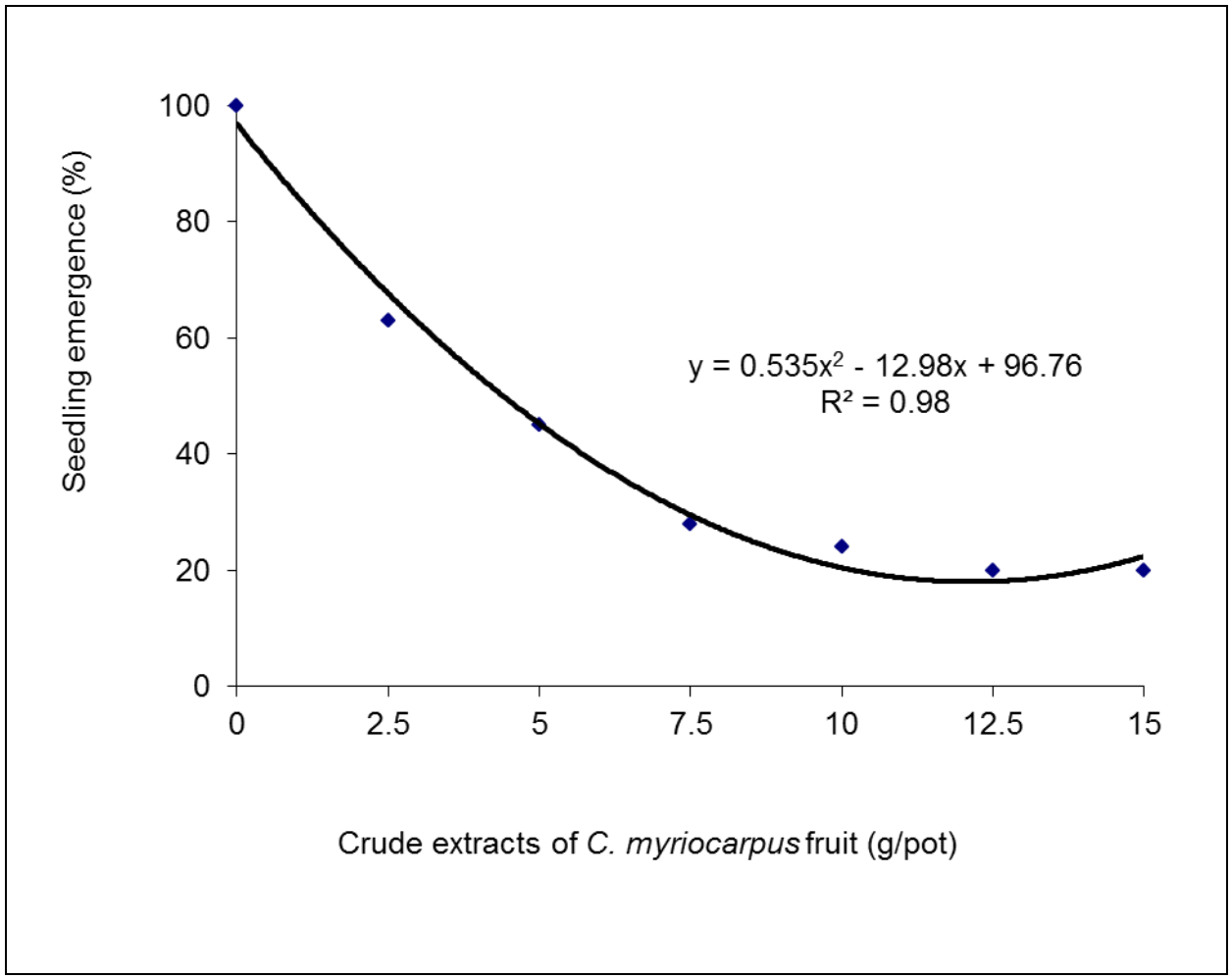

Figure 3.2 Quadratic relationship between chilli seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

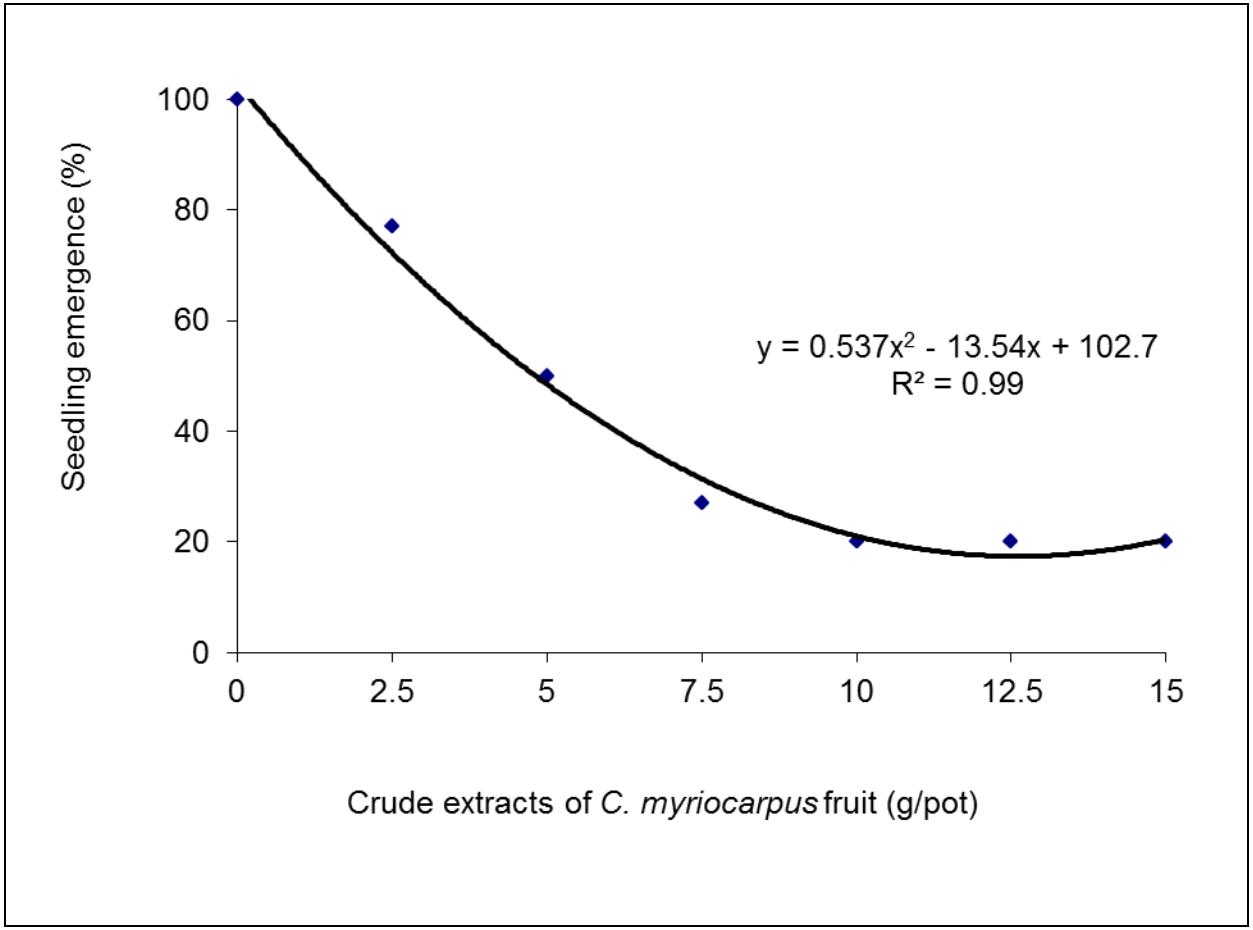

Figure 3.3 Quadratic relationship between cucumber seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

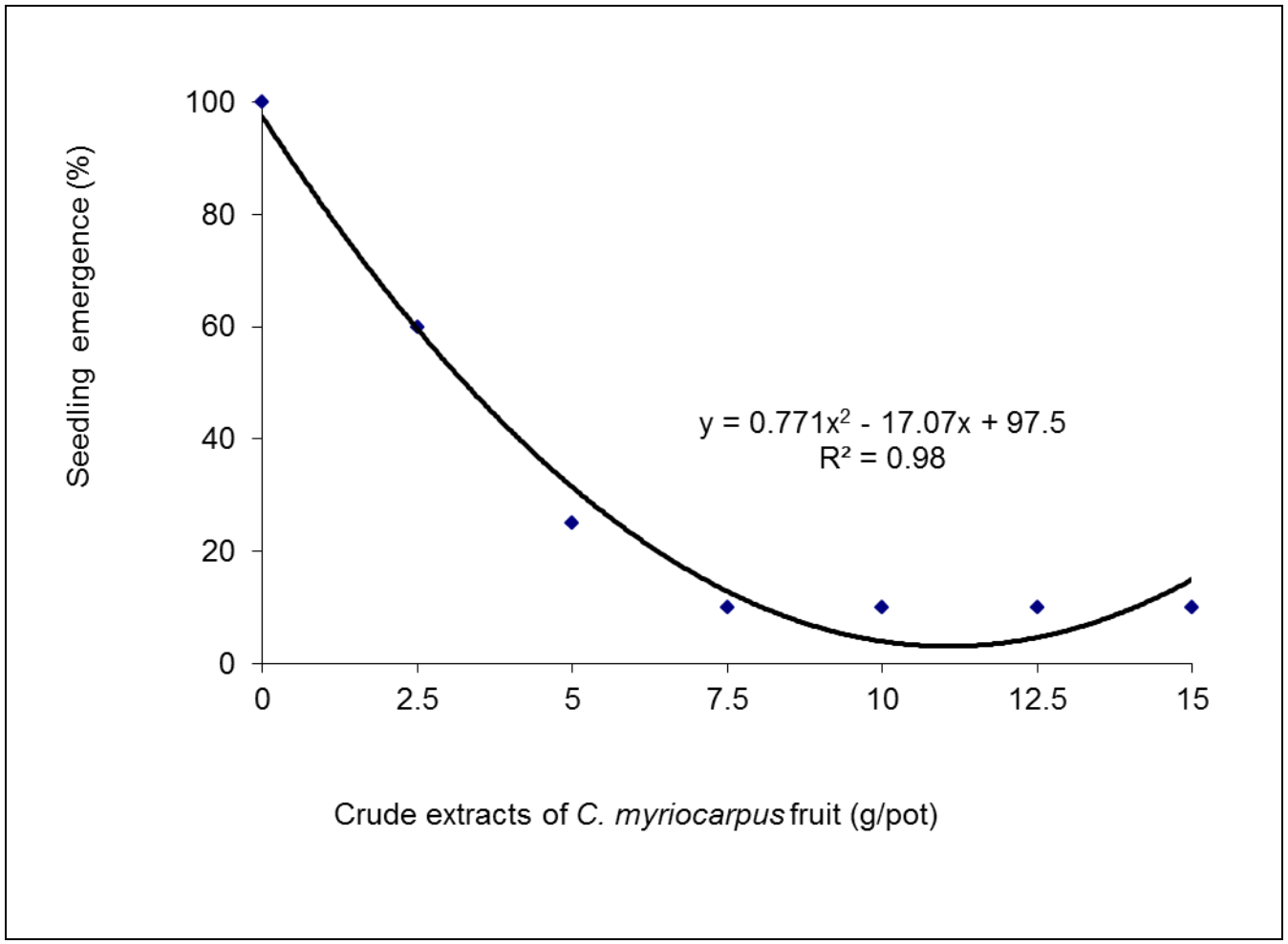

Figure 3.4 Quadratic relationship between eggplant seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

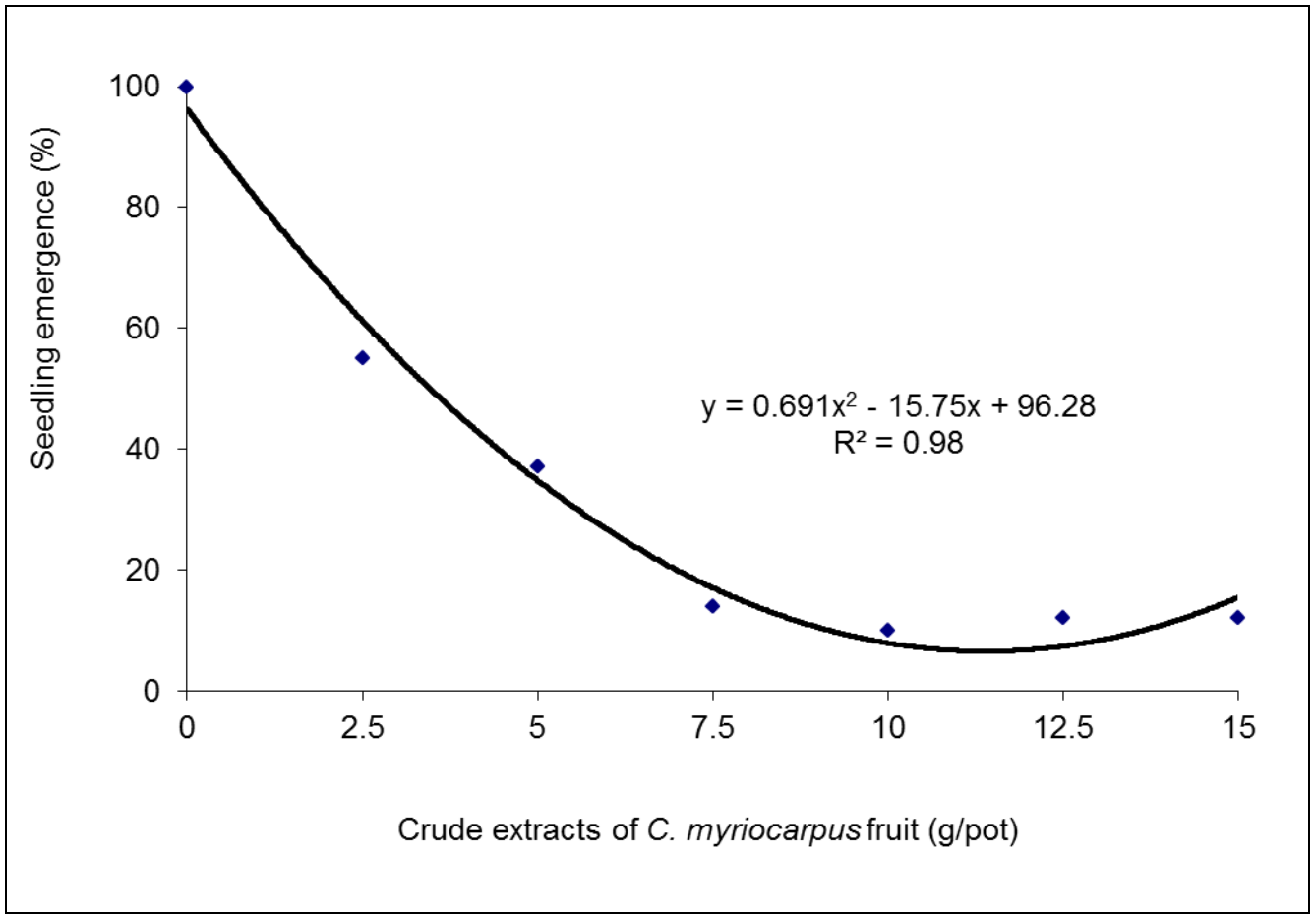

Figure 3.5 Quadratic relationship between lettuce seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

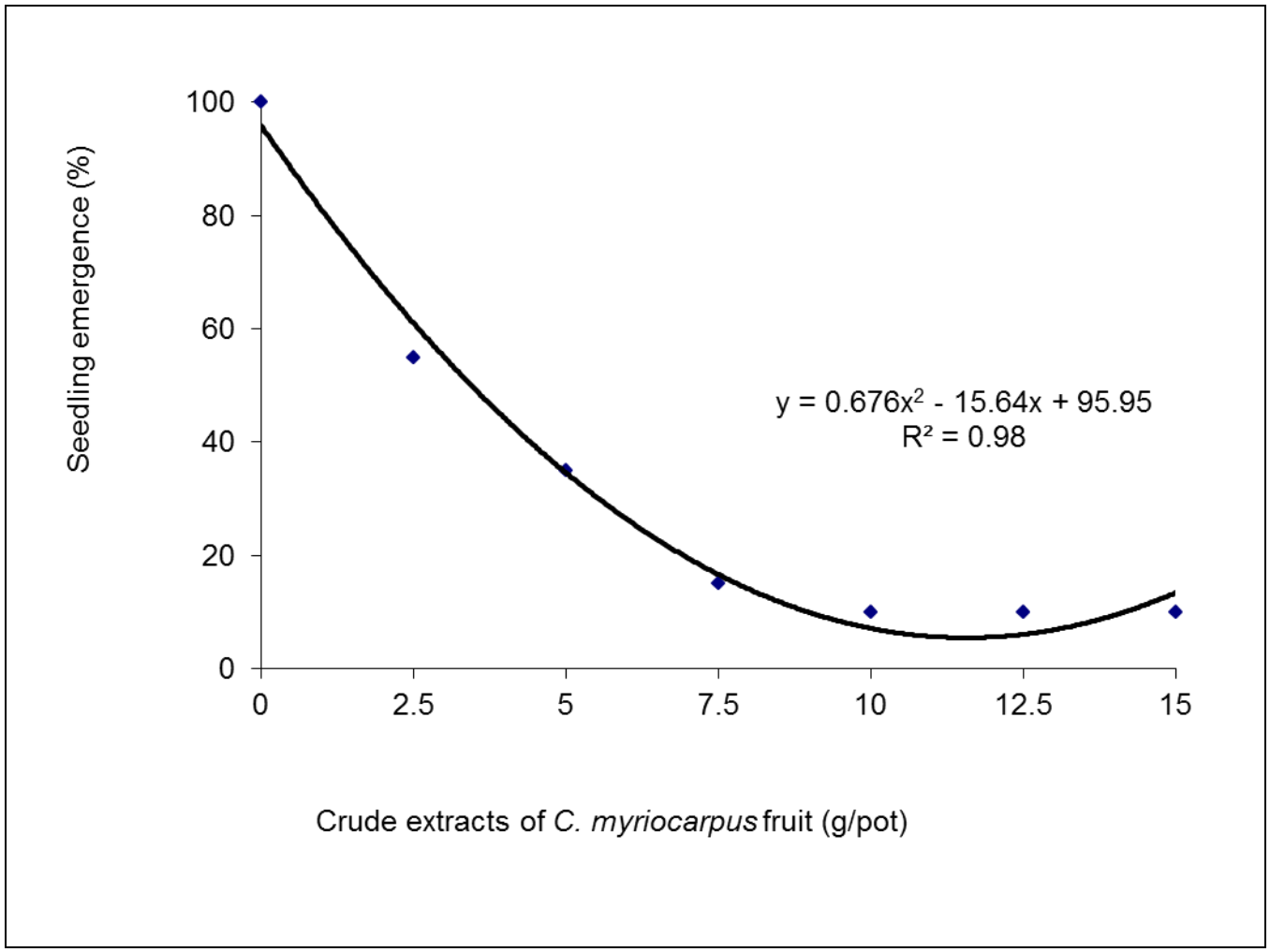

Figure 3.6 Quadratic relationship between pea seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

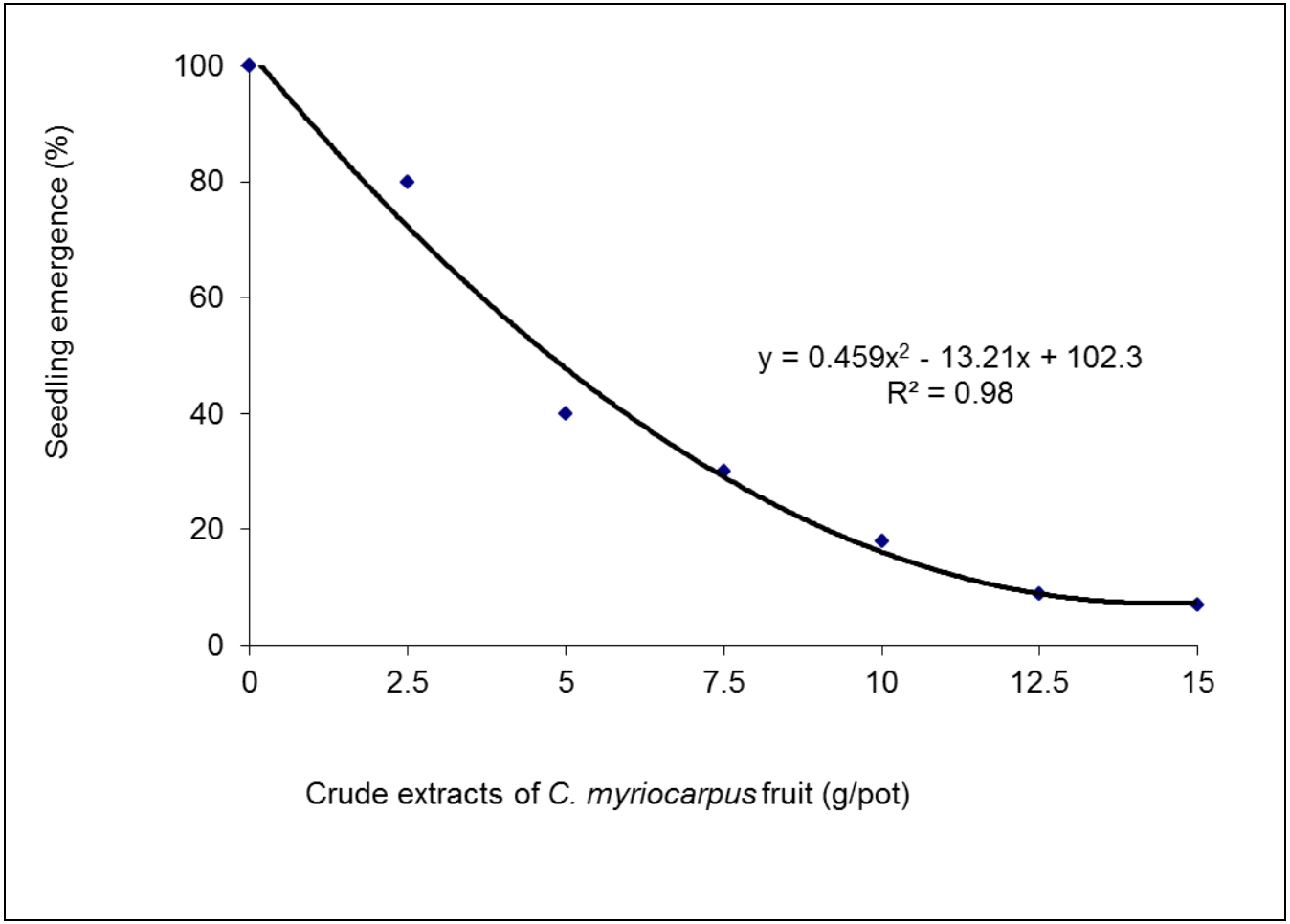

Figure 3.7 Quadratic relationship between pepper seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

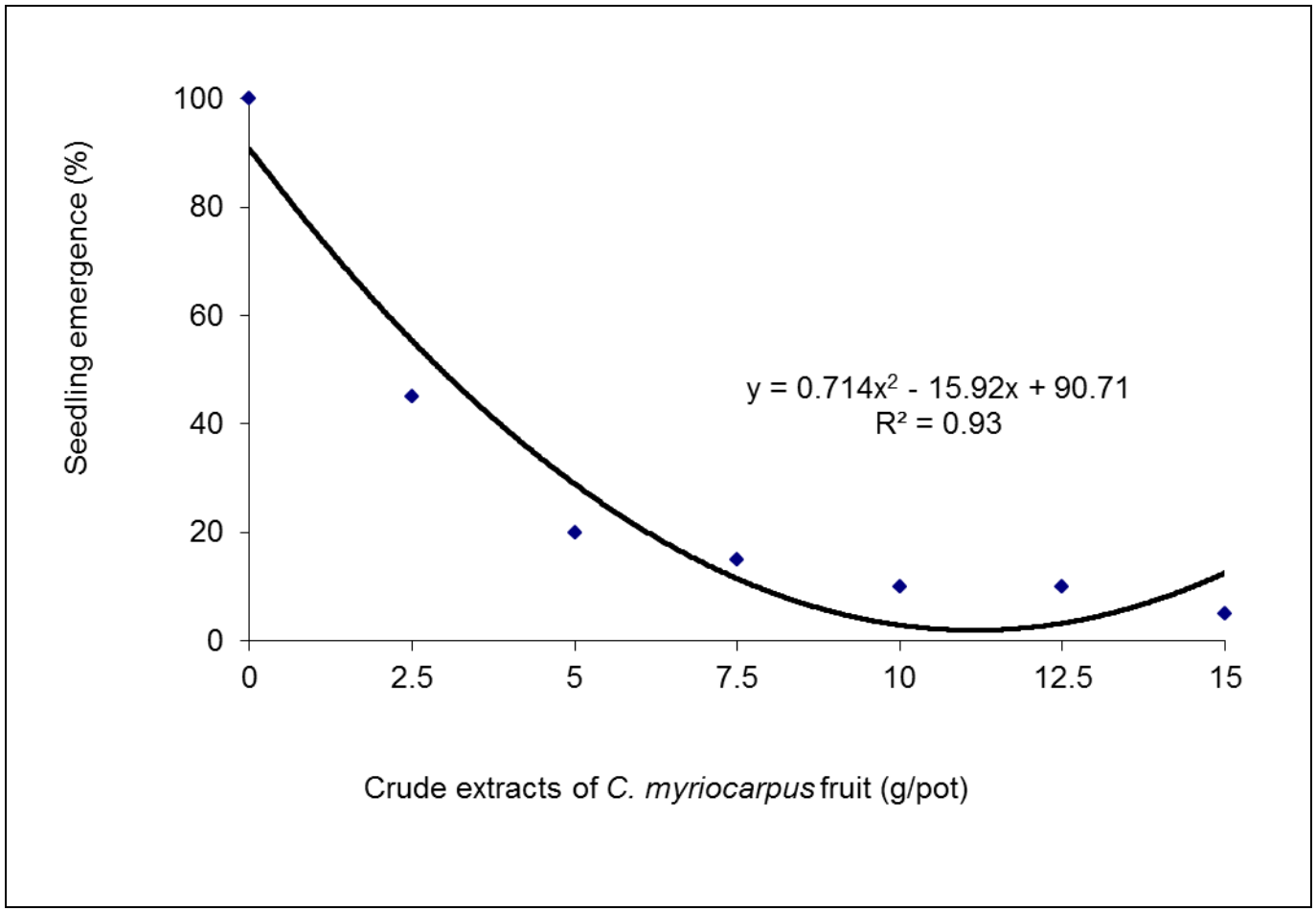

Figure 3.8 Quadratic relationship between sunflower seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

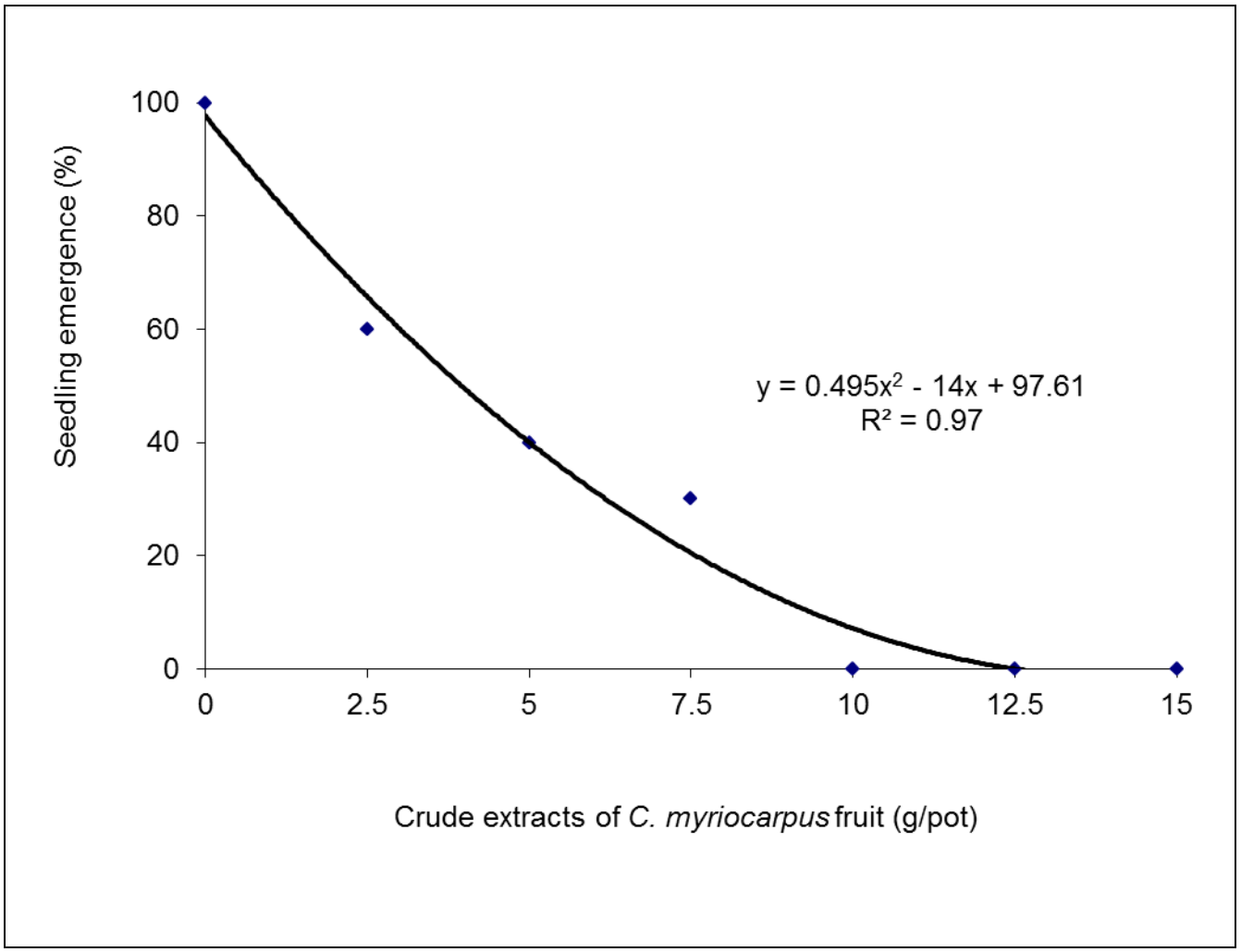

Figure 3.9 Quadratic relationship between tomato seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

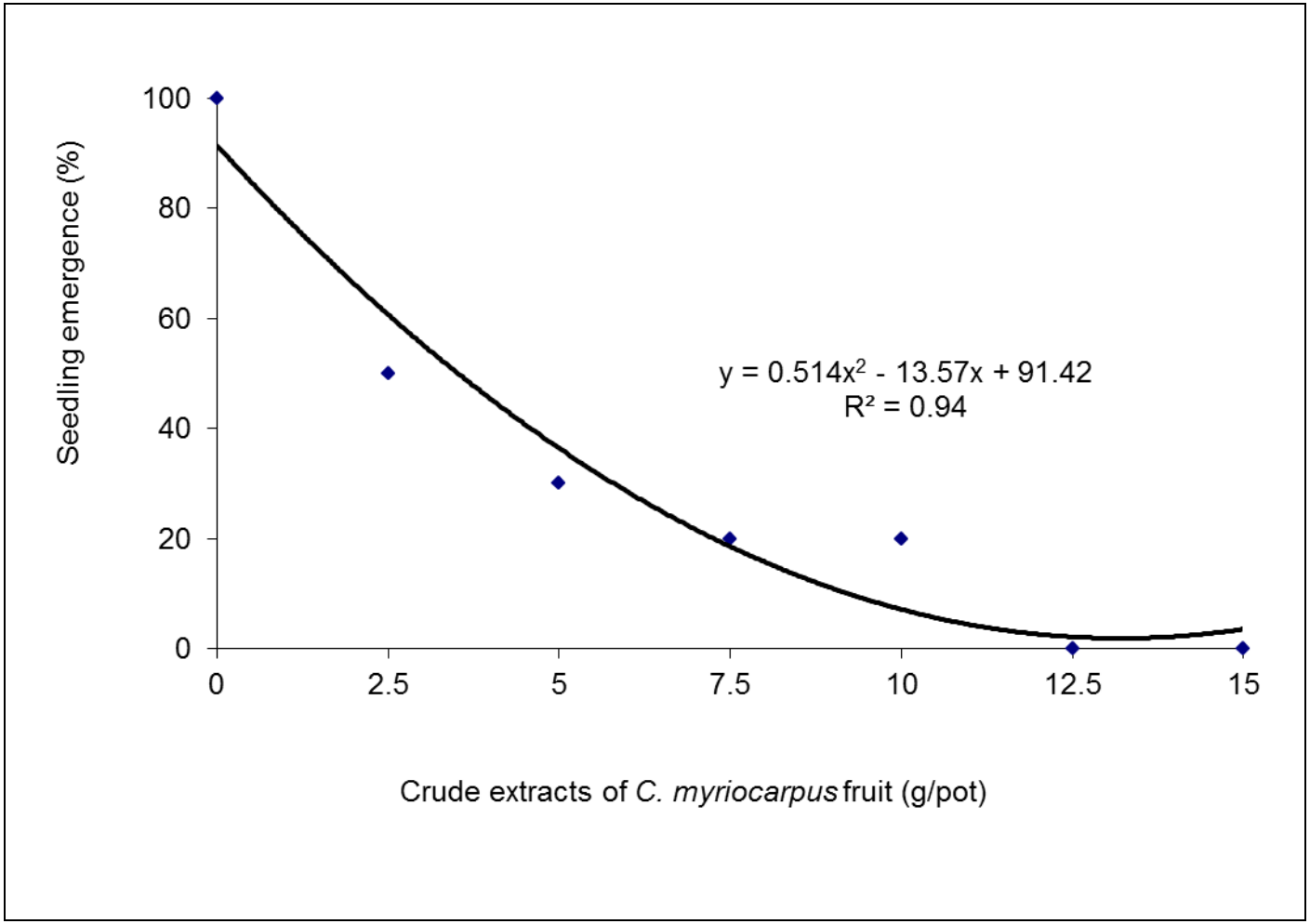

Figure 3.10 Quadratic relationship between watermelon seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

FIGURES 3.11 - 3.18: MONOCOTYLEDONOUS CROPS (PAGES 41 - 48)

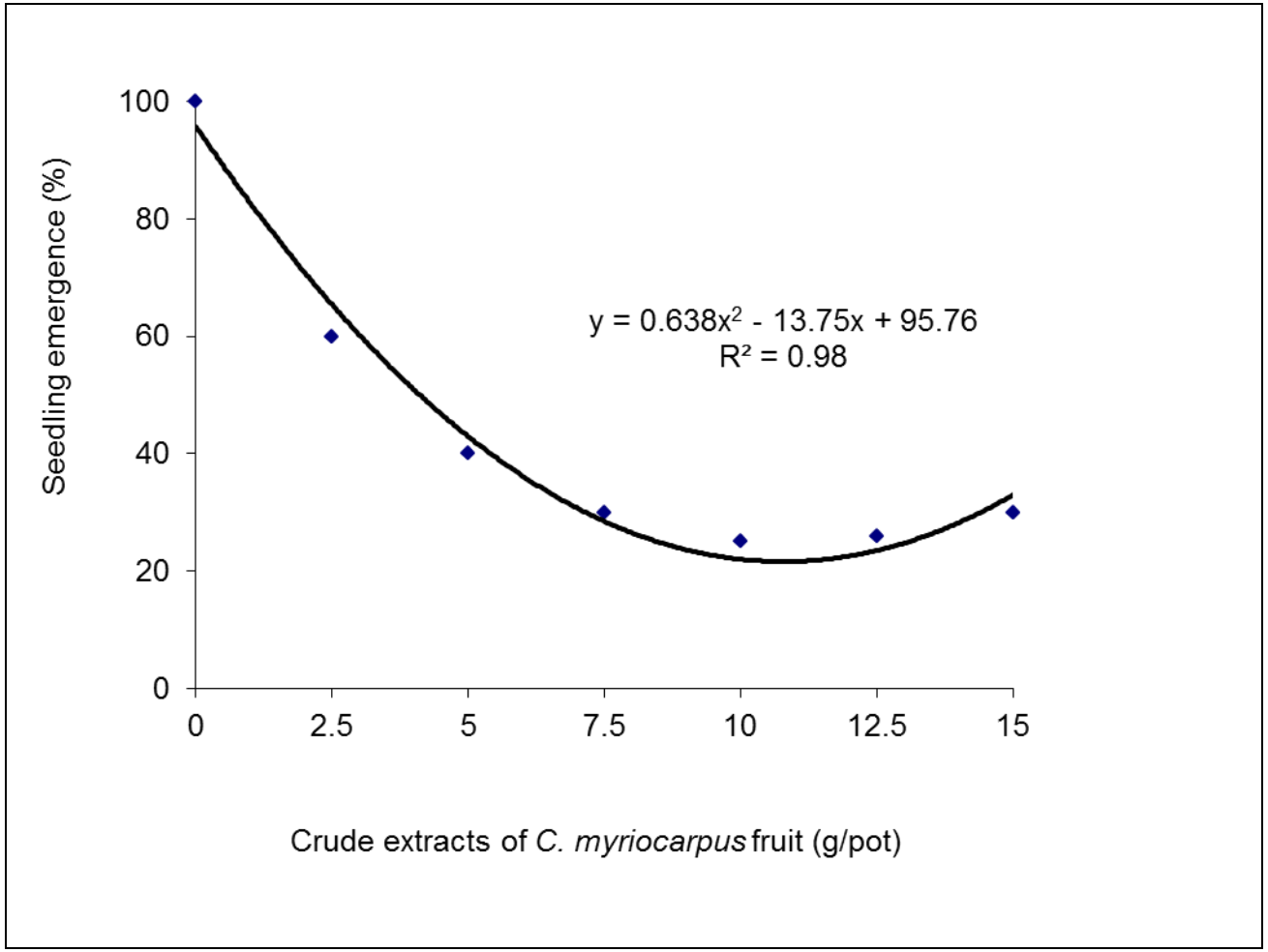

Figure 3.11 Quadratic relationship between chive seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

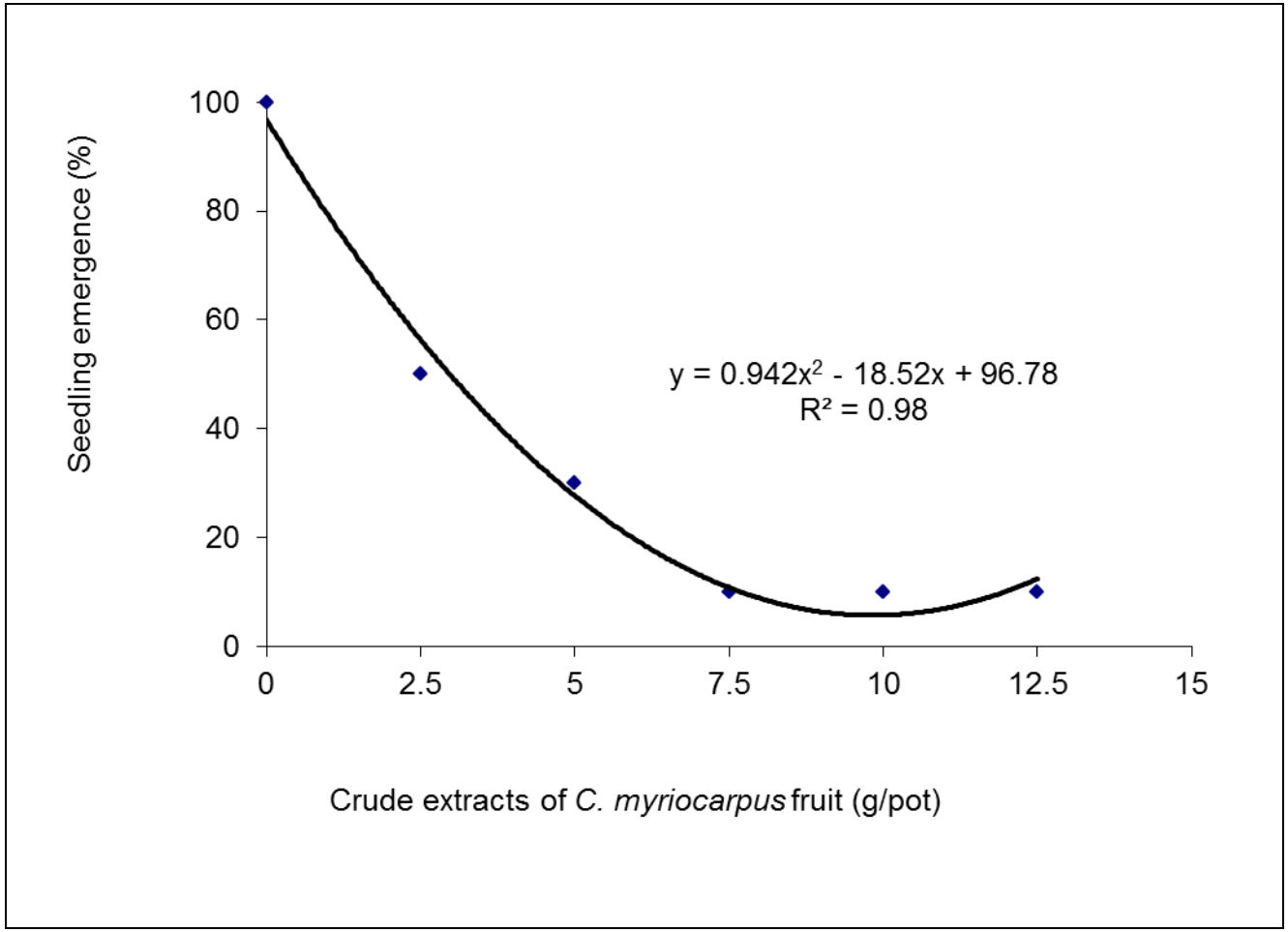

Figure 3.12 Quadratic relationship between leek seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

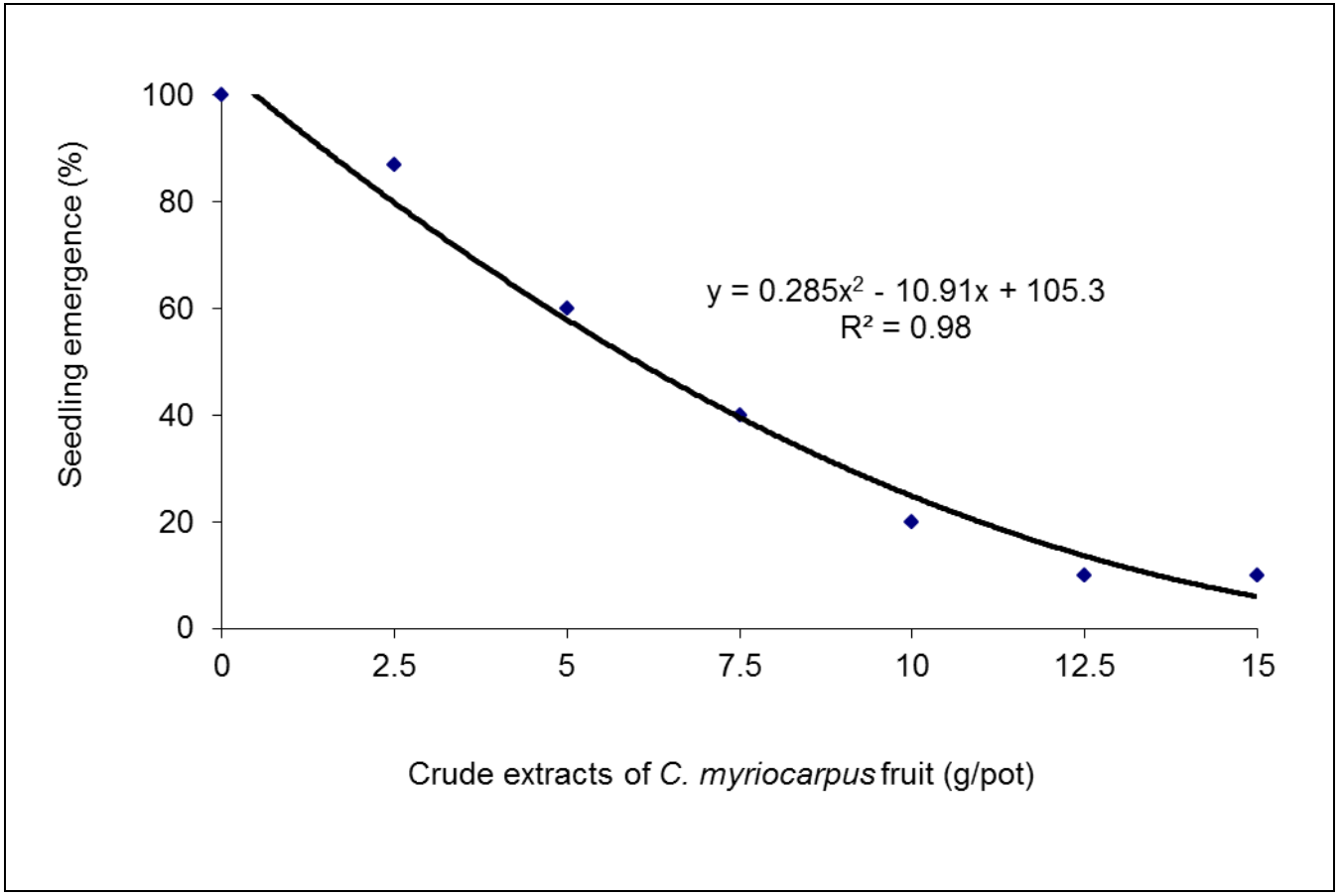

Figure 3.13 Quadratic relationship between maize seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

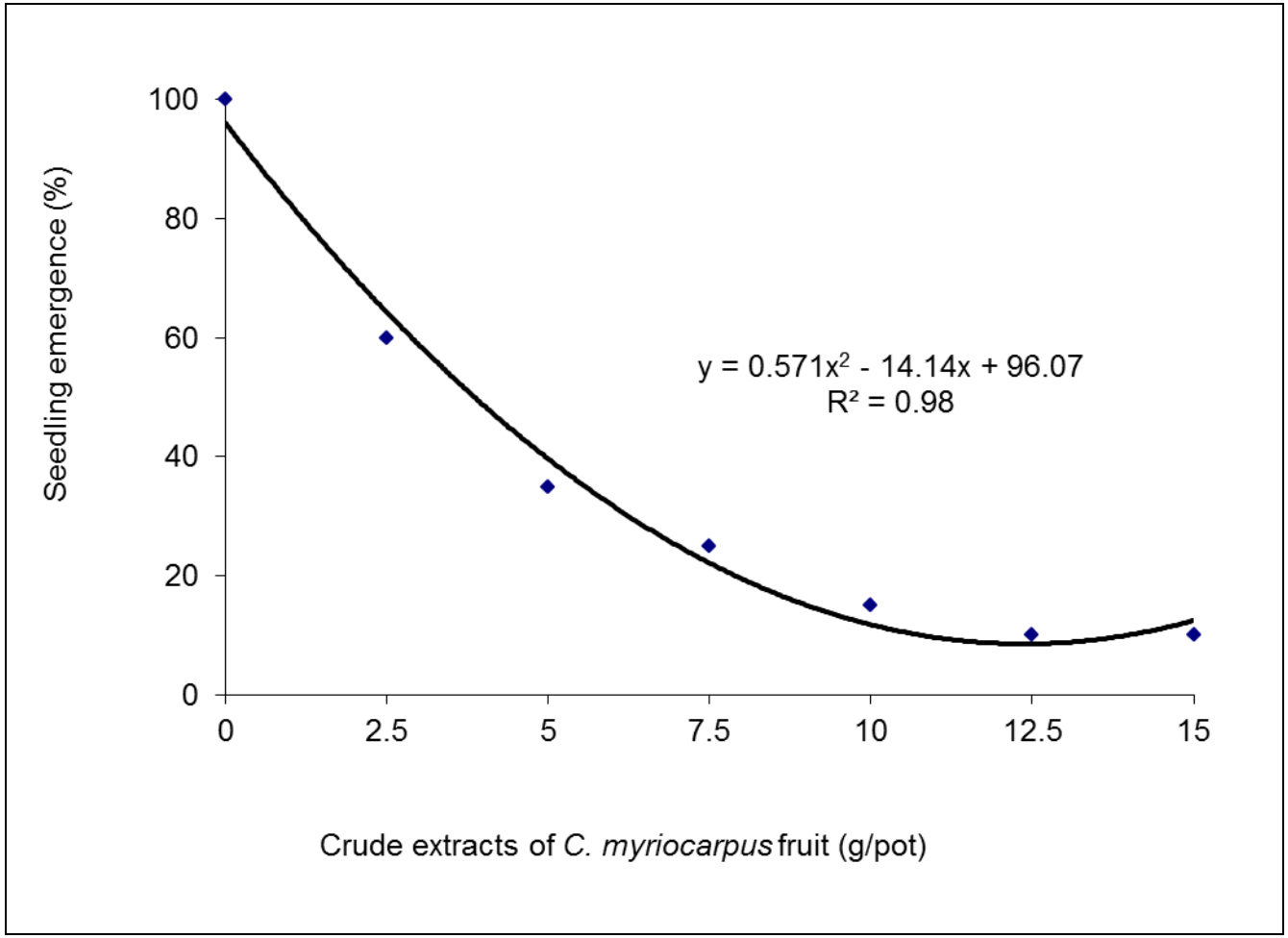

Figure 3.14 Quadratic relationship between millet seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

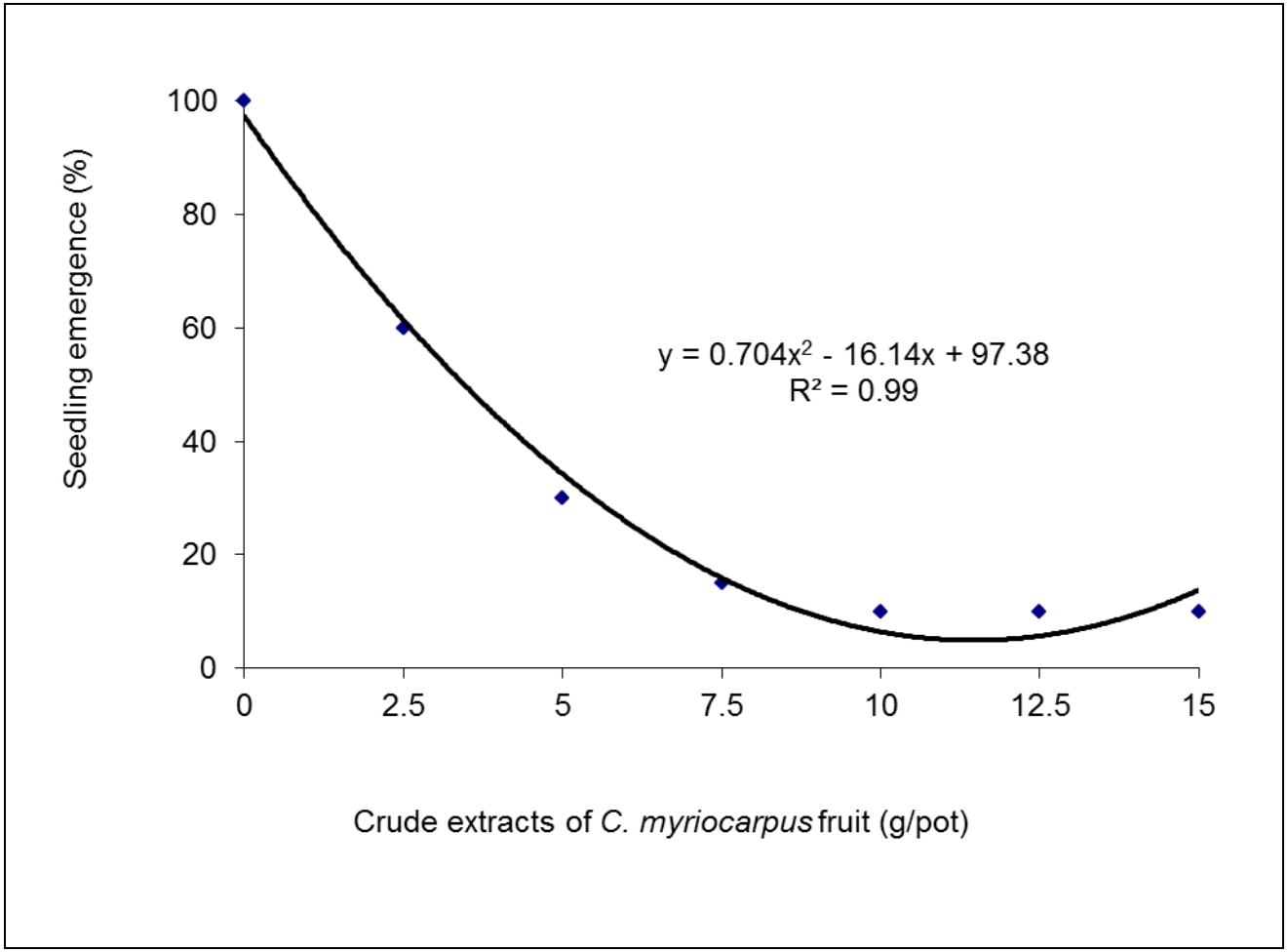

Figure 3.15 Quadratic relationship between onion seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

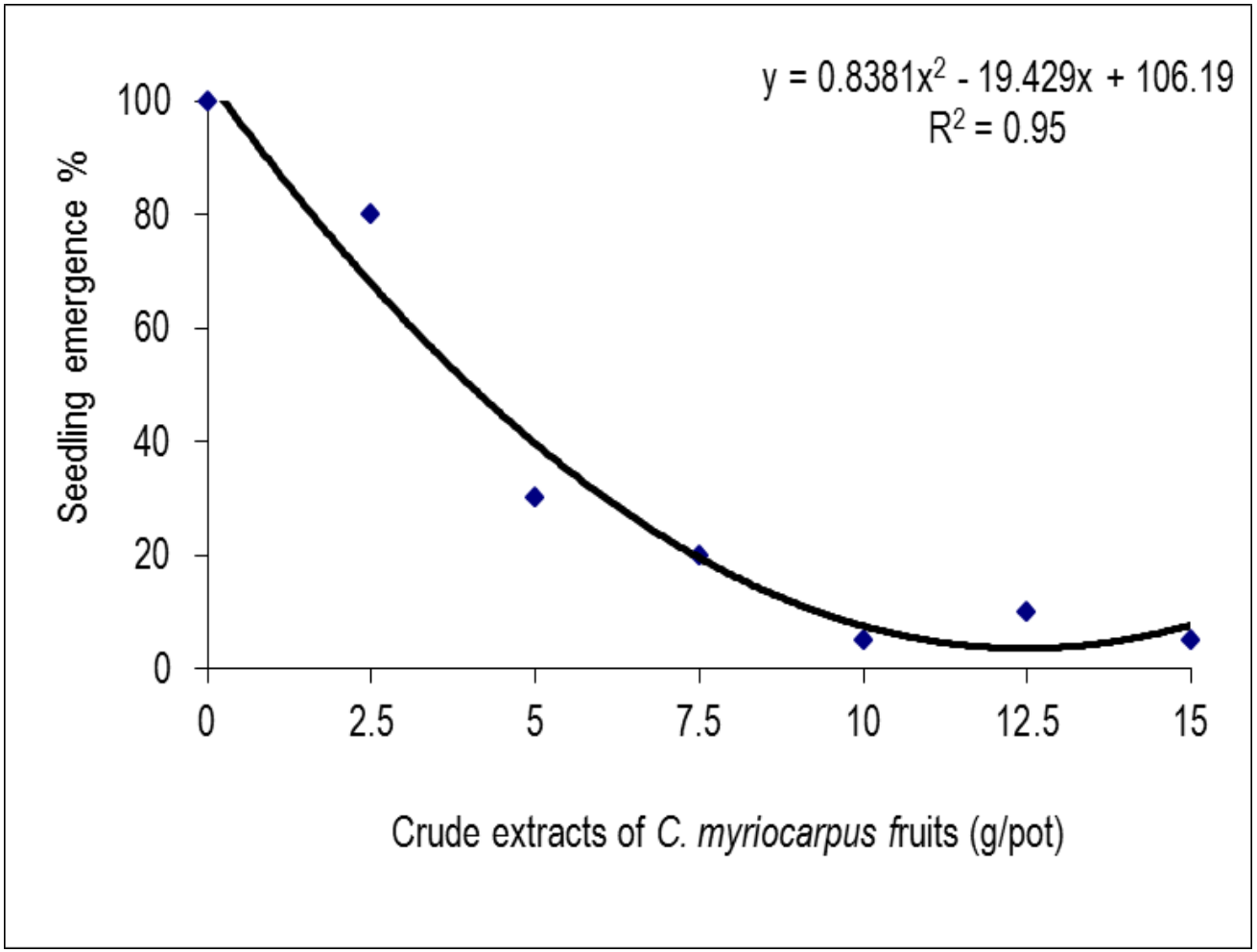

Figure 3.16 Quadratic relationship between rye seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

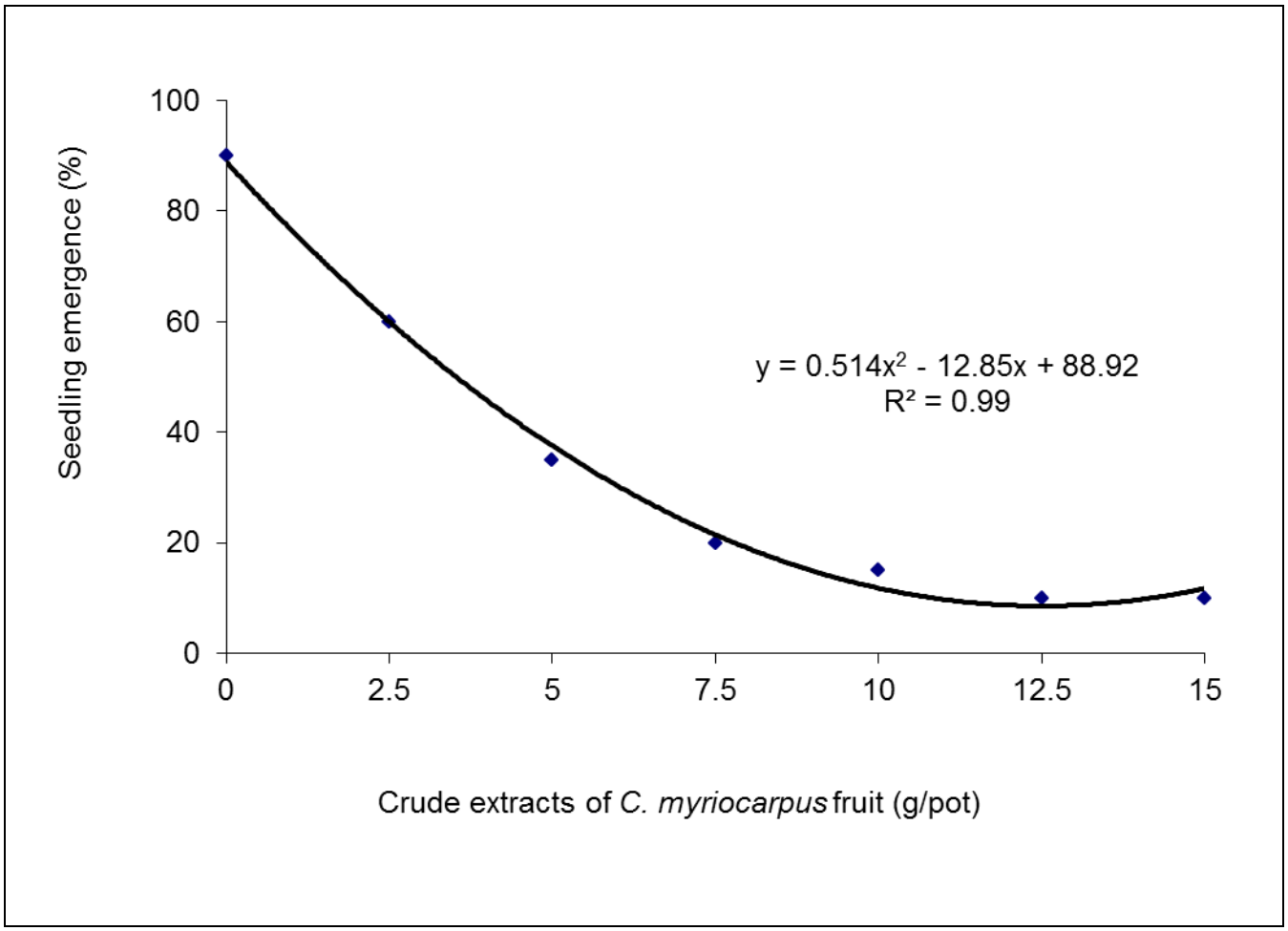

Figure 3.17 Quadratic relationship between sorghum seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

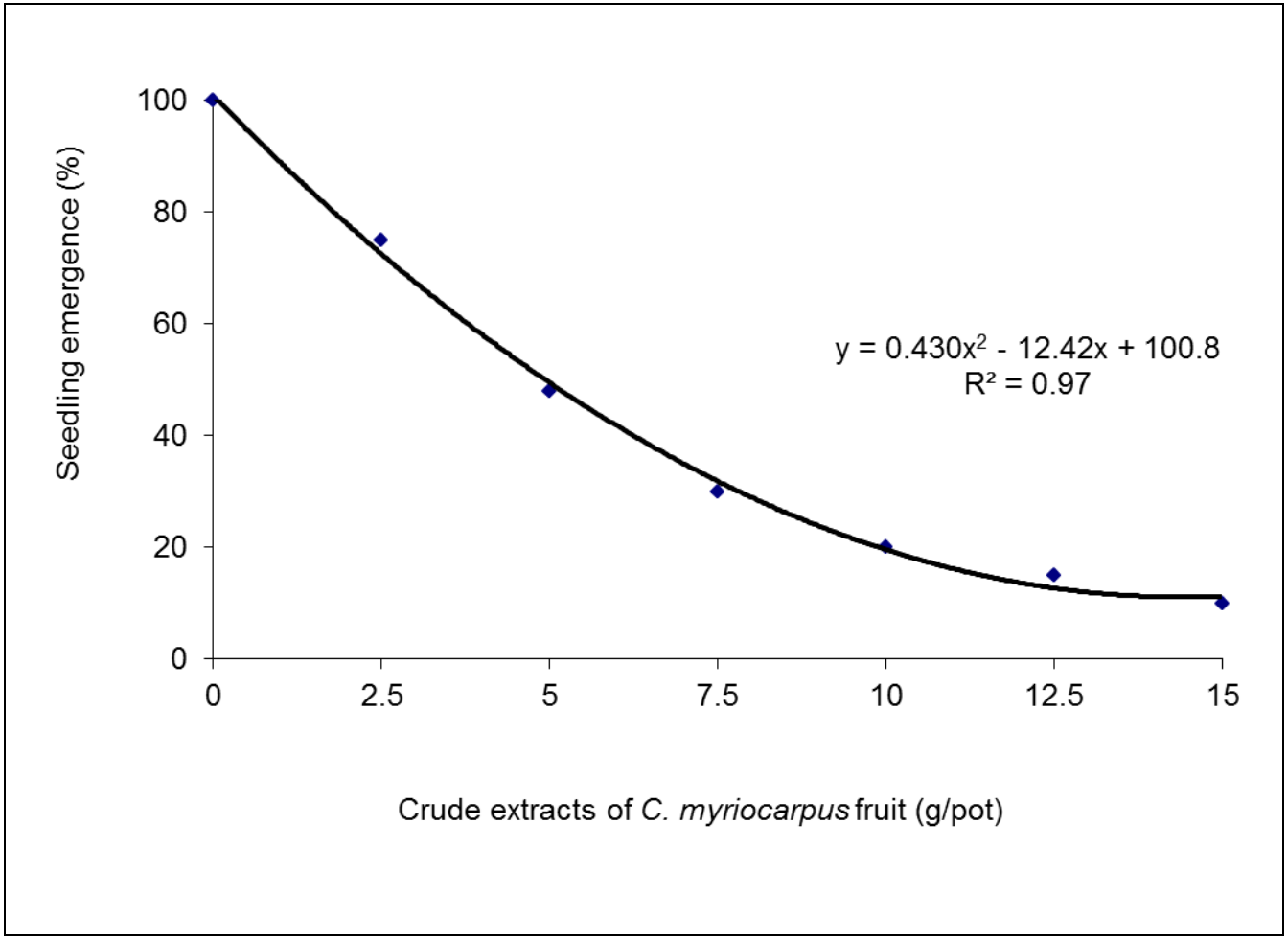

Figure 3.18 Quadratic relationship between wheat seedling emergence and crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 35).

3.4 Discussion

The Baermann method is used to extract nematodes that are alive (Kleynhans *et al*., 1996). The zero to negligible nematode counts suggested that the material killed the test plant-parasitic nematodes. Crude extracts of *C. myriocarpus* fruit reduced seedling emergence in all test crops. Certain widely used bio-nematicides have strong inhibition on seedling emergence. For instance, neem (*Azadirachta indica* A. Juss) had inhibitory effect on emergence of lettuce, mustard (*Sinapis arvensis* L.), bean, carrot (*Daucus carota* L.), radish (*Raphanus sativus* L.), alfalfa (*Medicago sativa* L.) and rice (*Oryza sativa* L.) (Xuan *et al*., 2004; Ashrafi *et al*., 2008b). Crude extracts from roots and leaves of catmint (*Nepeta meyeri* Benth.*)* inhibited seedling growth of barley (*Hordeum vulgare* L.) and sunflower by 87% and 67%, respectively (Mutlu and Atici, 2009). Soil amended with crude extracts of ryegrass leaves inhibited emergence of Korean lawn grass (*Zoysia japonica* L.*)* when used as an organic bio-pesticide (Zuk and Fry, 2006). Germination and subsequent seedling growth of eggplant, lettuce, spinach (*Spinacia oleracea* L.), leek, watermelon and tomato were inhibited by yuzu (*Citrus junos* L.), which explained more than 90% of the total treatment variation (Fujihara and Shimizu, 2003); a figure comparable to most results of crude extracts of *C. myriocarpus* fruit.

Allelochemicals that have been implicated in inhibiting seedling emergence include terpenoids, flavonoids and phenolic compounds (Marcias *et al*., 2002). In *Cucurbitaceae* family, most plant species contain cucurbitacins (Chen *et al*., 2005), with certain genera in this family, including the *Cucumis* genus, having auto-allelopathy with strong inhibition of germination (Martin and Blackburn, 2003). In their review of the chemical

structures of 12 cucurbitacins in *Cucurbitaceae* family, Chen *et al*. (2005) indicated that cucurbitacin A, which occurs in large quantities in fruit and roots of *C. myriocarpus,* was the only cucurbitacin that was water-soluble. Cucurbitacin A comprises two toxic compounds, *viz*. cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$), which are known as the tetracyclic triterpenoids (Chen *et al*., 2005). Cucurbitacin A confers auto-allelopathy on seeds of *C. myriocarpus*, but when removed through exposing seeds to 55˚C for 24 hours or to running water for 24 hours, seeds germinated within seven days (Mafeo and Mashela, 2006).

In biological systems, quadratic relationships are an indication of density-dependent or concentration-dependent growth patterns (Mamphiswana *et al*., 2010; Pofu *et al*., 2010 a,b). Generally, the density-dependent growth patterns suggest that there is, depending on the concentration, the stimulation growth phase, followed by the levelling off phase and then the inhibition growth phase (Salisbury and Ross, 1992; Liu *et al*., 2003). In the current study, the quadratic relationships for all the crops tested were already in the inhibition growth phase, suggesting that the crude extracts of *C. myriocarpus* fruit were already excessive for seedling emergence and perhaps, for seed germination as well in the selected test crops.

Generally, the degree of allelopathy on plants, in addition to being density-dependent, also depends on the stage of growth of the plant (Einhellig, 1985). This view was also supported by lack of phytotoxicity when the crude extracts of *C. myriocarpus* fruit were used at transplanting as a post-emergent bio-nematicide (Mashela, 2002; Mashela *et*

al., 2008). The observed quadratic relationships between emergence and dosage of crude extracts of *C. myriocarpus* fruit suggested that there might be dosages that stimulate seedling emergence, if indeed, the observed relationships prescribe to conditions of density-dependent growth patterns as described for most biological systems (Salisbury and Ross, 1992; Liu *et al*., 2003).

Generally, allelopathic inhibitors interfere with key physiological processes in receptor plants, resulting in reduction of plant growth and development (Inderjit and Duke, 2003; Ashrafi *et al*., 2008a). Results of this study suggested that processes involved in inhibition of emergence were having similar pathways, as described in seed germination (Campbell, 1990). Crude extracts of *C. myriocarpus* consistently reduced seedling emergence in all plant species when used within the range suitable for transplants in suppression of plant-parasitic nematodes.

3.5 Conclusions

Similar high R-squared values in negative quadratic relationships of variables measured with dosages of crude extract of *C. myriocarpus* fruit suggested that the inhibiting allelochemical in crops tested targeted the processes which have similar physiological activities. In this study, the targeted process might have occurred during seed germination, since seedling emergence is a physical process. Detailed bioassay studies on responses of seed germination to crude extracts of *C. myriocarpus* fruit in vitro were necessary to substantiate results observed in *vivo*.

CHAPTER 4

INFLUENCE OF C*UCUMIS* BIO-NEMATICIDE ON SEED GERMINATION OF SELECTED DICOTYLEDONOUS AND MONOCOTYLEDONOUS CROPS

4.1 Introduction

Seed germination is a chemical process, starting from imbibition of water and ending when the radicle raptures the testa (Starr and Taggart, 1987; Campbell, 1990; Bewley, 1997). Chemically, after imbibition the embryo releases gibberellic acid (GA) as a signal to the aleurone layer, which then synthesises and secretes alpha-amylase and other hydrolytic enzymes that digest stored food in the endosperm and produce products that are absorbed by the cotyledons (Starr and Taggart, 1987; Campbell, 1990). The embryo uses the absorbed products for growth, which starts with the growth of the radicle and germination ending when this embryonic root raptures the testa.

Movement of GA from the embryo to the aleurone layer entails diffusion from a high concentration to a lower concentration through the endosperm (Campbell, 1990). Any chemical that counters the arrival of GA at the aleurone layer and/or prevent the synthesis of hydrolytic enzymes or digestion of the endosperm or absorption of digested materials by the radicle, would obviously inhibit seed germination. In this study, the intention was not to investigate the mechanism involved in the interaction between seed germination and crude extracts of *Cucumis myriocarpus* fruit, but to eliminate the buffering effect of soil and its microbes on interaction between seedling emergence and the dosages of the material as observed previously (Chapter 3). The objective of this study was to investigate if germination of selected dicotyledonous and

monocotyledonous crops would have density-dependent growth responses to aqueous solutions of crude extracts of *C. myriocarpus* fruit.

4.2 Materials and methods

Seed germination trials using crops similar to those used in emergence trials (Chapter 3) were conducted at the University of Limpopo, Republic of South Africa (23°53'10"S, 29°44'15"E), under laboratory conditions. Fruit of *C. myriocarpus* were collected locally, prepared and stored as described previously (Chapter 3).

4.2.1 Experimental design and cultural practices

Seven levels of crude extracts of *C. myriocarpus* fruit, *viz*. 0, 25, 50, 75, 100, 125 and 150 g material/ℓ distilled water, were mechanically shaken for 12 hours on a LABCON shaker (Model 3100U) at 200 rpm. The mixture was sieved through a double-layered muslin cloth to remove debris and then filtered through Whatman No. 1 filter paper, with filtrates used as test solutions soon thereafter. The remaining test solutions were each sealed in 50 mℓ containers and stored at room temperature.

Ten seeds of tomato (*Solanum lycopersicum* L.*)* cv. 'Floradade', watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) cv. 'Crimson Giant' and butternut squash (*Cucurbita moschata* [Duch.]) cv. 'Waltham', lettuce (*Latuca sativa* L.) cv. 'Great Lakes', sunflower (*Helianthus annaus* L.) cv. 'PAN 7033', pea (*Pisum sativum* L.) cv. 'Hygrotech J12082', bean (*Phaseolus vulgaris* L.*)* cv. 'Contendor', eggplant (*Solanum melongena* L.*)* cv. 'Black Beauty', chilli (*Capsicum frutescence* L.) cv. 'Long Slim Cayenne' and

pepper (*Capsicum annum* L.*)* cv. 'Capistrano' and ten seeds of chive (*Allium schoenoprasum* L.) cv. 'Hygrotech J03940', leek (*Allium fistosum* L.) cv. 'Hygrotech G07157), maize (*Zea mays* L.) cv. 'SNK 2147', millet (*Panicum miliaceum* L.*)* cv. 'Babala [OPV]', onion (*Allium cepa* L.) cv. 'Texas Grano', rye (*Secale cereal* L.*)* cv. 'ARC-FRI PBR', sorghum (*Sorghum bicolor* (L.) Moench) cv. 'Pannar 8609' and wheat (*Triticum aestivum* L.*)* cv. 'Caledon' were separately primed in 25 mℓ of each concentration in growth chamber at 25^0C and 75% RH for 8 hours.

Since normal bioassay for seed germination-allelopathy interactions are contaminated by test solutions, two layers of Whatman No. 1 filter paper were placed in 90-mm-diameter glass petri dishes (Appendix 4.1), each seeded with 10 seeds and 10-mℓ test solutions added. The seven treatments were arranged in a completely randomised design (CRD) inside the LABCON (Model: L.T.G.C.) growth chamber in darkness, with four replications. The temperature in the growth chamber varied in accordance with the requirements of the crop (Hygrotech, 2009), whereas relative humidity was kept constant at 75%. A 5-mℓ test solution per treatment was re-applied on the seventh day after initial application.

4.2.2 Data collection

Successful seed germination, viewed as testa-ruptured by the radicle, was daily recorded for 10 days, with counts being removed to eliminate re-counting.

4.2.3 Data analysis

Data were expressed as percentage germination [(germinated seeds/total seeds) x 100] and subjected to analysis of variance (ANOVA) with SAS program (SAS Institute Inc. 2004). Mean separation when treatments were significant ($P \leq 0.05$) was achieved using Waller-Duncan multiple-range test. Lines of the best fit between germination percentage and dosages of *C. myriocarpus* fruit were generated, with the coefficients of determination (R^2) serving as an indicator of the best fit. Unless stated otherwise, only treatment means that were significant at the probability level of 5% were discussed.

4.3 Results

Aqueous crude extracts of *C. myriocarpus* fruit significantly ($P \leq 0.05$) reduced germination of all test plants. Partitioning of the sum of squares for dicotyledonous crops indicated that the treatments explained 75%, 86%, 99%, 97% and 97% of the total treatment variation in seed germination of bean, butternut squash, chilli, eggplant and lettuce, respectively (Appendices 4.2 – 4.6), as for pea, pepper, sunflower, tomato and watermelon, the total treatment variation in seed germination was explained by 90%, 95%, 93%, 99% and 97%, respectively (Appendices 4.7 – 4.11).

Similarly, on monocotyledonous seeds the treatments explained 85%, 94%, 90% and 97% of the total treatment variation in seed germination of chive, leek, maize and millet, respectively (Appendices 4.12 – 4.15), while for onion, rye, sorghum and wheat, the total treatment variation in seed germination was explained by 95%, 98%, 73% and 97%, respectively (Appendices 4.16 – 4.19).

Seed germination of dicotyledonous crops (Figures 4.1 – 4.10) had negative quadratic relationships with aqueous crude extracts of *C. myriocarpus* fruit. In the quadratic relationships, the crude extract levels contributed 86%, 91%, 98%, 90%, 98%, 93%, 83%, 99%, 93% and 96% of the total treatment variation in mean germination of bean (Figure 4. 1), butternut squash (Figure 4. 2), chili (Figure 4. 3), eggplant (Figure 4. 4), lettuce (Figure 4. 5), pea (Figure 4. 6), pepper (Figure 4. 7), sunflower (Figure 4. 8), tomato (Figure 4. 9) and watermelon (Figure 4. 10), respectively.

Similarly, in monocotyledonous crops the aqueous crude extract levels contributed 98%, 98%, 93%, 91%, 80%, 92%, 96% and 92% of the total treatment variation in mean germination of chive (Figure 4.11), leek (Figure 4.12), maize (Figure 4.13), millet (Figure 4.14), onion (Figure 4.15), rye (Figure 4.16), sorghum (Figure 4.17) and wheat (Figure 4.18), respectively.

FIGURES 4.1 – 4.10: DICOTYLEDONOUS CROPS (PAGES 58 – 67)

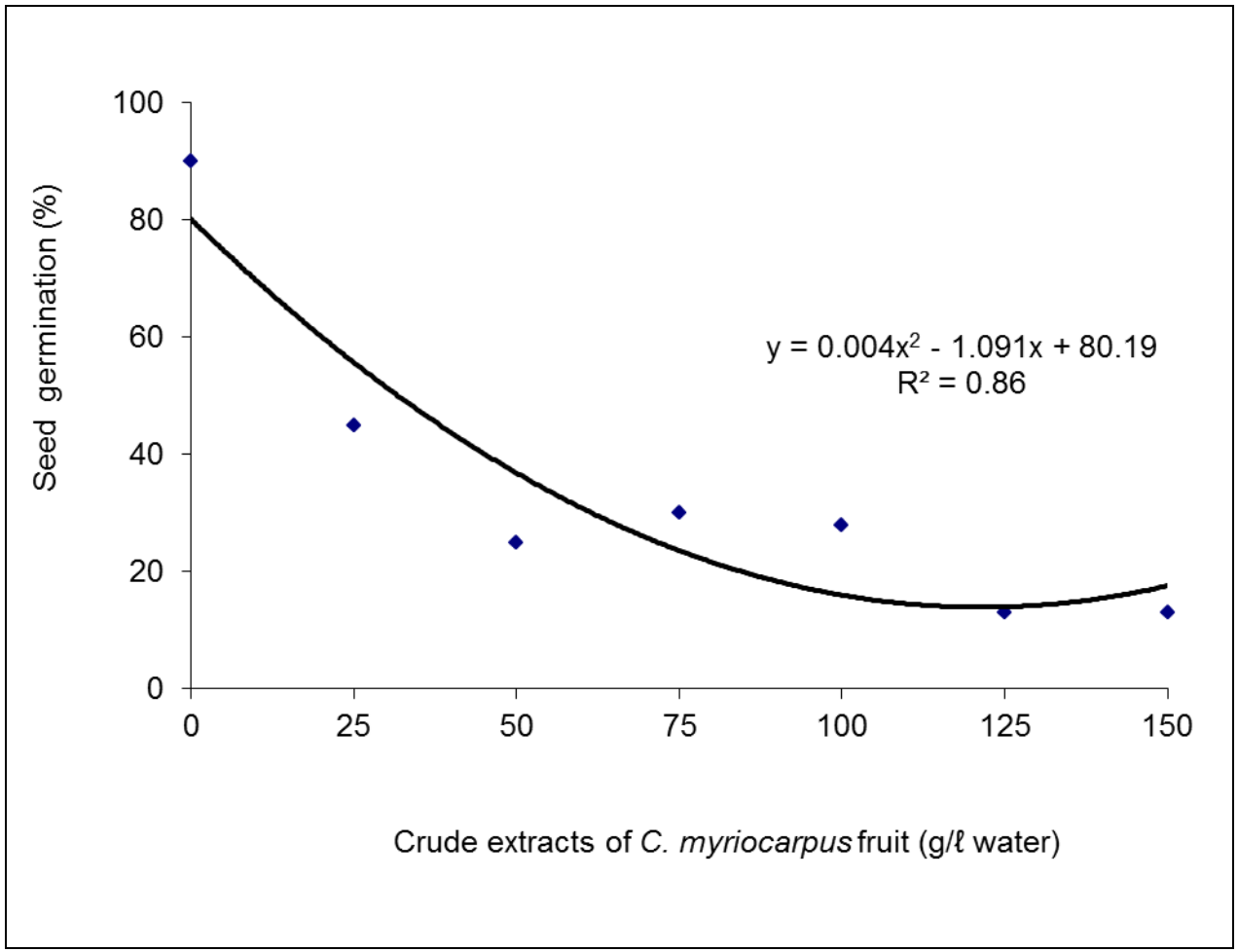

Figure 4.1 Quadratic relationship between germination of bean and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

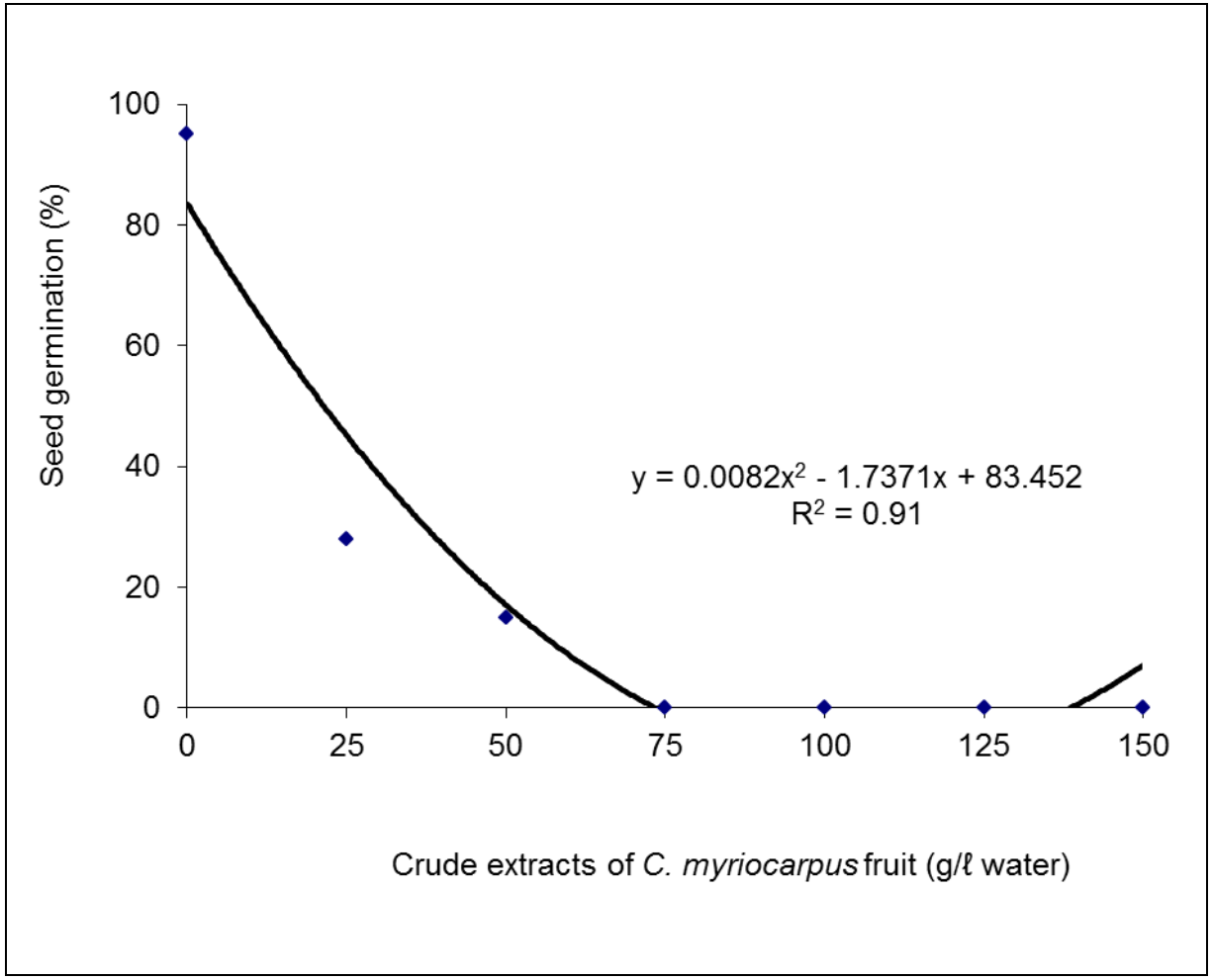

Figure 4.2 Quadratic relationship between germination of butternut squash seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

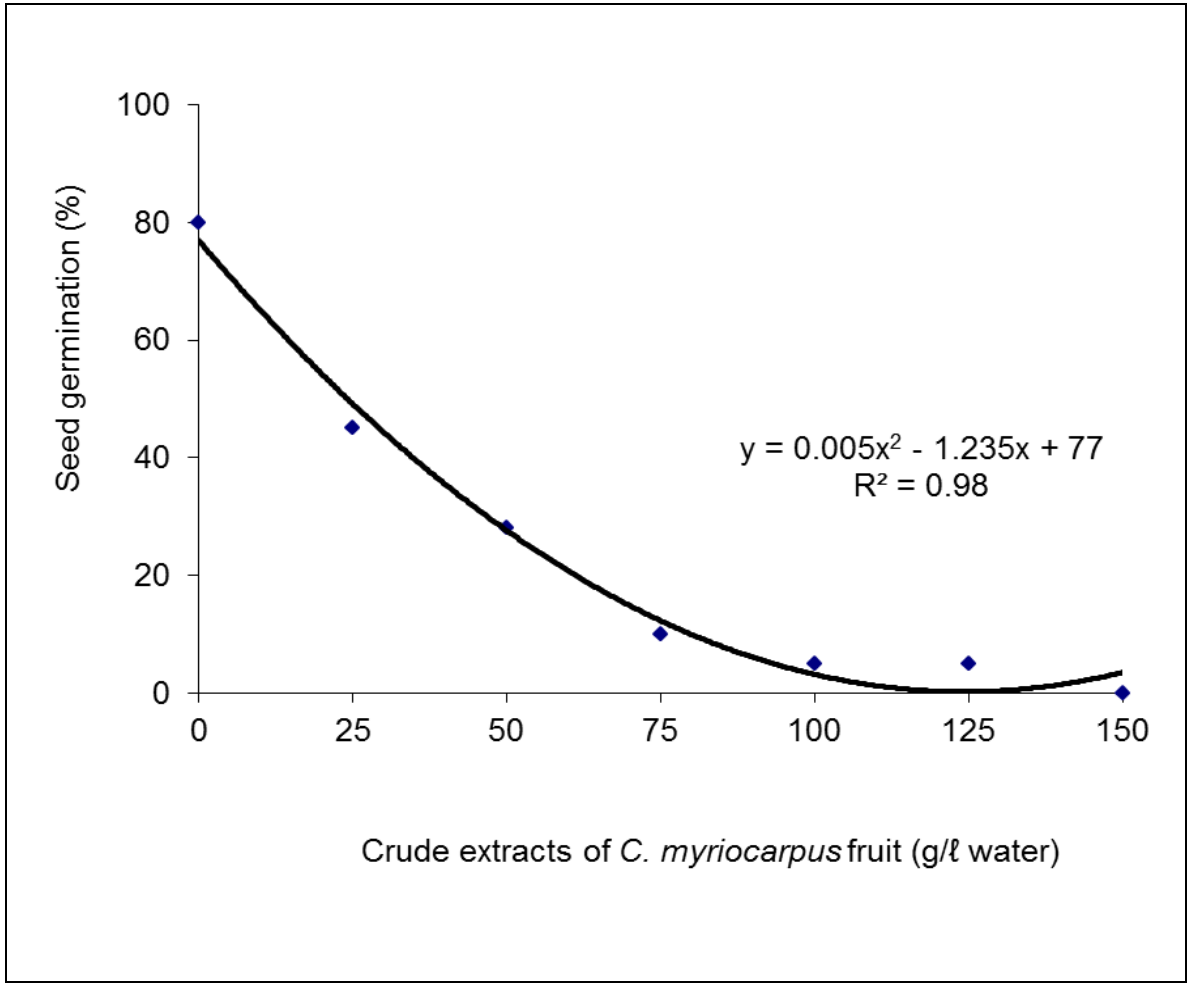

Figure 4.3 Quadratic relationship between germination of chili seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

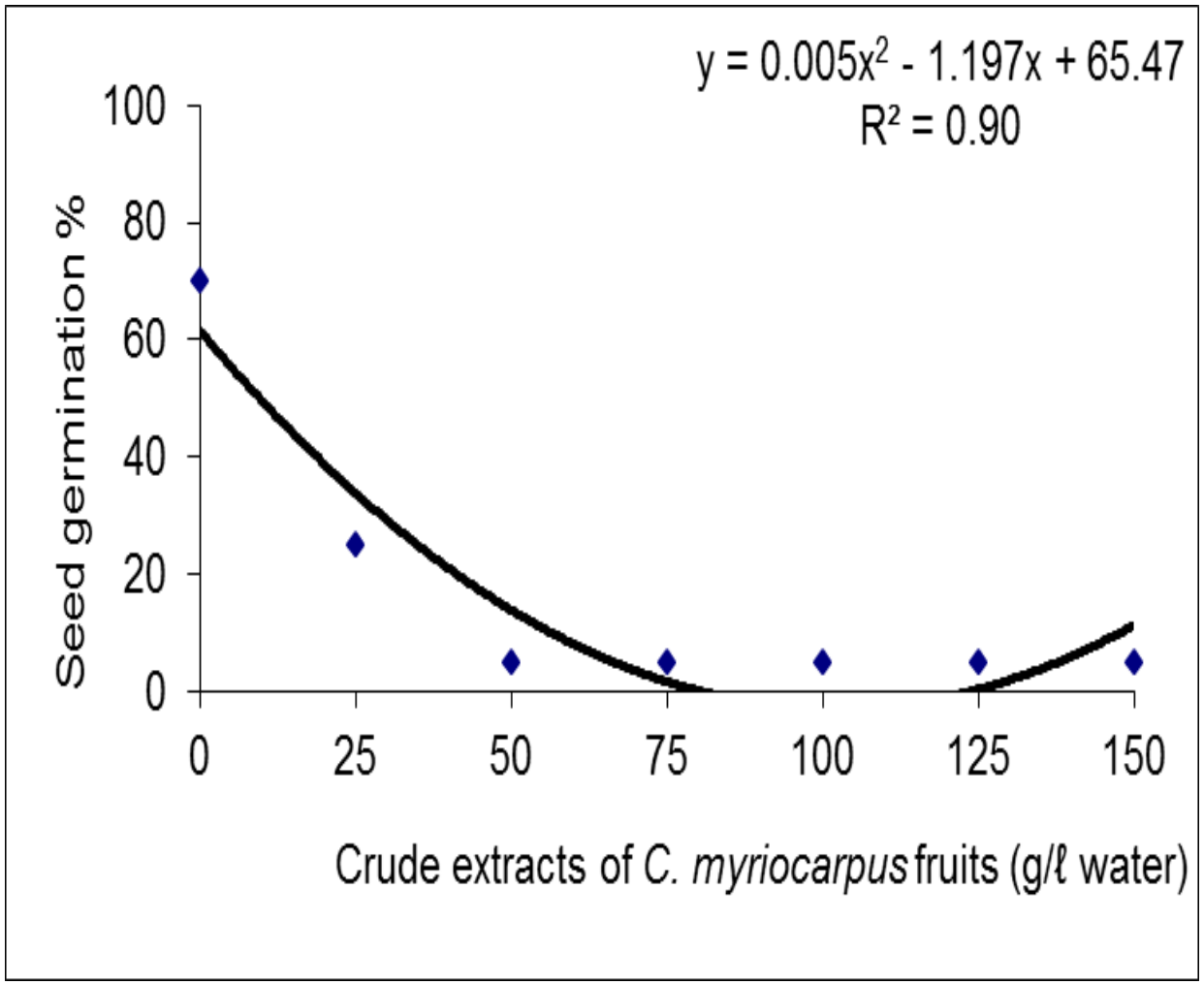

Figure 4.4 Quadratic relationship between germination of eggplant seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

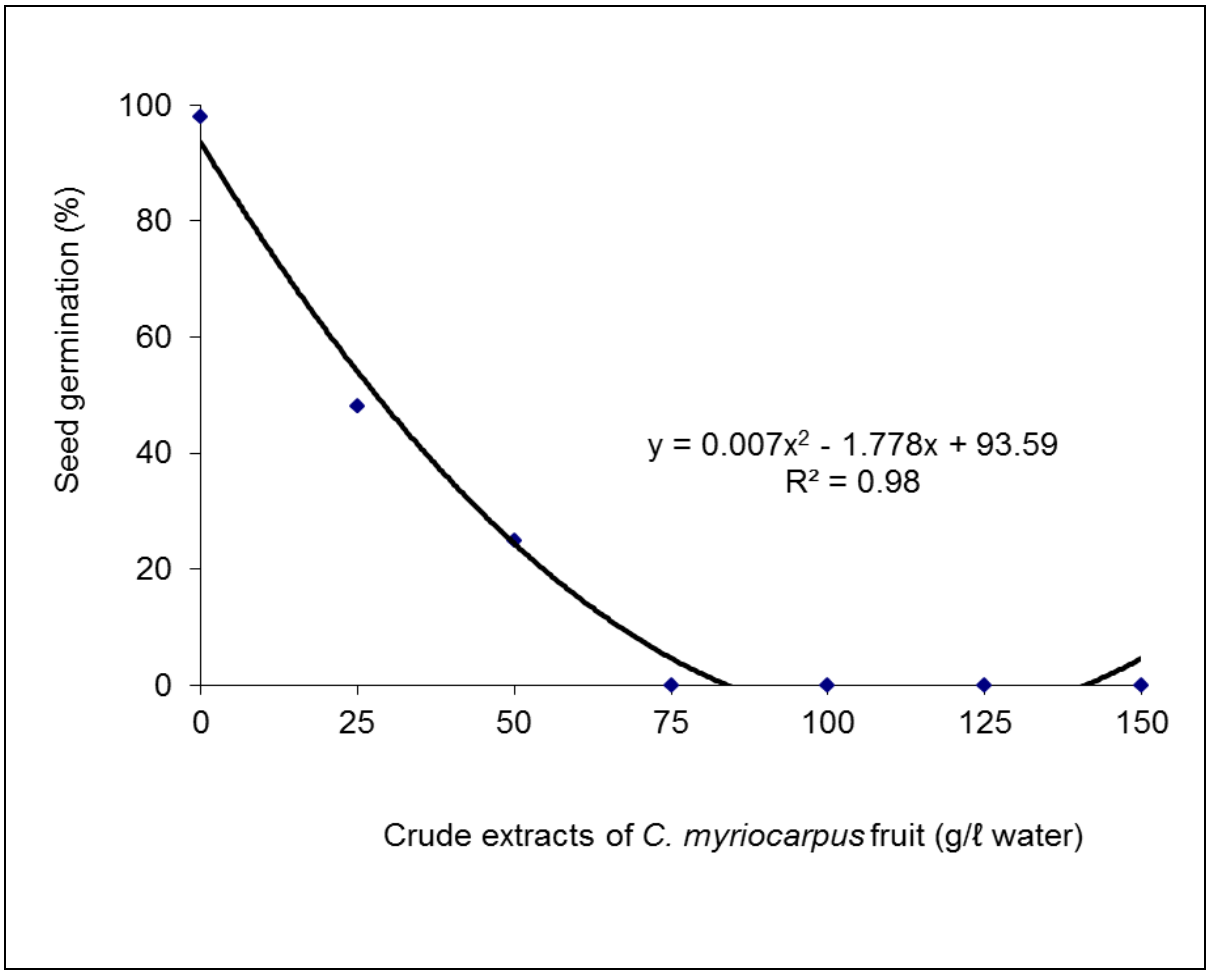

Figure 4.5 Quadratic relationship between germination of lettuce seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

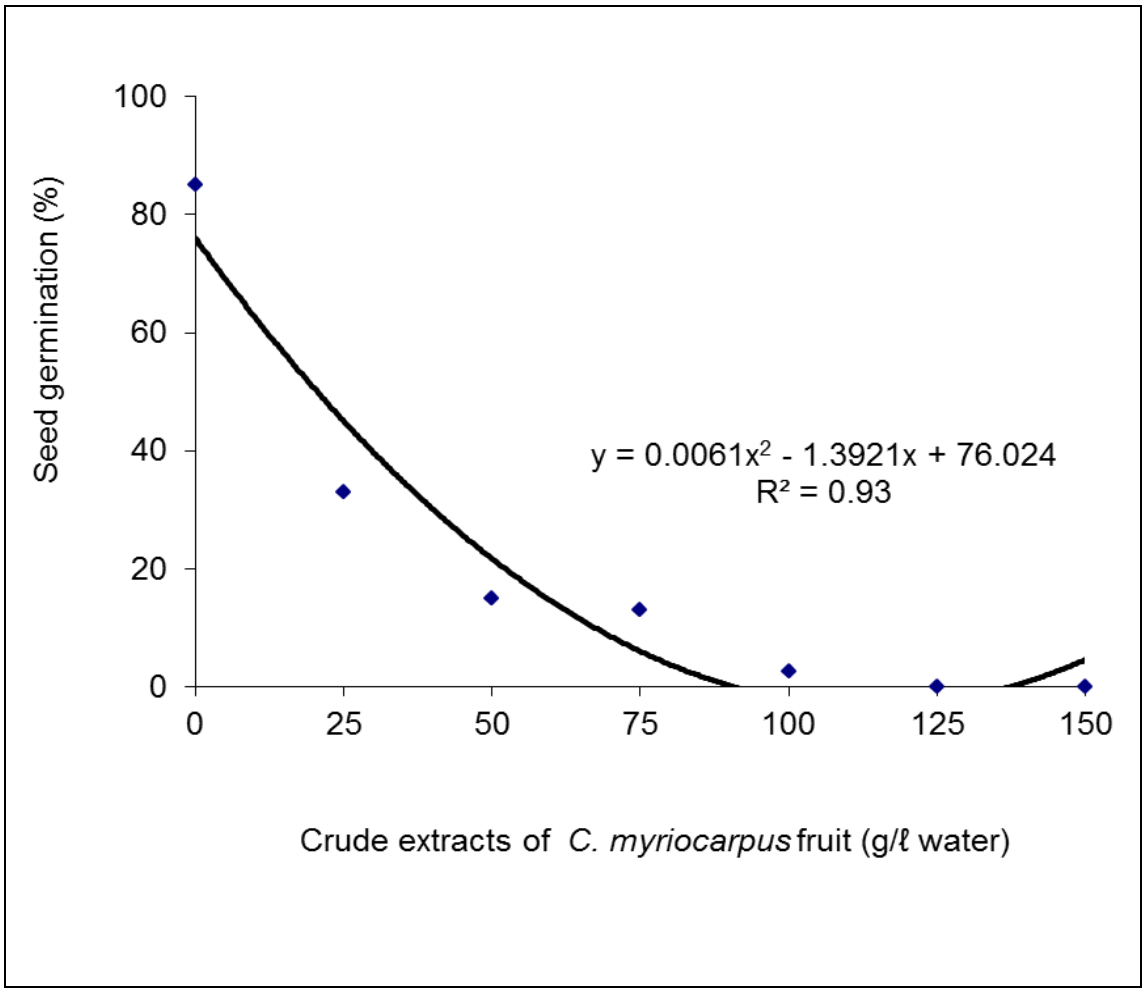

Figure 4.6 Quadratic relationship between germination of garden pea seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

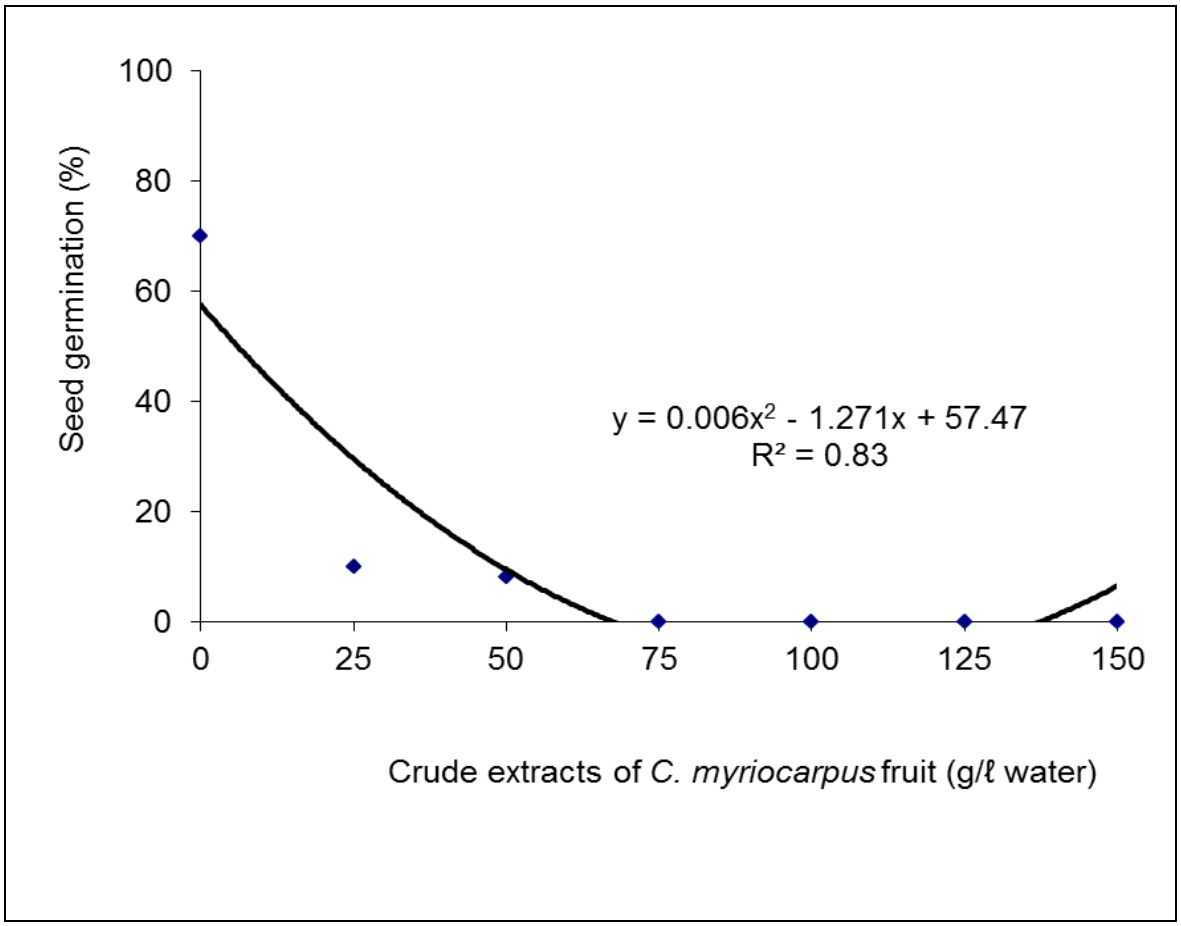

Figure 4.7 Quadratic relationship between germination of pepper seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

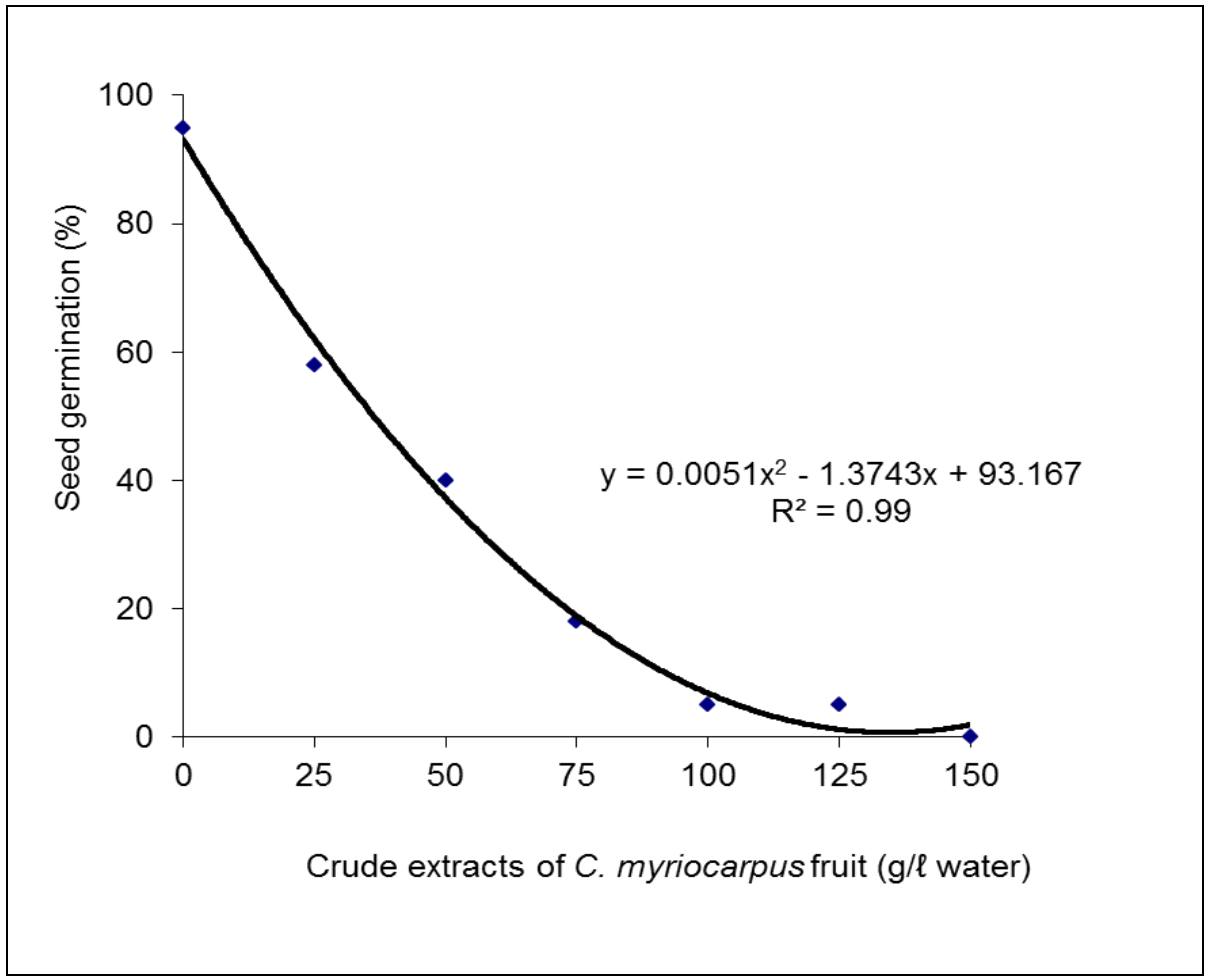

Figure 4.8 Quadratic relationship between germination of sunflower seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

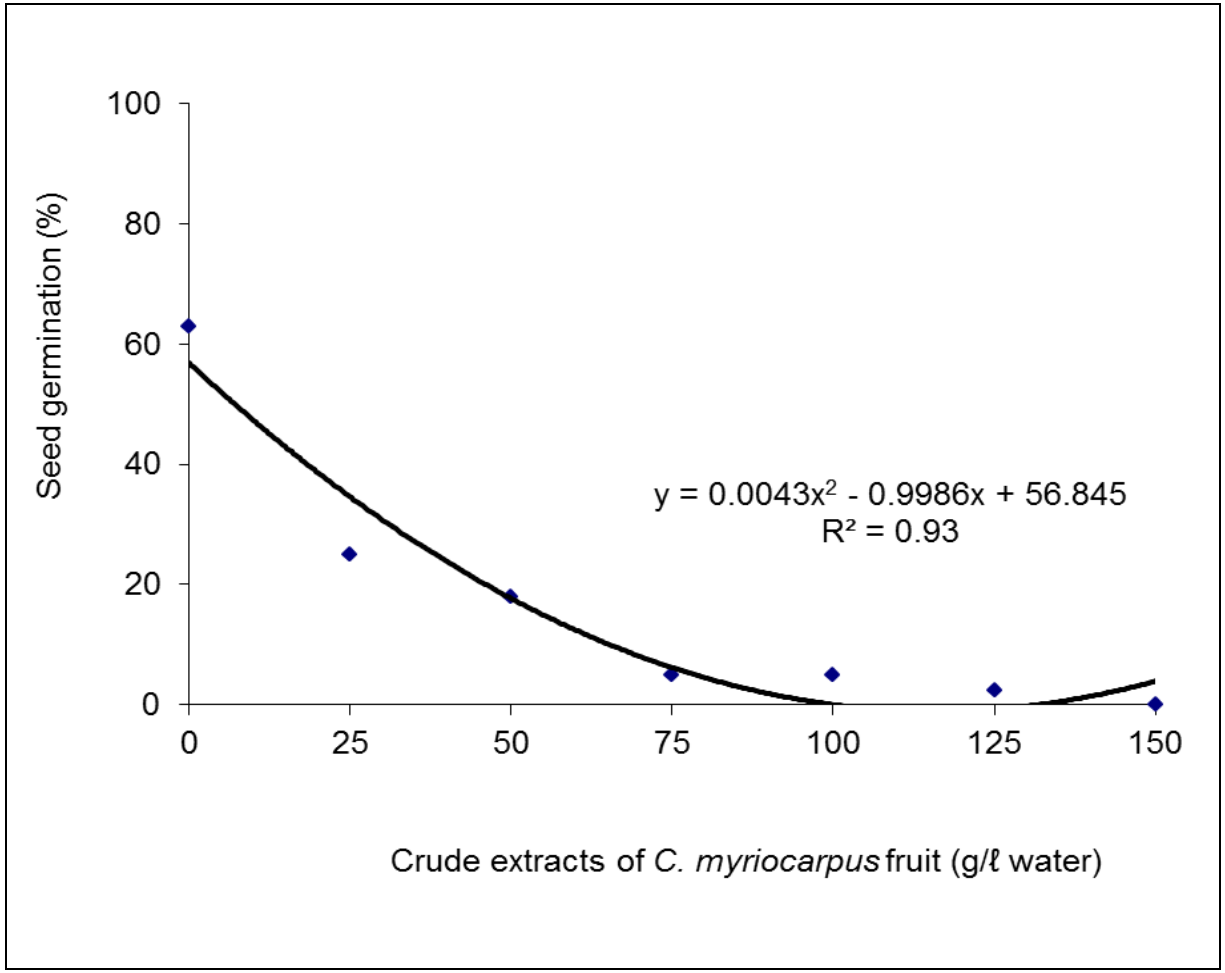

Figure 4.9 Quadratic relationship between germination of tomato seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

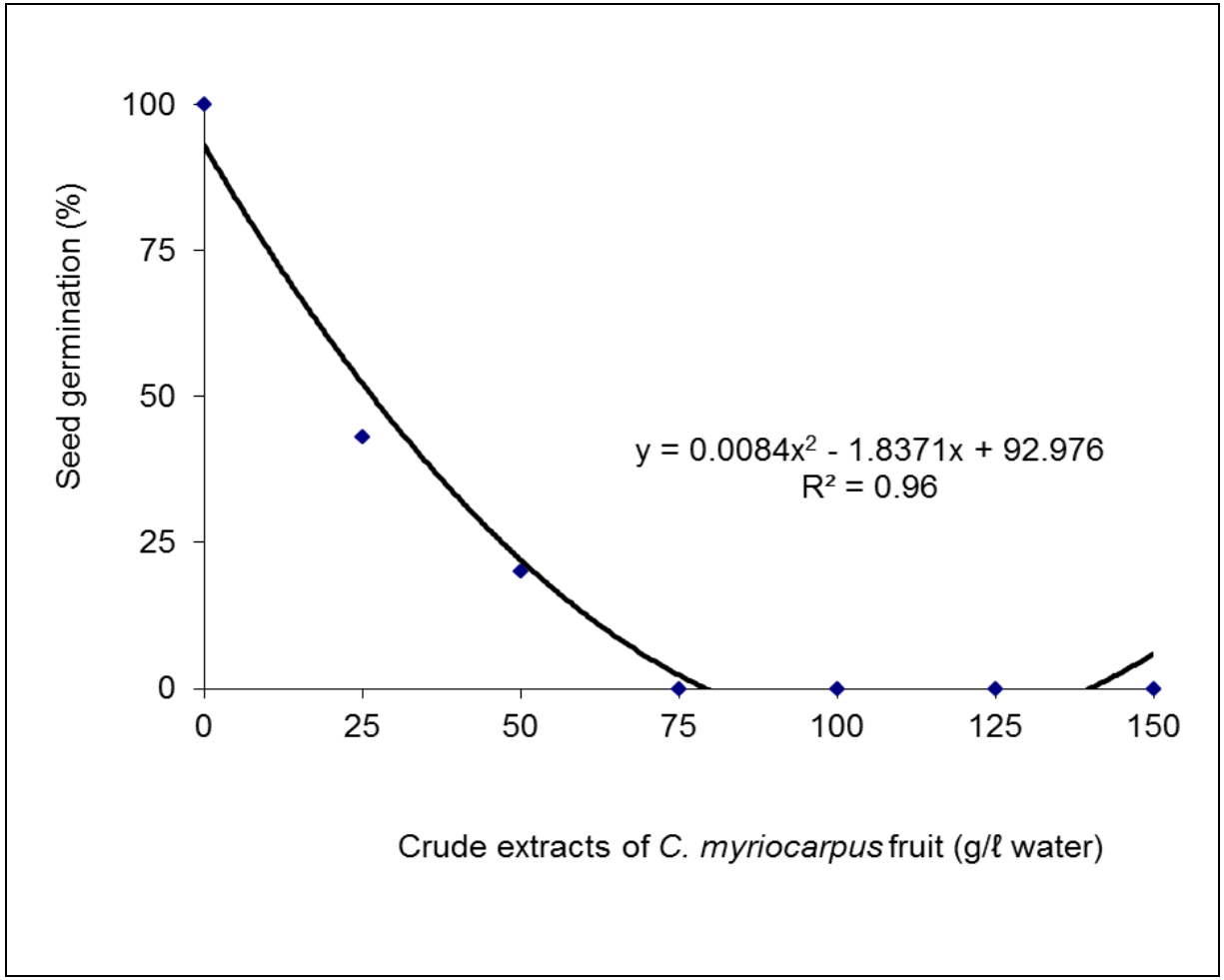

Figure 4.10 Quadratic relationship between germination of watermelon seeds and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

FIGURES 4.11 – 4.18: MONOCOTYLEDONOUS CROPS (PAGES 69 – 76)

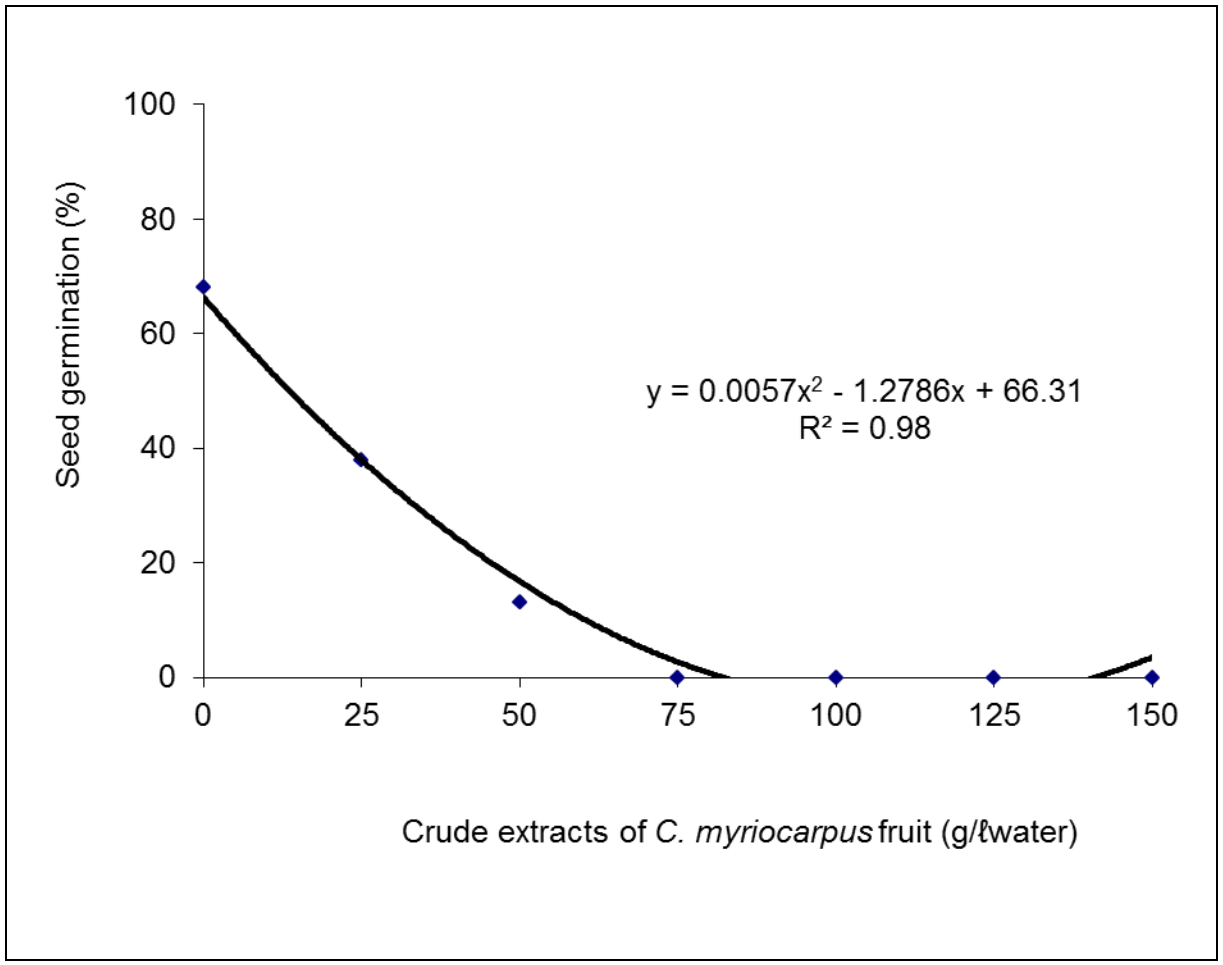

Figure 4.11 Quadratic relationship between germination of chive and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

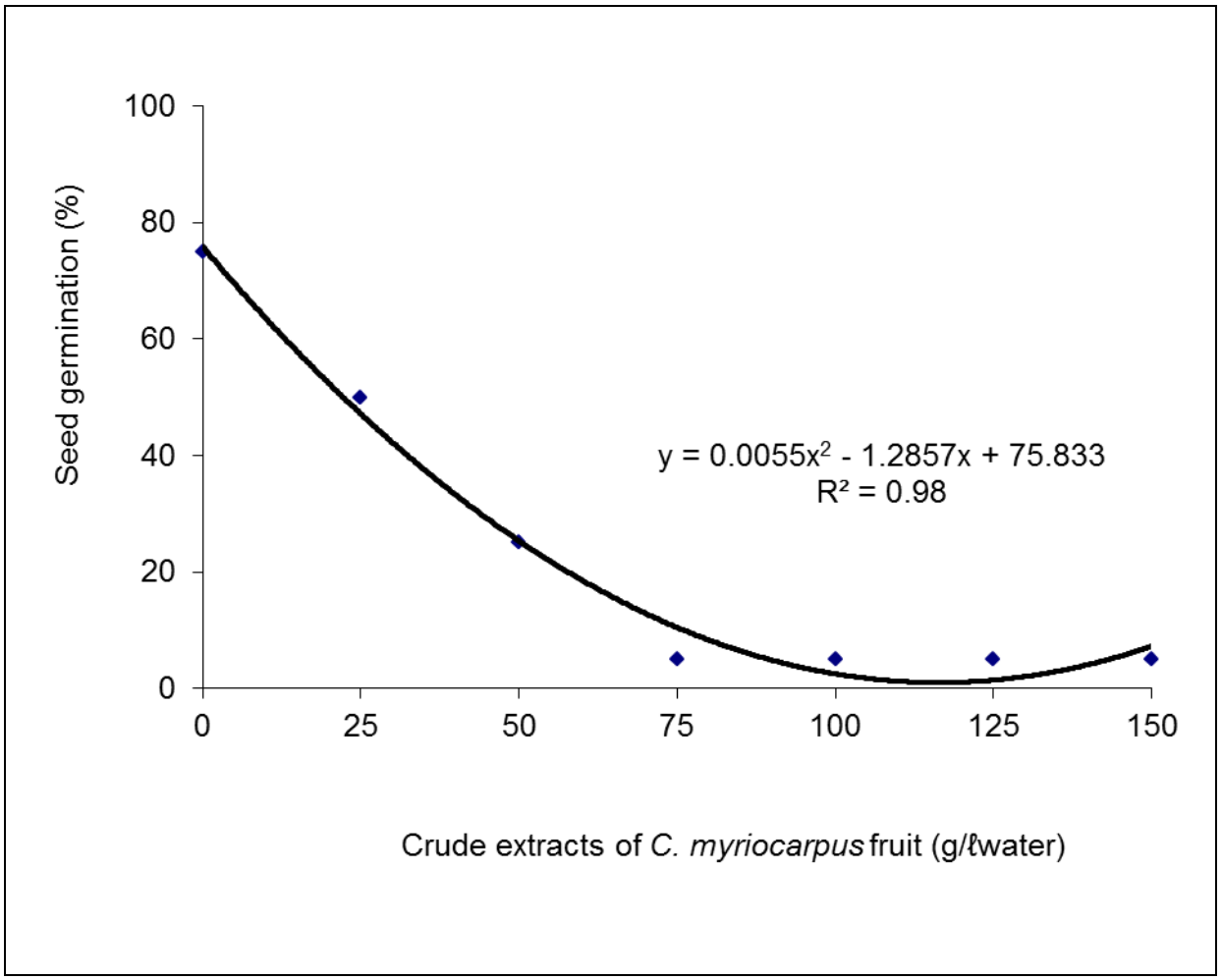

Figure 4.12 Quadratic relationship between germination of leek and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

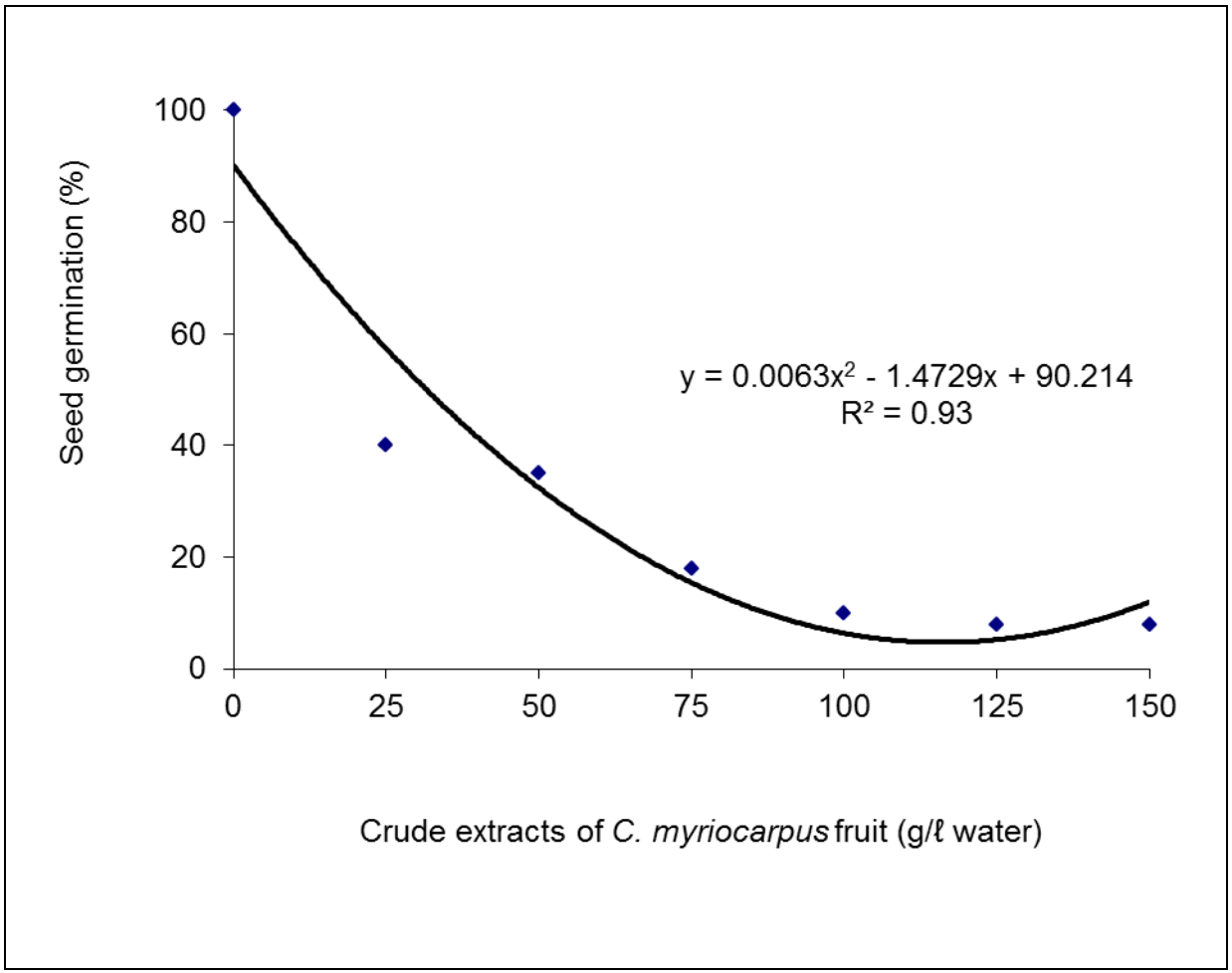

Figure 4.13 Quadratic relationship between germination of maize and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

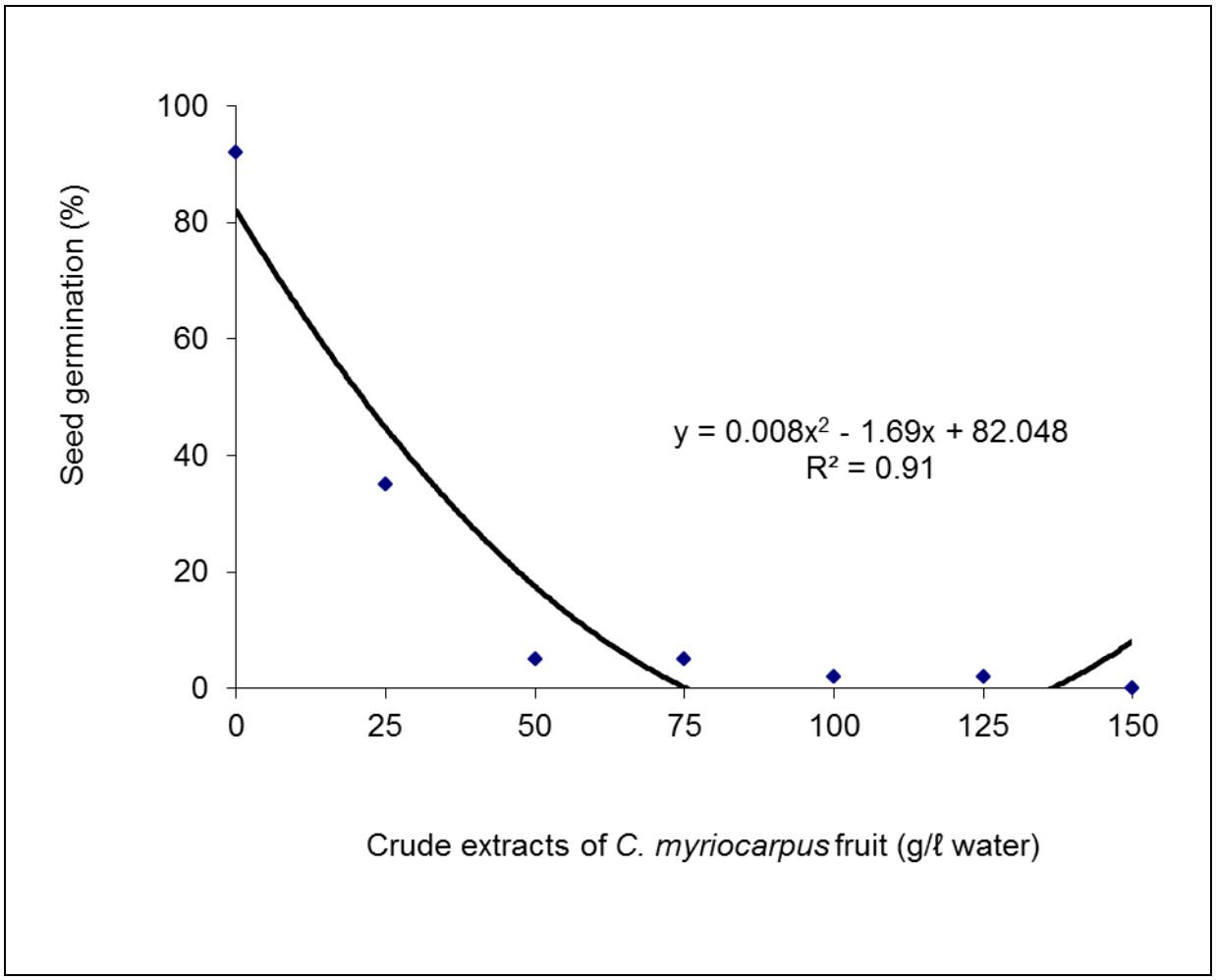

Figure 4.14 Quadratic relationship between germination of millet and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

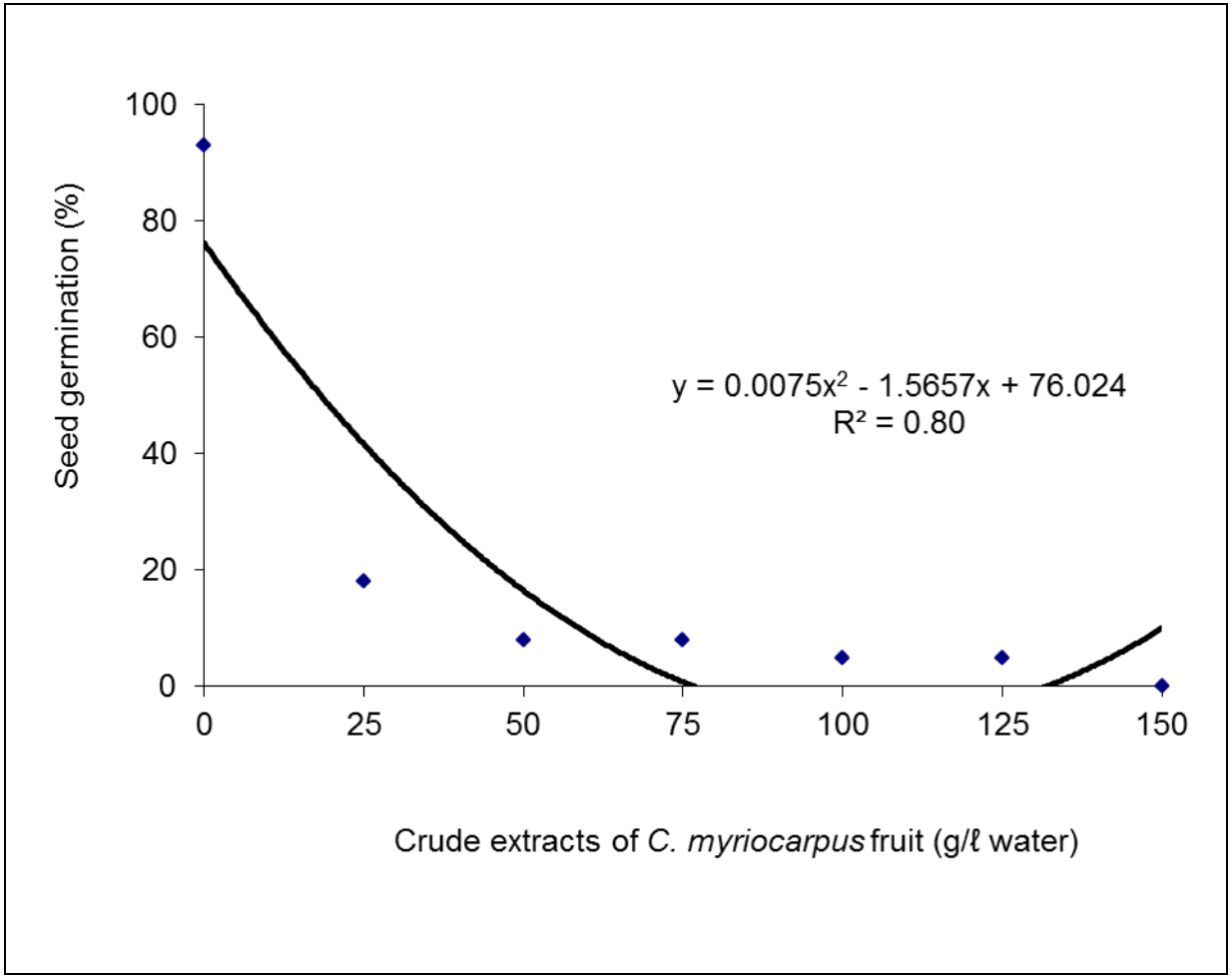

Figure 4.15 Quadratic relationship between germination of onion and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

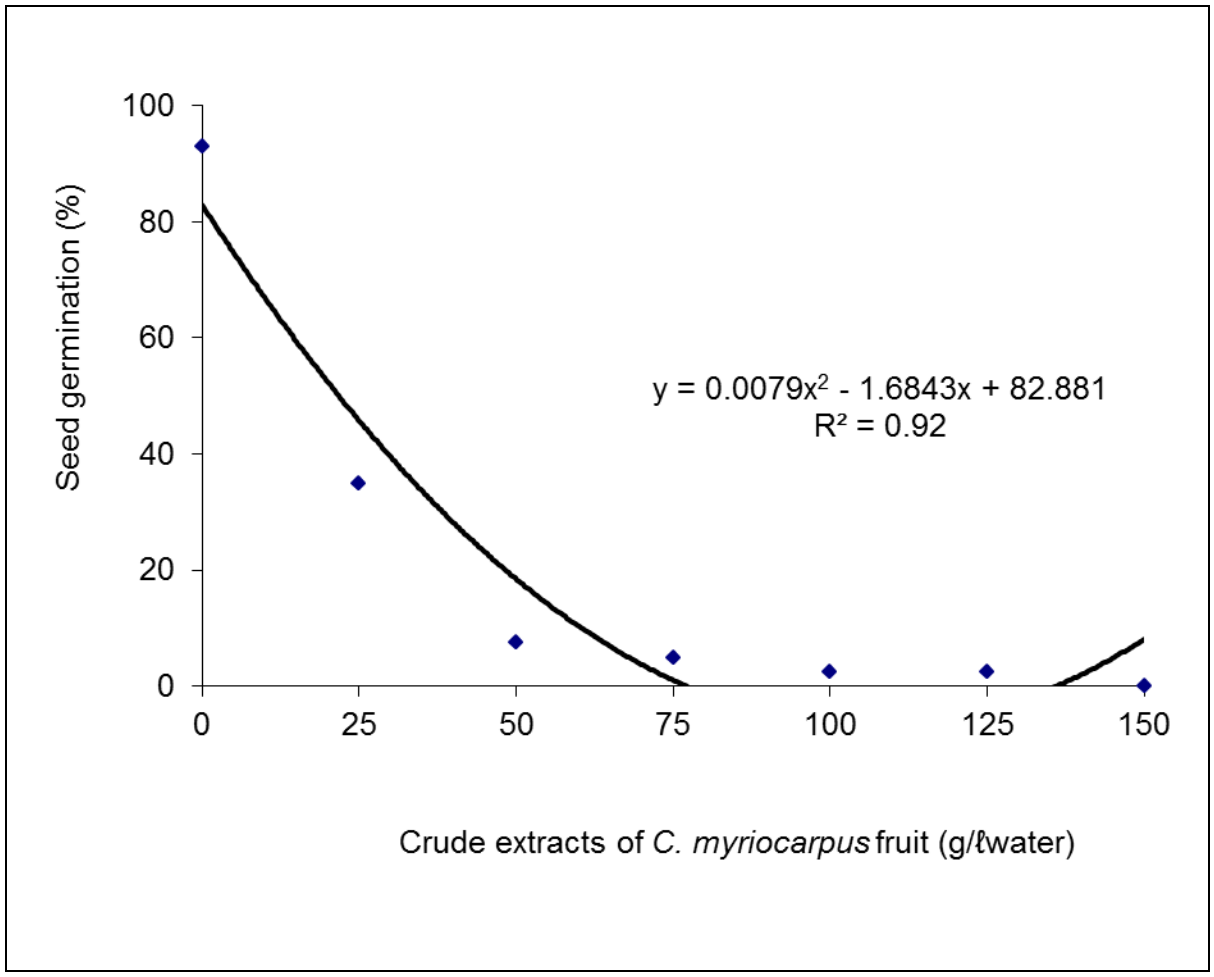

Figure 4.16 Quadratic relationship between germination of rye and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

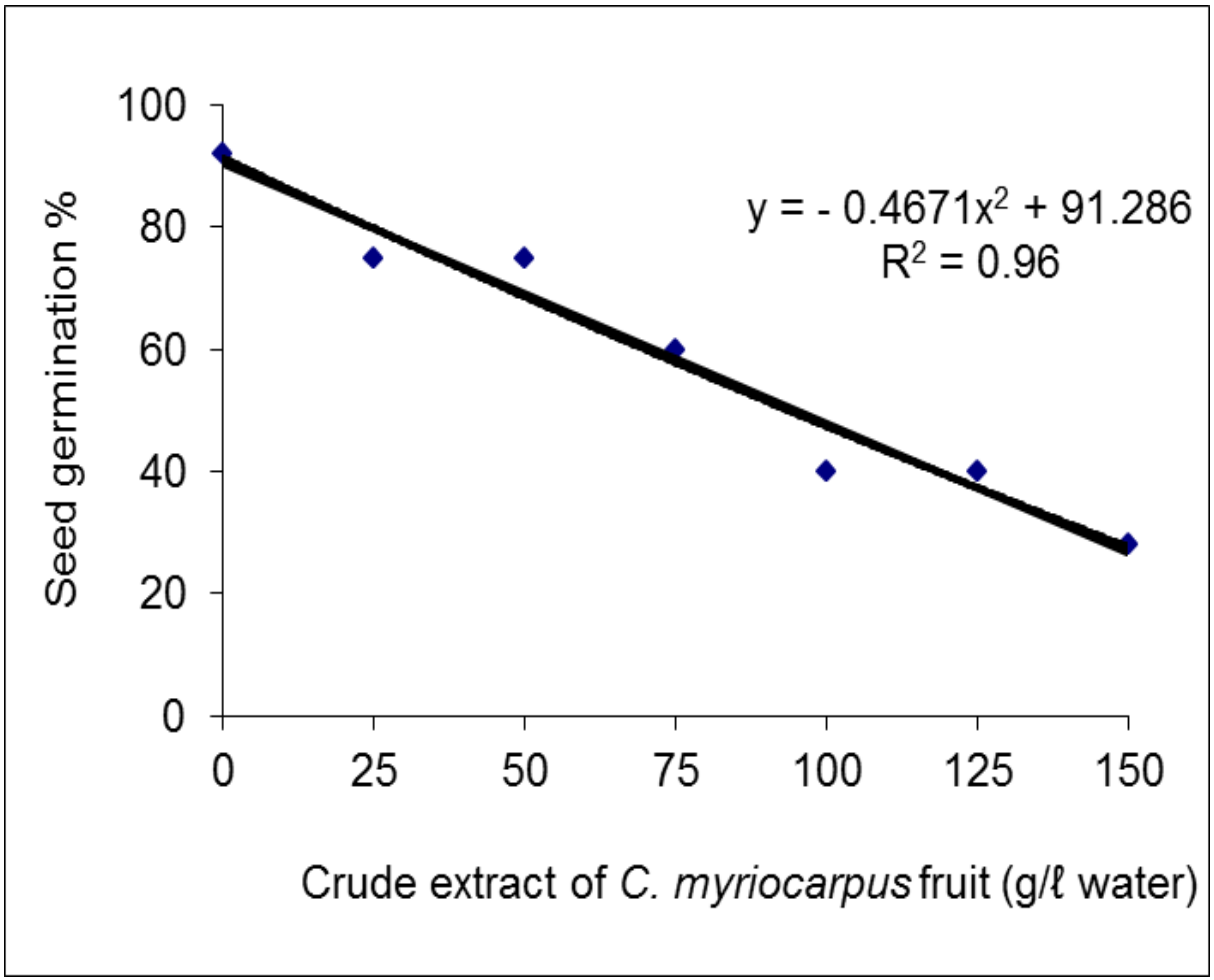

Figure 4.17 Linear relationship between germination of sorghum and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

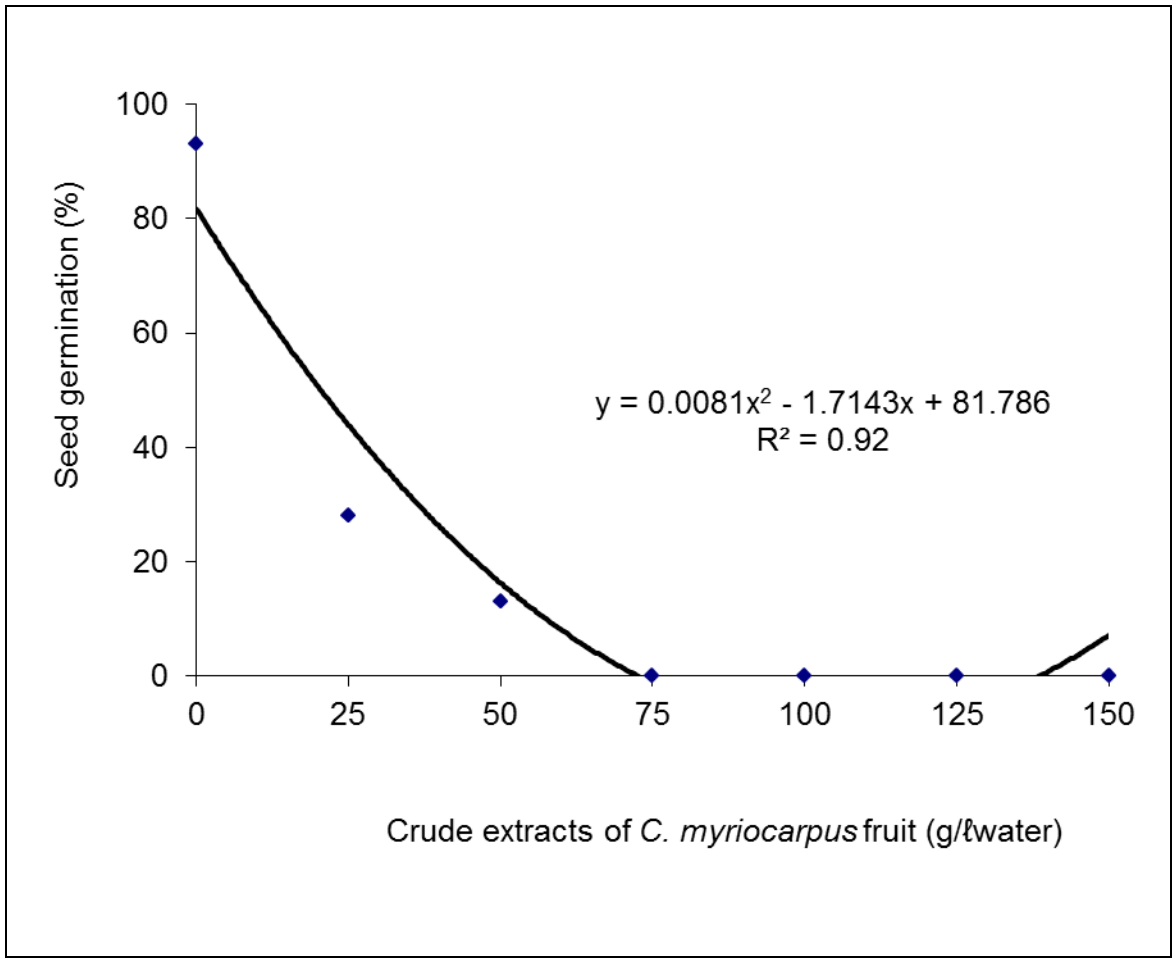

Figure 4.18 Quadratic relationship between germination of wheat and aqueous crude extracts of *Cucumis myriocarpus* fruit at 10 days after treatment (n = 28).

4.4 Discussion

Exposed to a series of aqueous crude extract solutions of *C. myriocarpus* fruit, seed germination, regardless of whether from dicotyledonous or monocotyledonous crops, exhibited more or less similar quadratic relationships, with the exception of sorghum. Results of this study confirmed those observed in the seedling emergence trials under greenhouse conditions (Chapter 3). Quadratic relationships in this study also suggested that the dosages of crude extract solutions of *C. myriocarpus* fruit used were already beyond the saturation level required to provide stimulation responses (Salisbury and Ross, 1992; Mamphiswana *et al*., 2010). In sorghum where linear relationships were depicted, the dosages of crude extract solutions of *C. myriocarpus* fruit used might have already been above the dosage for the saturation range (Mamphiswana *et al*., 2010).

In this study, the focus was not to demonstrate the allelopathic effects of crude extract of *C. myriocarpus* fruit to various crops, but to determine whether the material could be used as a pre-emergent bio-nematicide in the stimulation range. Germination comprises initiation of complex chemical processes which include hormone synthesis, enzyme activity, membrane permeability, absorption of sugars and minerals and cell division (Campbell, 1990). Allelopathic chemicals can affect any of these activities, and therefore, curtail germination.

Allelopathy from other sources prevented cell division of embryos, whereas in other cases inhibited GA or hydrolytic enzyme activities (Einhellig, 1985; Putnam and Tang, 1986; Won and Kil, 1997; Martin and Blackburn, 2003; Inderjit and Duke, 2003; Jeronimo *et al*., 2005). In white mustard (*Sinapis alba* L.), juglone and

sorgoleone potent allelochemicals from crude extracts of black walnut (*Junglans nigra* L.) leaves, inhibited the primary action of ATP production in germinating seeds by inhibiting chloroplast-oxygen evolution in the cotyledons and thereby affecting mitochondrial functions (Einhellig, 1985). Lovett *et al*. (1989) demonstrated that radicle protrusion and elongation in linseed (*Linum utatissimum* L.) was inhibited by benyl-amine, an allelochemical produced from leaf washings of camelina weed (*Camelina sativa* L.). In thorn-apple (*Datura stramonium* L.), alkaloids from winter wheat (*Triticum aestivum* L.) straws interfered with metabolism of food reserves in the endosperm of germinating seeds (Mazloom *et al*., 2009).

A glance at the coefficients of determination in the quadratic relationships within the crops in both seedling emergence (Chapter 3) and seed germination in this study showed that they were more or less similar. The observation was true among different crops in both dicotyledonous and monocotyledonous crops, which suggested that the site of reaction of the allelochemicals from the crude extracts of *C. myriocarpus* fruit in various seeds had similar receptors in the germination process.

In the Ground Leaching Technology system, 2 g/plant crude extracts of *C. myriocarpus* fruit when applied at transplanting consistently supressed numbers of *M. incognita* with fertiliser effect on crops (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2007). The negative quadratic relationships between the variables measured and the concentration of crude extracts of *C. myriocarpus* fruit in all trials do not imply that the material was not suitable as a pre-emergent bio-nematicide at all levels. The relationship simply implied that the quantities currently

used for suppressing nematodes when applied at planting, were at the toxic ranges for seed germination and seedling emergence of the test crops. Allelopathic effects differ with the age of the plant (Rice, 1984; Einhellig and Leather, 1988), suggesting that dosages exist for seed germination or seedling emergence and transplanting stages. Also, since the coefficients of determination were more or less similar for both germination and emergence (Chapter 3), it appears that crops within the same family may require similar dosages of the test material.

4.5 Conclusions

Results of this and the previous study (Chapter 3) suggested that the quantities of crude extracts of *C. myriocarpus* fruit were not compatible with germination and emergence of all the test crops. However, due to the observed density-dependent relationships, there could be dosages of crude extracts of *C. myriocarpus* fruit that could stimulate growth of various seedlings. The average between the starting points of stimulation and saturation could be the point where the material would be suitable as a pre-emergent bio-nematicide. In ensuing studies, computer models would be used to determine the distinguishing features of density-dependent growth patterns.

CHAPTER 5
DOSAGE-DEPENDENT GROWTH RESPONSES OF SELECTED CROPS TO CRUDE EXTRACTS OF *CUCUMIS MYRIOCARPUS* FRUIT

5.1 Introduction

Quadratic relationships are indicative of biological systems that interact with either extrinsic or intrinsic factors in accordance to the density-dependent growth patterns, which are characterised by stimulation, saturation or inhibition responses (Salisbury and Ross, 1992). Maximum rates of reproduction versus initial nematode population density (Pi) of the southern root-knot nematode (*Meloidogyne incognita*) under various conditions (Pofu *et al*., 2010a,b) and the concentration of antioxidants versus those of phenolic compounds in organs of *Monsonia burkeana* Planch (Mamphiswana *et al*., 2010), exhibited strong density-dependent growth patterns. The observed growth patterns might provide some reasons why literature is replete with inconsistent results for measurements of similar responses under various environmental conditions (Mamphiswana *et al*., 2010).

Germination and emergence of selected monocotyledonous and dicotyledonous crops versus a series of crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit had negative quadratic relationships (Chapters 3 and 4), which were interpreted to imply that the concentrations of crude extracts of *C. myriocarpus* fruit were already beyond the saturation points for germination and emergence in the tested crops as described elsewhere (Mamphiswana *et al*., 2010). Using the density-dependent growth patterns, it was shown that cucumin, one of the active constituents of cucurbitacin A in crude extracts of *C. myriocarpus* fruit (Rimington, 1938) inhibited division of cancer cells but at concentrations where the material was toxic to healthy cells, whereas at lower concentrations division of cancer cells was stimulated (Van

Wyk *et al*., 1997). Stimulation dosages of the material would be ideal in crop production. However, the quantities cannot be determined using conventional statistical methods.

The Curve-fitting Allelochemical Response Data (CARD) computer model was developed previously to quantify responses in biological systems to extrinsic factors in relation to density-dependent growth patterns (Liu *et al*., 2003). In this model, the degree of sensitivity in stimulation, saturation or inhibition was determined through six biological indices, *viz*. (1) threshold stimulation (D_m) - the dosage at which the independent factor begins to have a measurable effect on the dependent variable, (2) saturation level (R_h) - the dosage at which the response remains constant prior to decreasing, (3) 0% inhibition (D_0) - the end-point dosage of R_h where the independent factor has zero effect on the dependent factor, (4) 50% inhibition (D_{50}) - the dosage level where the independent factor inhibits the dependent factor by 50% and (5) 100% inhibition (D_{100}) - the dosage level where the independent factor inhibits the dependent factor by 100% and (6) transformation level (k) – degree of sensitivity to test material (Liu *et al*., 2003).

Characteristically, CARD model does not provide the quadratic equation and the coefficient of determination (R^2) on the graphs, as is the case when using Excel computer programme. However, the model provides the two indices separately as analytical outputs in a summary format (Appendix 5.1). Properly designed, the CARD model would probably provide insight on appropriate dosages of crude extracts of *C. myriocarpus* fruit within the stimulation range, where the material could serve as a pre-emergent bio-nematicide. The objective of this study was to determine whether

selected crops in the families Alliaceae, Gramineae and Solanaceae would respond to crude extracts of *C. myriocarpus* fruit in density-dependent growth patterns, which are characterised by stimulation, saturation and inhibition. This information would enable computation of mean dosage response for stimulation when using crude extracts of *C. myriocarpus* fruit as a pre-emergent bio-nematicide in test crops.

5.2 Materials and methods

Nine separate experiments with three crops each from the families Alliaceae, Gramineae and Solanaceae, representing economically important crops in Limpopo Province, South Africa, were conducted at the Horticultural Skills Centre of the University of Limpopo (23º53′10″S, 29º44′15″E), with ambient day/night temperatures averaging 27°C/18°C. Relative humidity, photosynthetically active radiation and solar radiation were not measured. Each crop constituted a separate experiment, where fifty 15-cm-diameter plastic pots were placed on greenhouse benches and filled with 5 ℓ growing mixture, comprising 3:1 (volume/volume) steam-pasteurised sand and Hygromix (Hygrotech, North Tshwane, South Africa). Fruit of *C. myriocarpus* were collected locally, prepared and stored as described previously (Chapter 3).

In the family Alliaceae, selected test crops included onion (*Allium cepa* L.) cv. 'Texas Grano', leek (*Allium fistosum* L.) cv. 'Hygrotech G07157' and chive (*Allium schoenoprasum* L.) cv. 'Hygrotech J03940', with individual experiments running from May through July in 2009. In the family Gramineae, maize (*Zea mays* L.) cv. 'SNK 2147', millet (*Panicum miliaceum* L.*)* cv. 'Babala [OPV]' and sorghum (*Sorghum bicolour* L.) cv. 'Pannar 8609' tested from November 2009 through February 2010. In

the family Solanaceae, trials for eggplant (*Solanum melongena* L.) cv. 'Black Beauty', pepper (*Capsicum annum* L.) cv. 'Capistrano' and tomato (*Solanum lycopersicum* L.) cv. 'Floradade' were conducted from mid-February through April in 2010.

5.2.1 Experimental design and cultural practices

In each experiment, 10 treatments, *viz*. 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.25 g crude extracts of *C. myriocarpus* fruit per pot, were arranged in a randomised complete block design, with five replicates. Pots were irrigated to field capacity prior to planting and then with 250 mℓ tapwater every other day. Two seeds per pot were planted at commercially prescribed depths (Hygrotech, 2009), with organic amendment applied in separate holes around the seeds at the same depths and covered with growing mixture. Plants were thinned to one per pot soon after emergence.

5.2.2 Data collection

Eighteen days after planting, for monocotyledonous crops, seedling height (cm), radicle length (cm), coleoptile length (cm) and coleoptile diameter (mm) were measured, whereas for dicotyledonous crops, hypocotyl diameter (mm), seedling height (cm), hypocotyl length (cm) and epicotyl length (cm) were measured. Hypocotyl and coleoptile diameters were measured using a digital vernier caliper below the axis of the primary leaf.

5.2.3 Data analysis

Data were subjected to analysis of variance using the SAS programme (SAS Institute Inc., 2004), with treatment means separated using the Waller-Duncan multiple-range test. Significant treatment means ($P \leq 0.01$) were subjected to CARD model to determine the biological indices, *viz*. (D_m), (R_h), (D_0), (D_{50}), (D_{100}), and k. The output summary of eggplant epicotyl length data was exhibited to provide information on how the model operates (Appendix 5.1). Mean dosage stimulation response (MDSR) was computed for various crops as half of the sum of threshold stimulation (D_m) and saturation level (R_h) [$MDSR = (D_m + R_h)/2$].

5.3 Results

Results of each family were separately recorded in order to enhance clarity, whereas those of mean dosage stimulation responses were integrated at the end of this section to allow for validation.

5.3.1 Alliaceae family

In all organs measured R^2 averaged at least 0.97 (range 0.94 - 0.99), suggesting the existence of strong density-dependent growth patterns among variables and test dosages for chive (Table 5.2), leek (Table 5.4) and onion (Table 5.6). Relationships of the four variables and test dosages were graphically summarised for chive (Figures 5.1 – 5.4), leek (Figures 5.5 – 5.8) and onion (Figures 5.9 – 5.12).

Generally, at low dosages the material stimulated growth of all variables, while at high dosages the material inhibited growth of seedlings. In chive (Table 5.1), the transformation levels for seedling height increased from k = 0 (R^2 = 0.89) to k = 6 (R^2

= 0.97). Further increases in k values resulted in the decrease of R^2 to 0.95 at k = 10. Consequently, in chive the best fit to the data for seedling height was at k = 6. Similarly, for the radicle length, coleoptile length and coleoptile diameter in chive, the best fits to the data were at k = 5, k = 5 and k = 8, respectively, in leek at k = 7, k = 20 and k = 15, respectively (Table 5.3), and in onion at k = 3, k = 5 and k = 7, respectively (Table 5.5).

Radicle and coleoptile lengths for chive had the same k = 5 values, in leek seedling height and coleoptile length each had a k = 7 value, whereas in onion coleoptile length and diameter had the same k = 7 values. Among the crops, chive and onion had the same k = 5 values for radicle length, whereas leek and onion had the same k = 7 values for coleoptile length. In terms of the model, onion seedling height, with k = 3 value was the most sensitive to crude extracts of *C. myriocarpus* fruit, whereas leek radicle length with k = 20 was the least sensitive to the material. Overall, onion with ∑k = 22 was the most sensitive to the material, whereas leek with ∑k = 49 was the least sensitive to the material.

Table 5.1 Responses of four yield components of chive seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.16[Z]	0.23	0.22	0.15	0.19
Saturation point (R_h)	1.72	5.81	0.78	0.50	2.20
0% inhibition (D_0)	0.56	0.91	0.85	0.75	2.51
50% inhibition (D_{50})	1.09	1.39	1.37	1.25	1.28
100% inhibition (D_{100})	2.00	2.10	2.10	2.10	2.08
K	**k = 6**	**k = 5**	**k = 5**	**k = 8**	**6.00**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 24					

[Z] Dosage in grams.

Table 5.2 Quadratic relationships of seedling height, radicle length, coleoptile length and coleoptile diameter of chive at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Seedling height	$y = 31.963x^2 - 148.568x + 4.484$	0.97
Radicle length	$y = 79.449x^2 - 271.694 + 7.536$	0.96
Coleoptile length	$y = 10.953x^2 - 38.420x + 1.222$	0.99
Coleoptile diameter	$y = 10.280x^2 - 52.670x + 0.604$	0.94

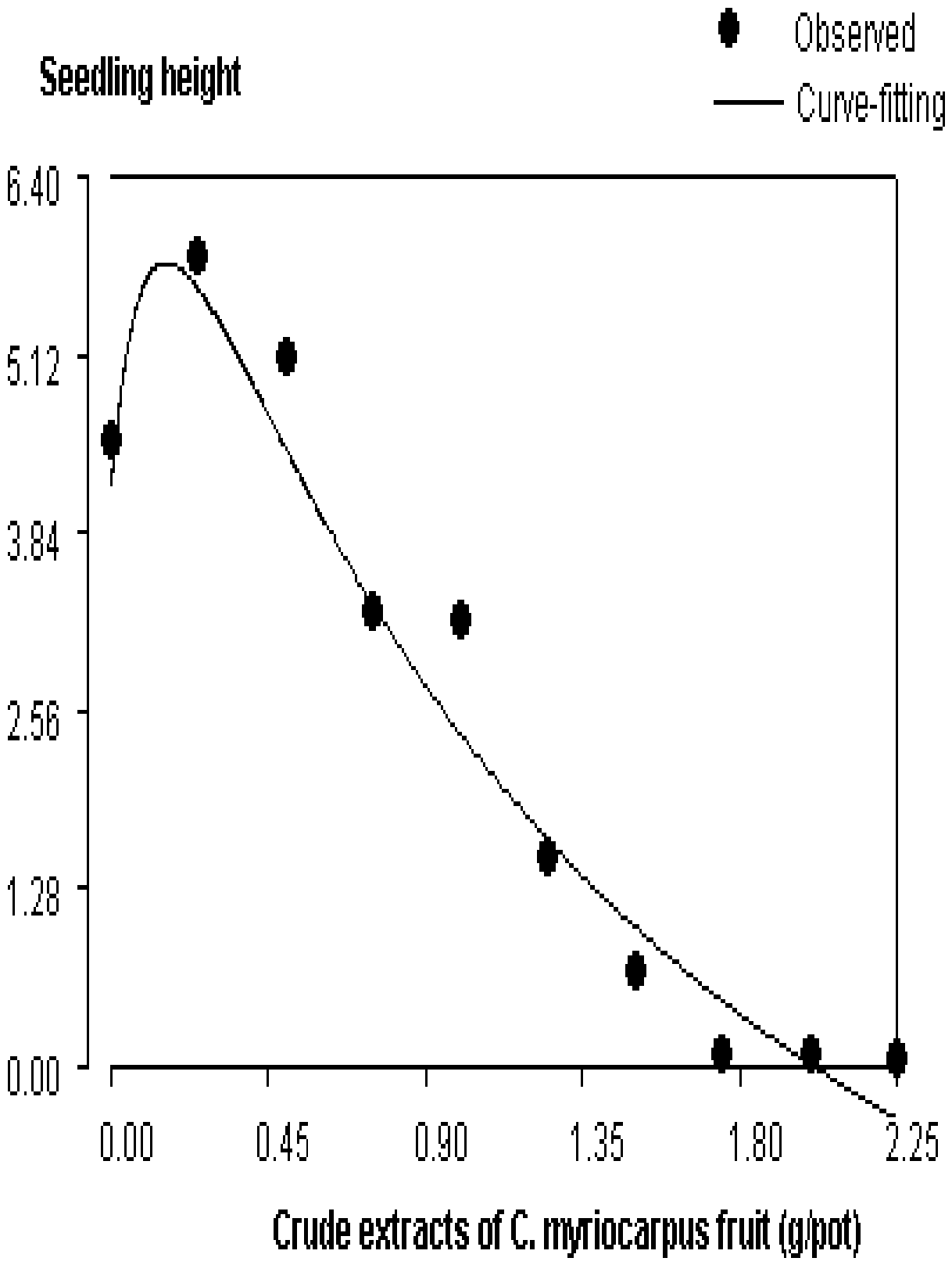

Figure 5.1 Response of seedling height of chive seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

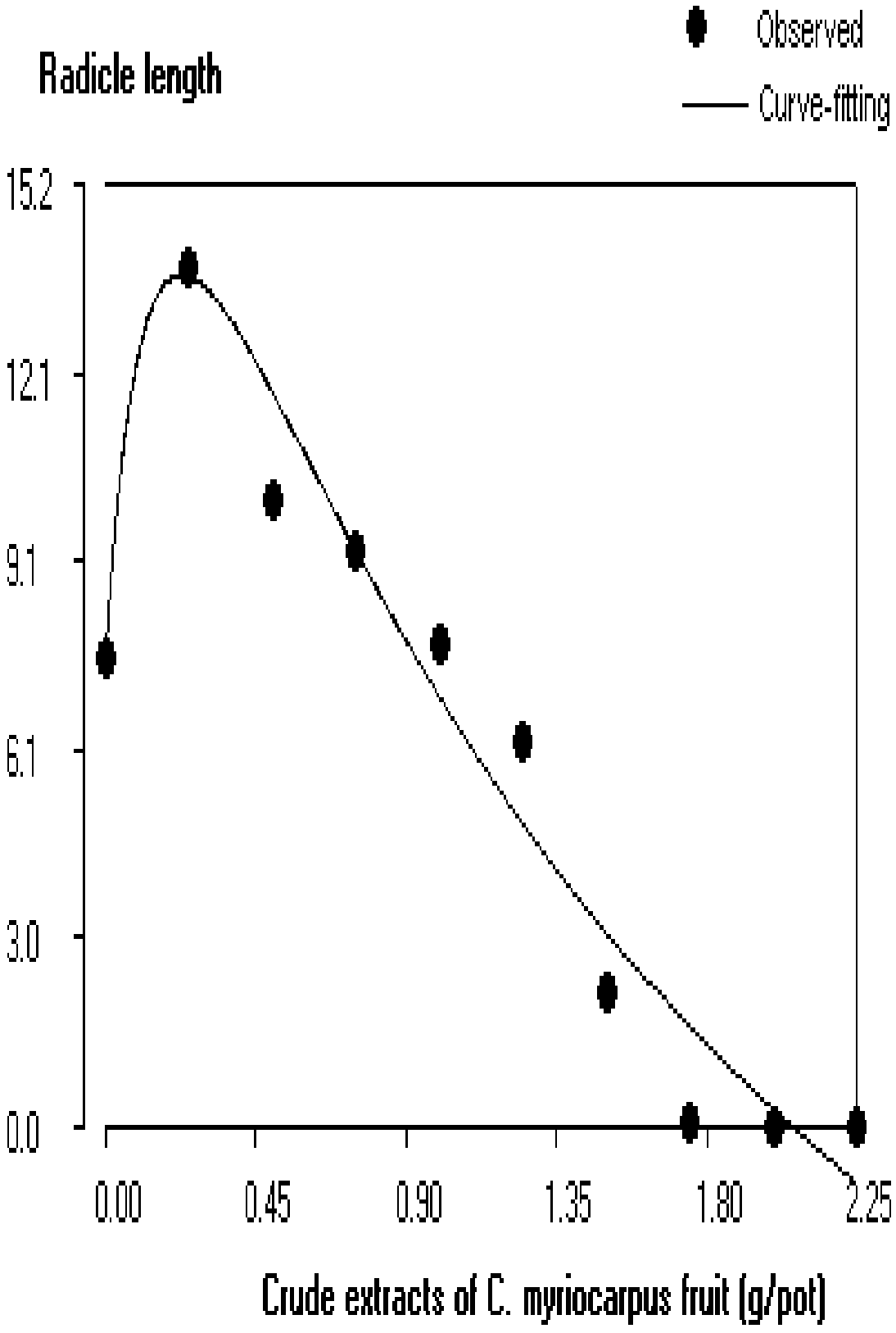

Figure 5.2 Response of radicle length of chive seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

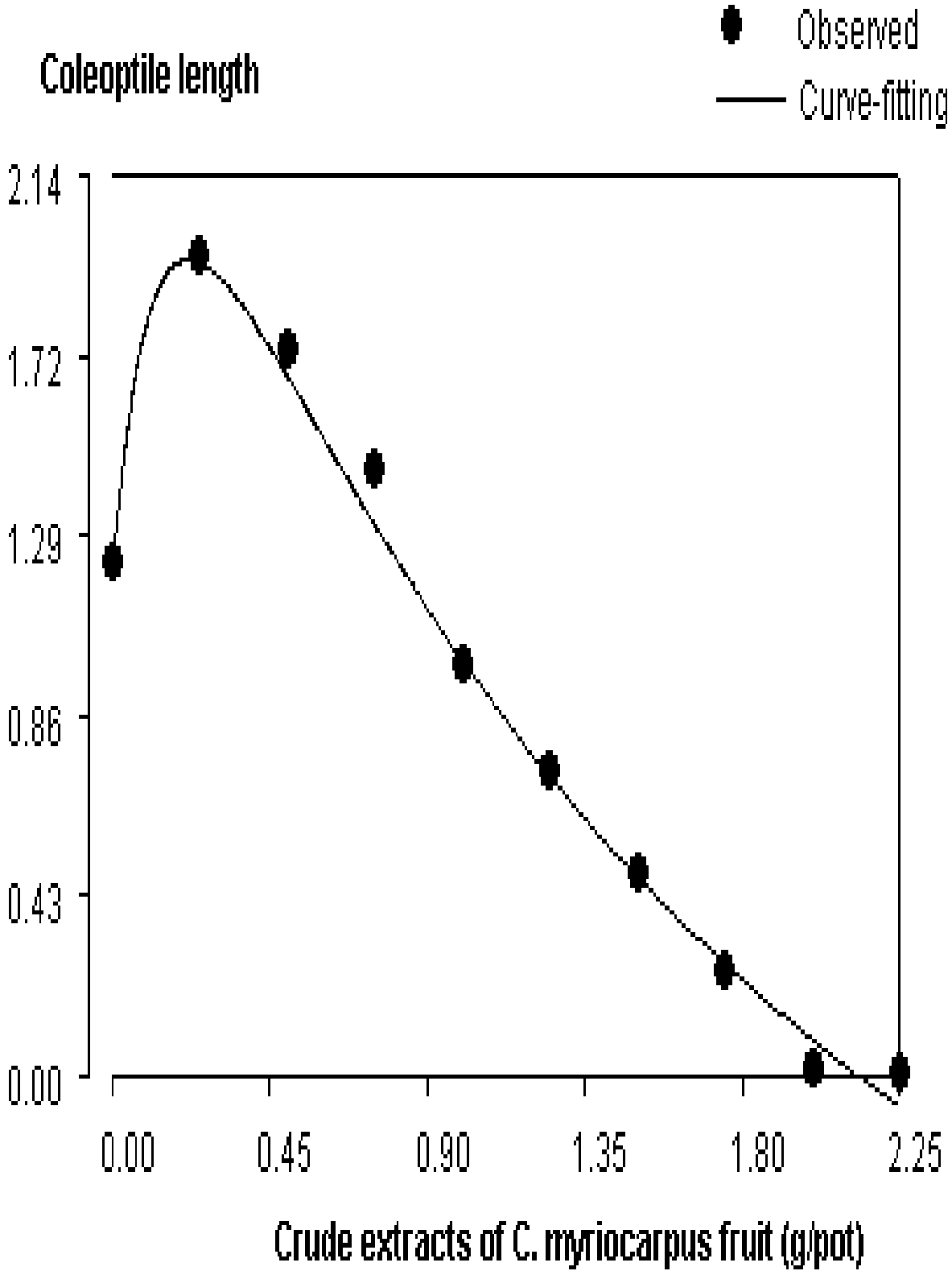

Figure 5.3 Response of coleoptile length of chive seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

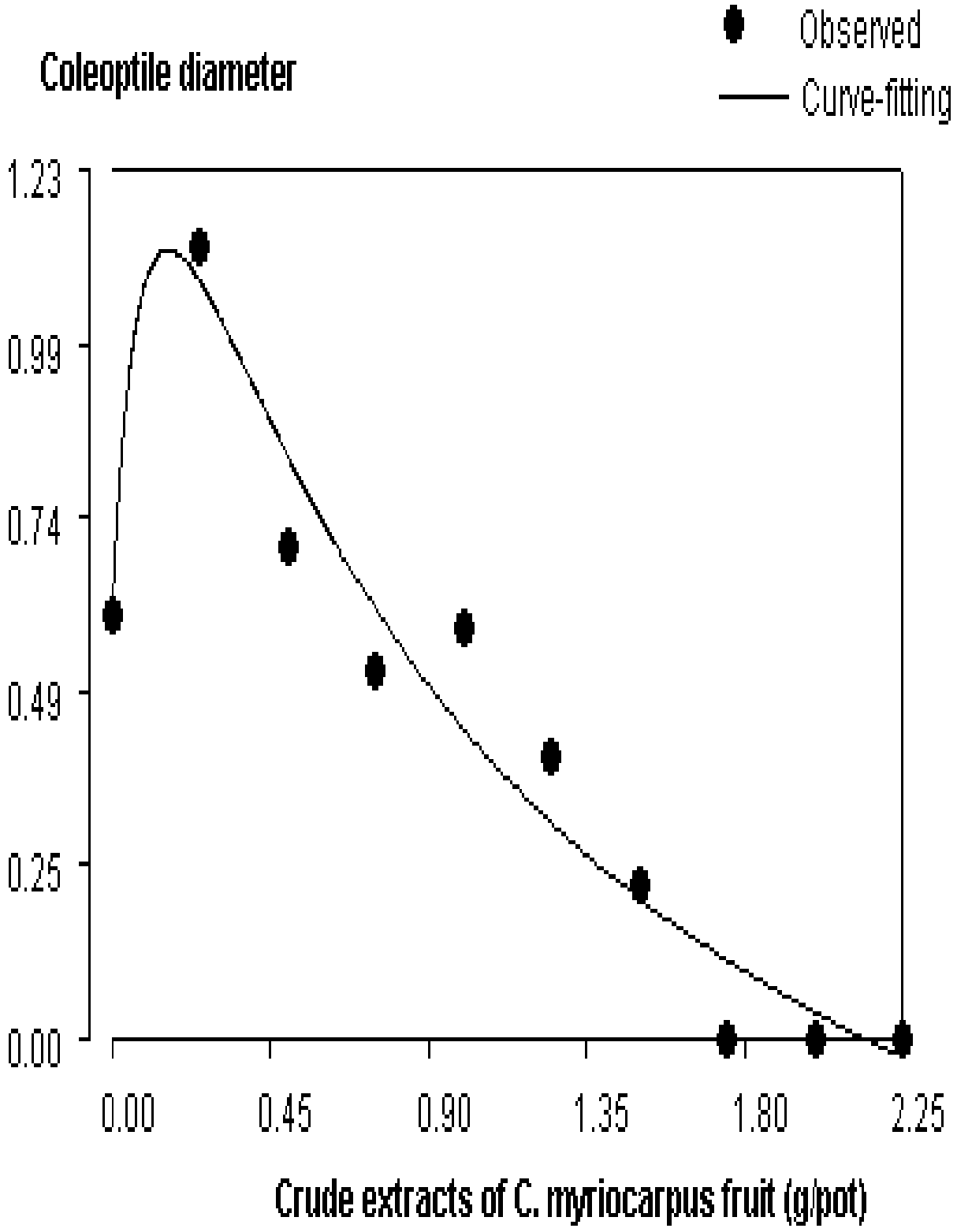

Figure 5.4 Response of coleoptile diameter of chive seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Table 5.3 Responses of four yield components of leek seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Means
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.22[Z]	0.08	0.15	0.08	0.53
Saturation point (R_h)	0.77	1.47	0.62	0.43	0.82
0% inhibition (D_0)	1.39	0.68	0.61	0.38	0.77
50% inhibition (D_{50})	1.66	1.23	1.21	0.87	1.24
100% inhibition (D_{100})	2.00	2.90	2.40	2.70	2.50
K	**k = 7**	**k = 20**	**k = 7**	**k = 15**	**12.25**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 49					

[Z] Dosage in grams.

Table 5.4 Quadratic relationships of seedling height, radicle length, coleoptile length and coleoptile diameter of leek at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Seedling height	$y = 75.311x^2 - 297.251x + 1.393$	0.97
Radicle length	$y = 466.942x^2 - 504.255x + 6.432$	0.99
Coleoptile length	$y = 12.268x^2 - 20.905x + 1.313$	0.96
Coleoptile diameter	$y = 17.176x^2 - 169.897x + 0.781$	0.99

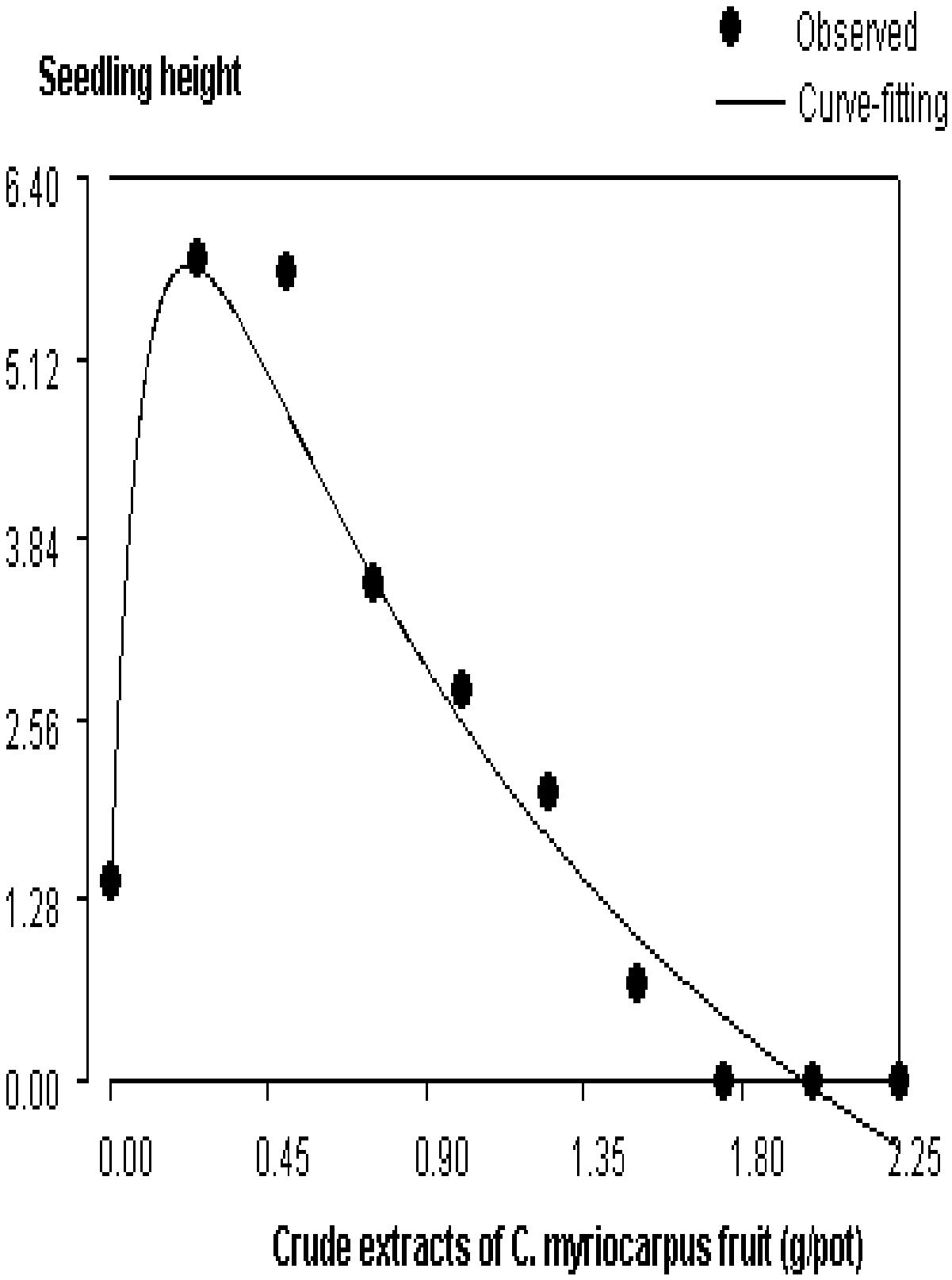

Figure 5.5 Response of seedling height of leek seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

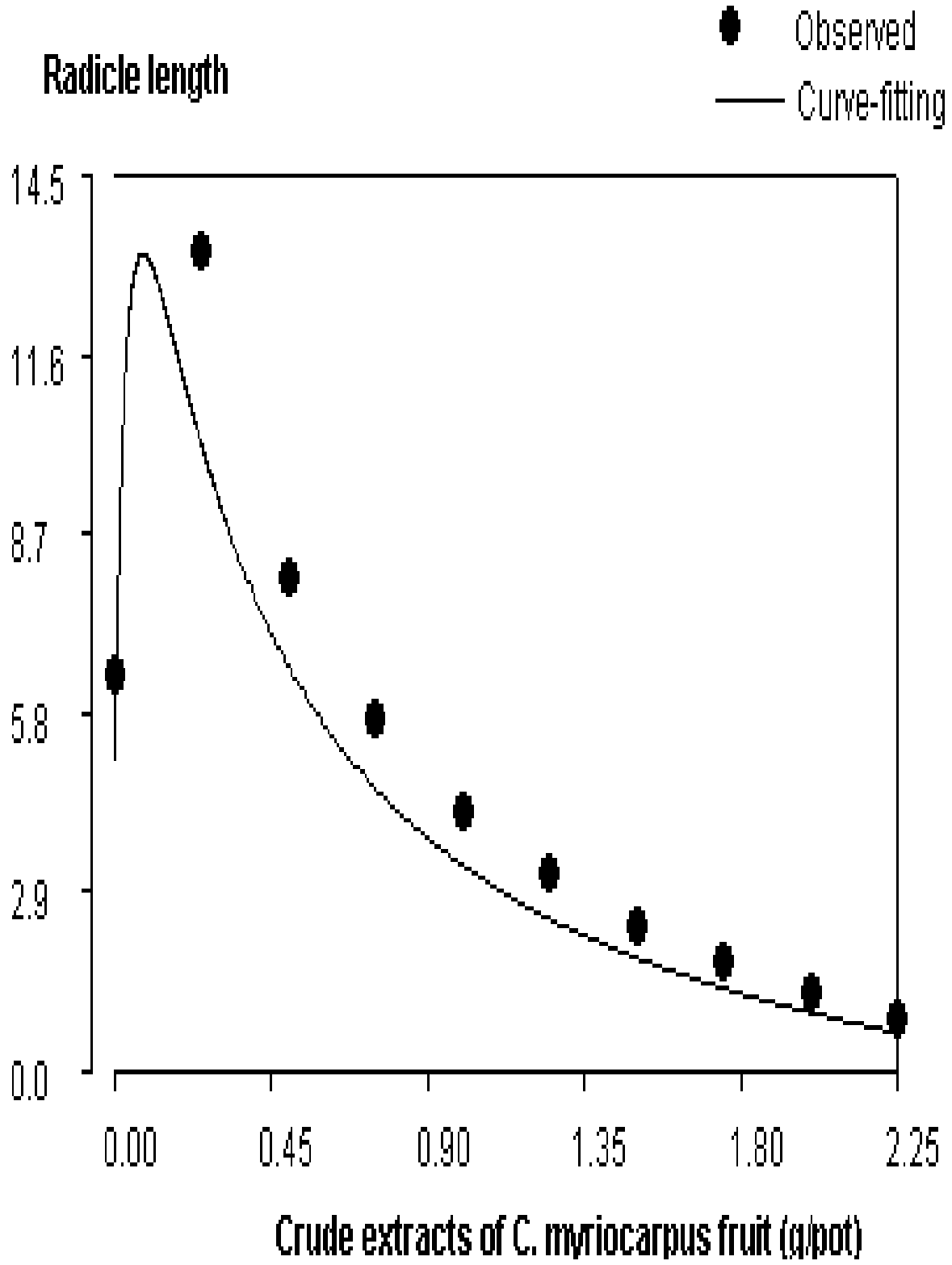

Figure 5.6 Response of radicle length of leek seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

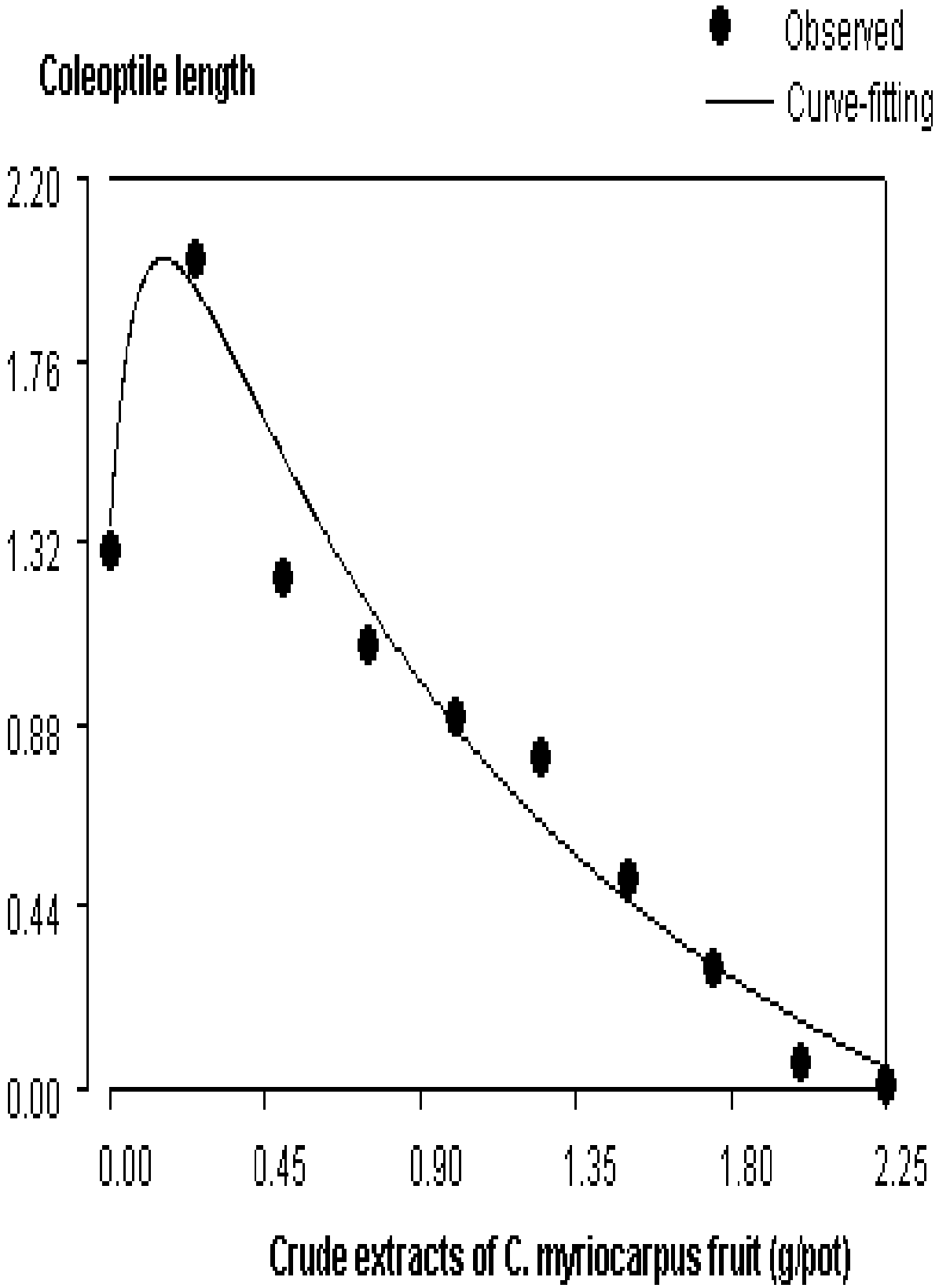

Figure 5.7 Response of coleoptile length of leek seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

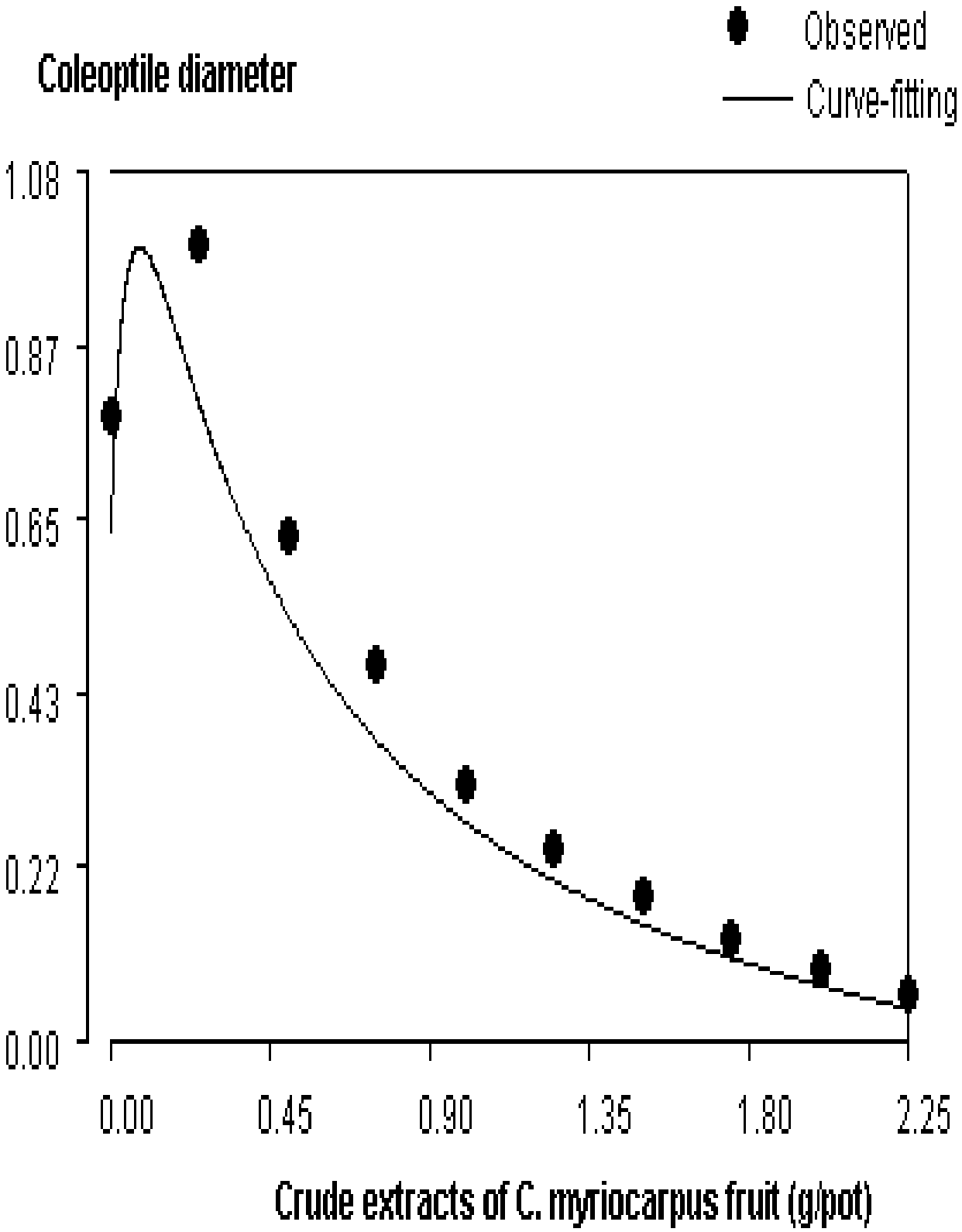

Figure 5.8 Response of coleoptile diameter of leek seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Table 5.5 Responses of four yield components of onion seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.34[2]	0.23	0.16	0.16	0.22
Saturation point (R_h)	0.69	0.97	0.68	0.34	0.67
0% inhibition (D_0)	1.17	0.95	0.67	0.62	0.85
50% inhibition (D_{50})	1.62	1.59	1.32	1.29	1.45
100% inhibition (D_{100})	2.10	2.60	2.60	2.70	2.5
K	**k = 3**	**k = 5**	**k = 7**	**k = 7**	**5.5**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 22					

[2] Dosage in grams.

Table 5.6 Quadratic relationships of seedling height, radicle length, coleoptile length and coleoptile diameter of onion at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Seedling height	$y = 23.775x^2 - 52.356x + 3.312$	0.97
Radicle length	$y = 66.962x^2 - 225.707x + 7.666$	0.95
Coleoptile length	$y = 12.989x^2 - 62.487x + 1.328$	0.98
Coleoptile diameter	$y = 6.807x^2 - 33.656x + 0.783$	0.99

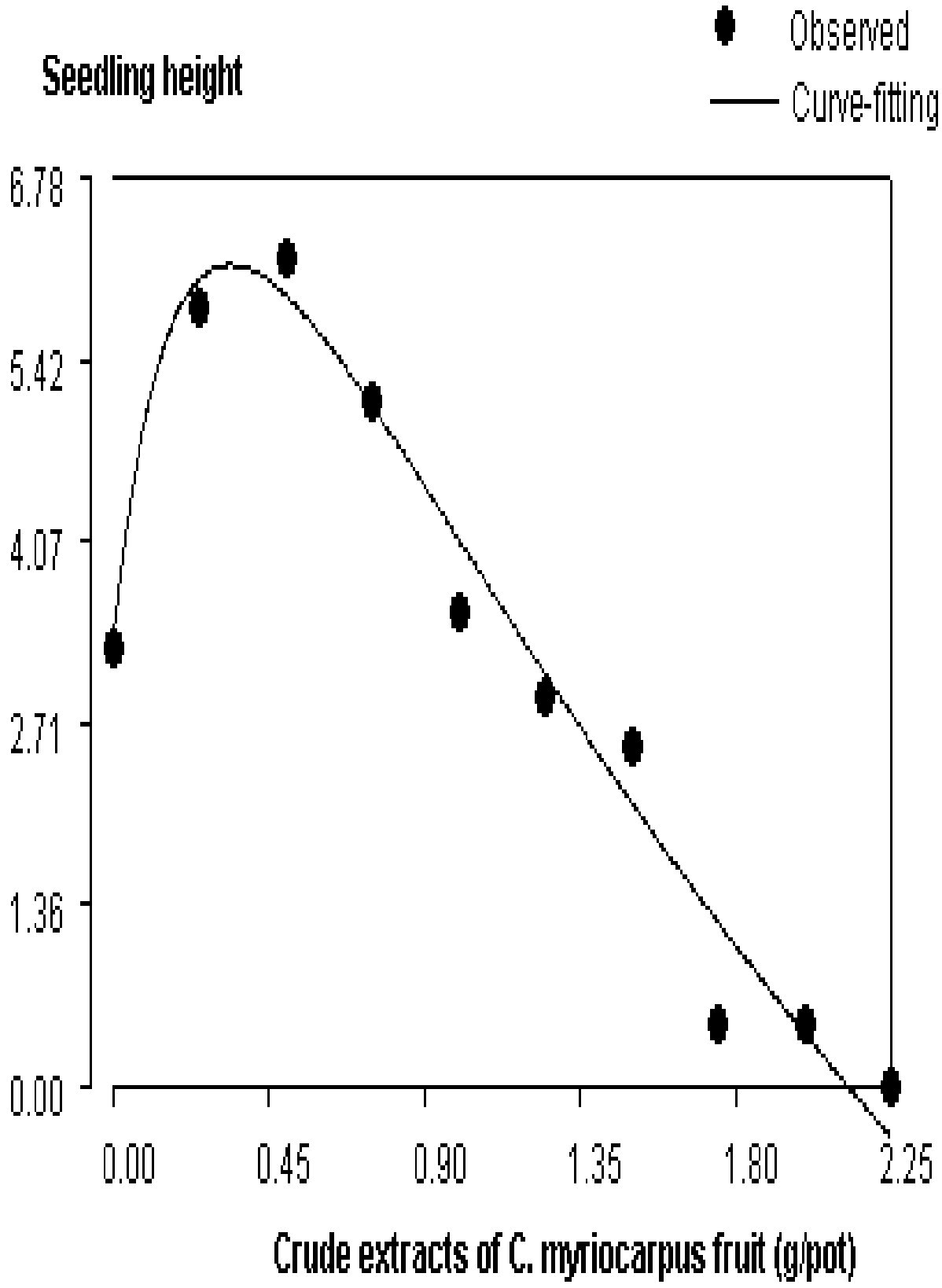

Figure 5.9 Response of seedling height of onion seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

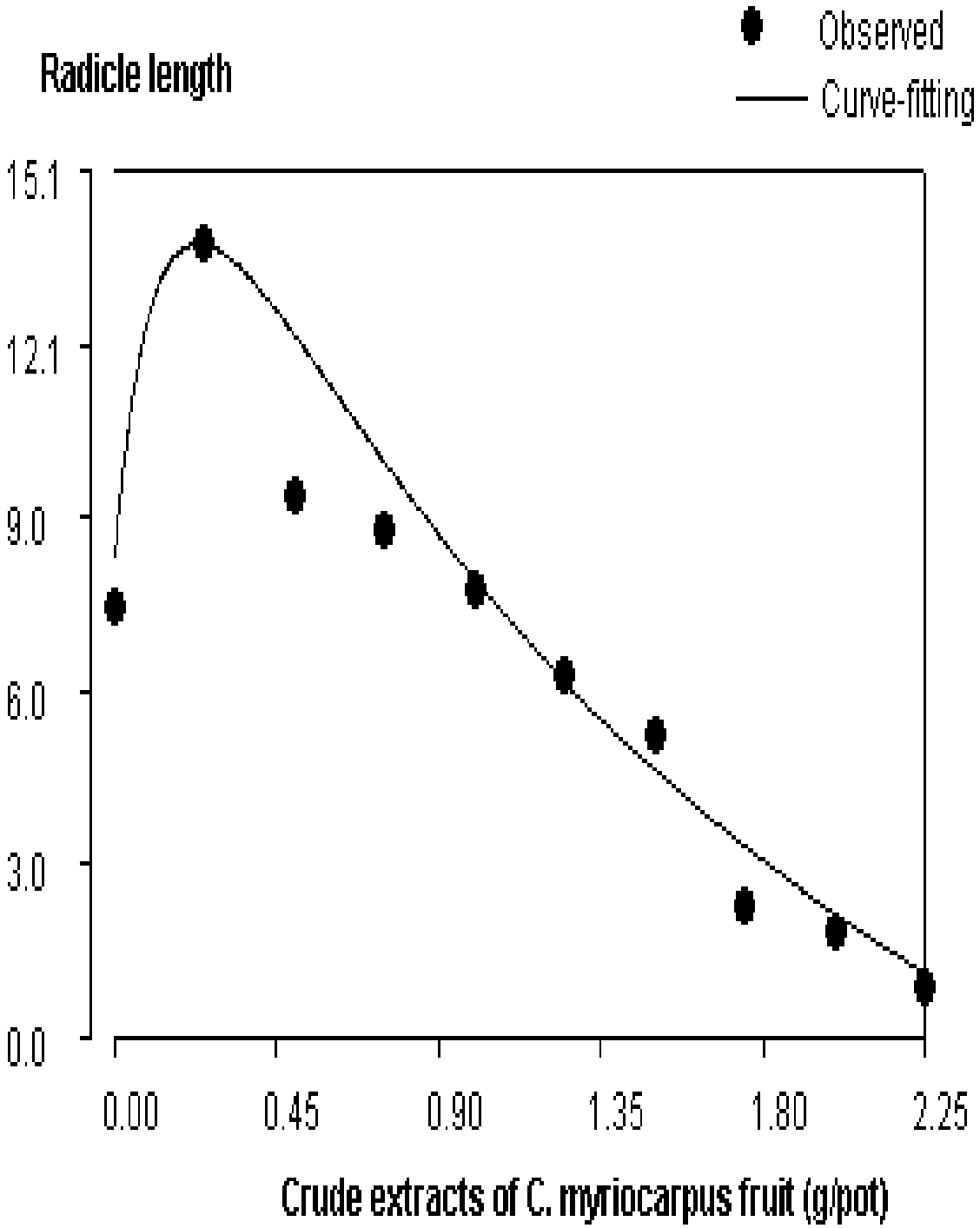

Figure 5.10 Response of radicle length of onion seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

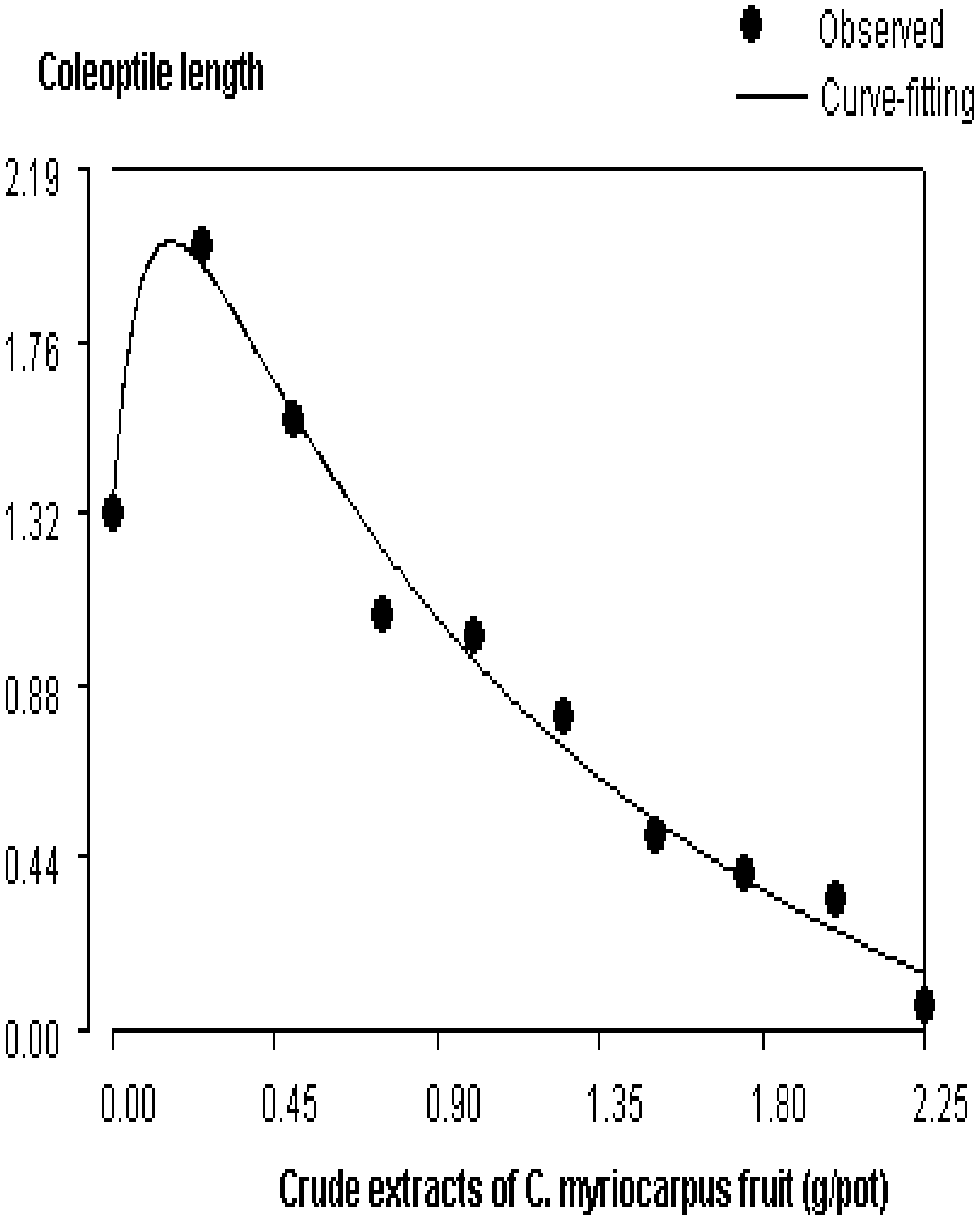

Figure 5.11 Response of coleoptile length of onion seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

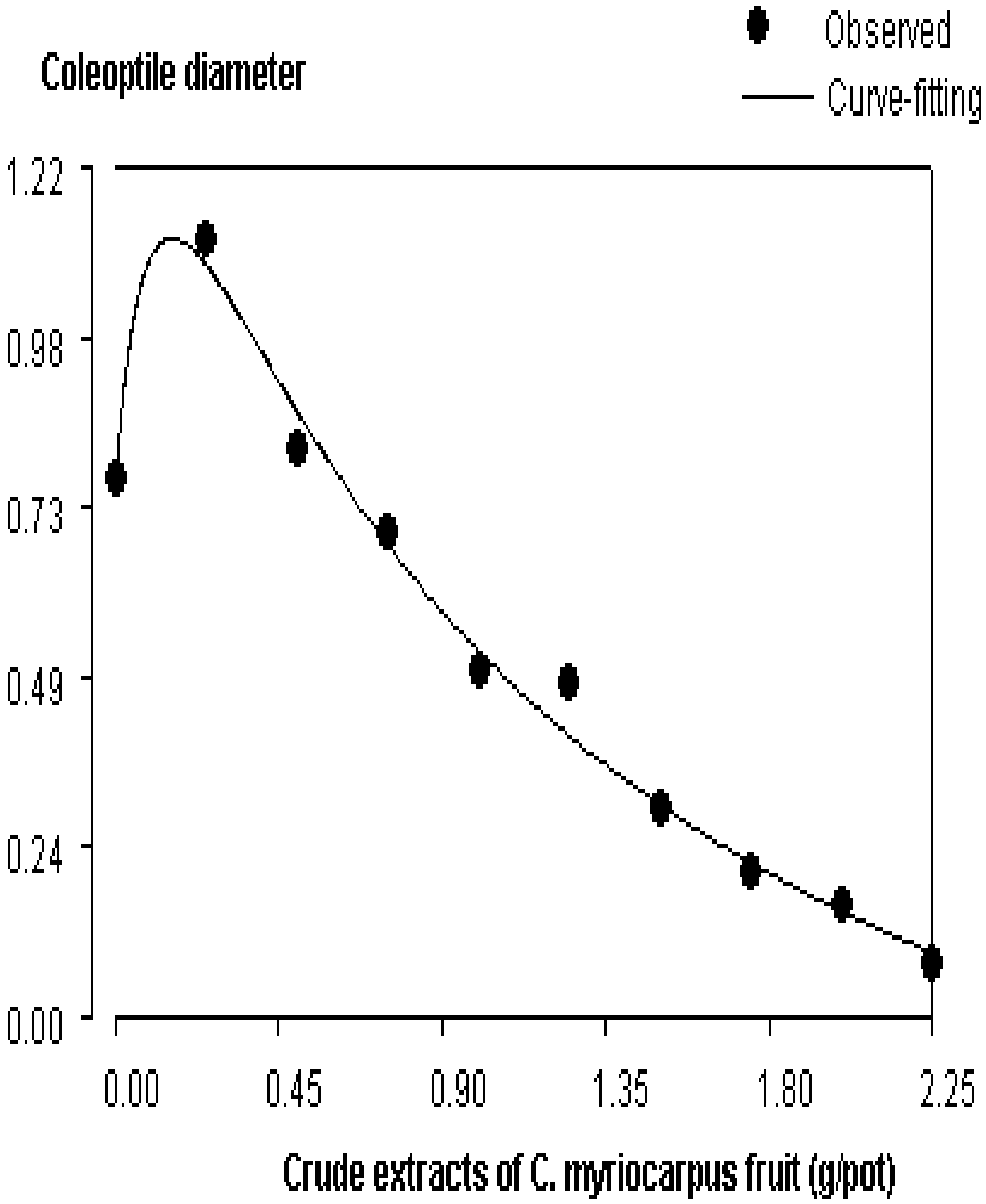

Figure 5.12 Response of coleoptile diameter of onion seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

5.3.2 Gramineae family

In all organs measured, the coefficients of determination (R^2) were averaging at least 0.94 (range 0.74 - 0.97), suggesting the existence of strong density-dependent interactions between the variables measured and test dosages for maize (Table 5.8), millet (Table 5.10) and sorghum (Table 5.12). The relationships of the four variables measured and the dosages of crude extracts of *C. myriocarpus* fruit were graphically summarised for maize (Figures 5.13 – 5.16), millet (Figures 5.17 – 5.20) and sorghum (Figures 5.21 – 5.24).

At low dosages the material stimulated growth of all tested organs, whereas at high dosages the material inhibited growth as observed previously in the family Alliaceae. In maize (Table 5.7), the transformation levels for seedling height increased from k = 0 (R^2 = 0.69) to k = 2 (R^2 = 0.84). Further increases in k values resulted in the decrease of R^2 to 0.60 at k = 6. Consequently, in maize the best fit to the data for seedling height was at k = 2. Similarly, for radicle length, coleoptile length and coleoptile diameter in maize, the best fits to the data were at k = 0, k = 2 and k = 7, respectively, in millet at k = 6, k = 8 and k = 2, respectively (Table 5.9), and in sorghum at k = 2, k = 1 and k = 2, respectively (Table 5.11).

Seedling height and coleoptile length of maize had the same k = 2 values, in millet, seedling height and coleoptile diameter each had a k = 2 value, whereas in sorghum radicle length and coleoptile diameter had k = 2 values. Among the crops, maize and millet had k = 2 values for seedling height, whereas millet and sorghum had k = 2 values for coleoptile diameter. In terms of the model, maize radicle length, with a k = 0 value was the most sensitive to crude extracts of *C. myriocarpus* fruit, whereas

millet coleoptile length with k = 8 was the least sensitive to the material. Overall, sorghum with ∑k = 9 was the most sensitive to the material, whereas millet with ∑k = 18 was the least sensitive to the material.

Table 5.7 Responses of four yield components of maize seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.63[Z]	0.62	0.32	0.26	0.48
Saturation point (R_h)	1.15	3.50	0.79	1.65	1.77
0% inhibition (D_0)	2.39	1.02	3.48	2.15	2.26
50% inhibition (D_{50})	2.89	2.27	7.08	4.65	4.22
100% inhibition (D_{100})	3.40	2.30	12.80	12.8	7.83
K	**k = 2**	**k = 0**	**k = 2**	**k = 7**	**2.75**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 11					

[Z] Dosage in grams

Table 5.8 Quadratic relationships of seedling height, radicle length, coleoptile length and coleoptile diameter of maize at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Seedling height	$y = 30.828x^2 - 38.665x + 4.034$	0.84
Radicle length	$y = -8.365x^2 + 1.196x + 25.647$	0.78
Coleoptile length	$y = 1.387x^2 - 1.514x + 0.724$	0.94
Coleoptile diameter	$y = 24.065x^2 - 87.725x + 1.503$	0.97

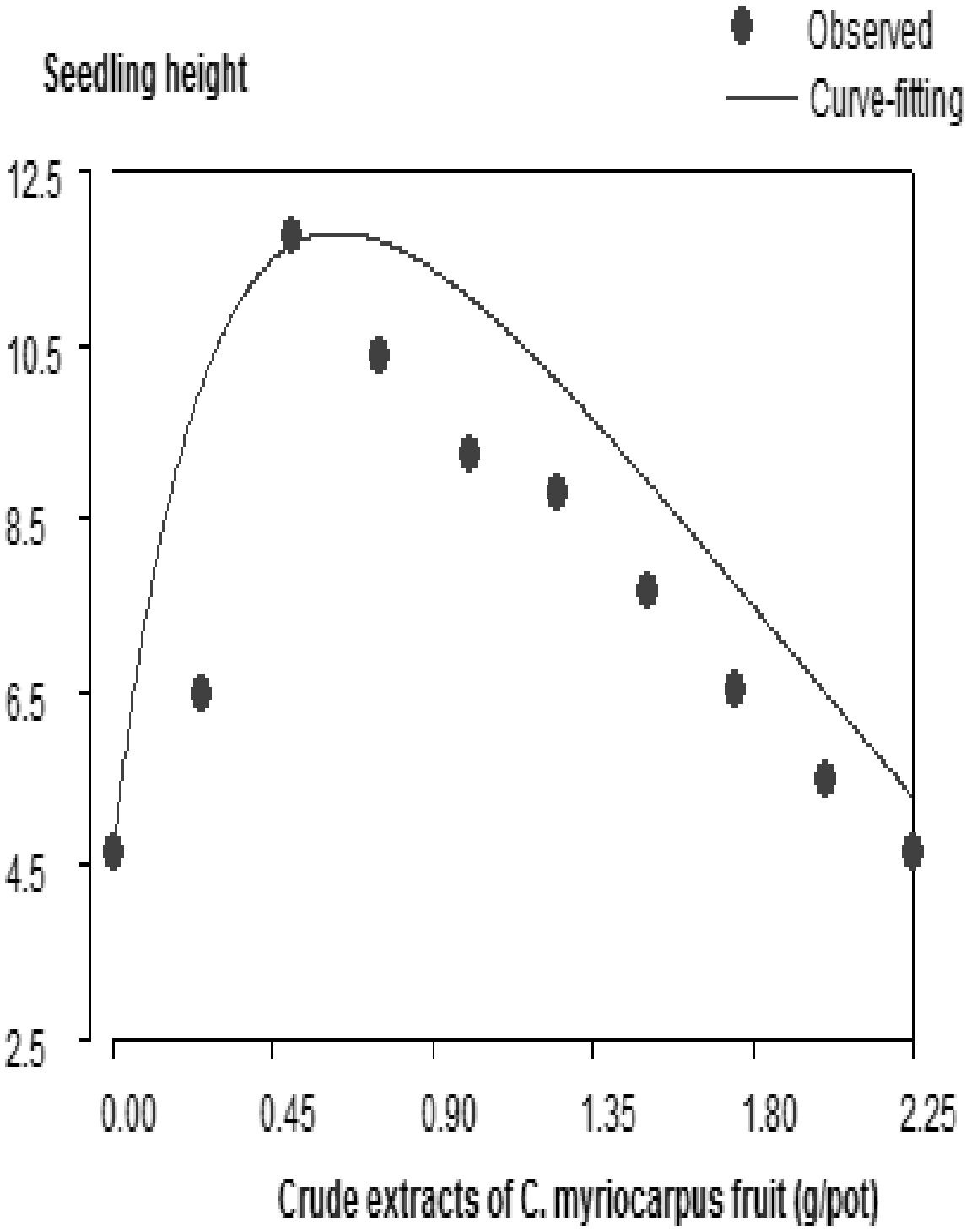

Figure 5.13 Response of seedling height of maize seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

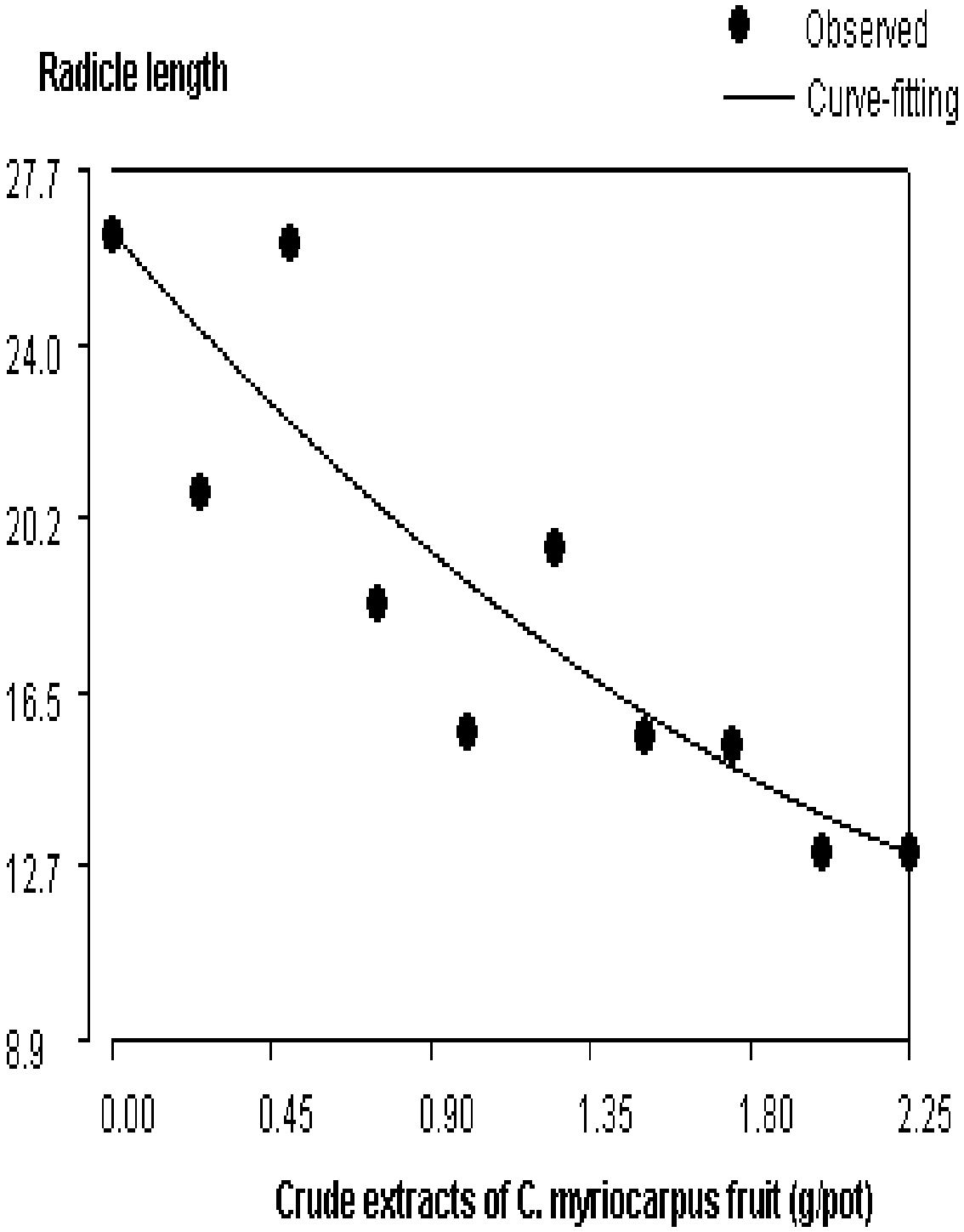

Figure 5.14 Response of radicle length of maize seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

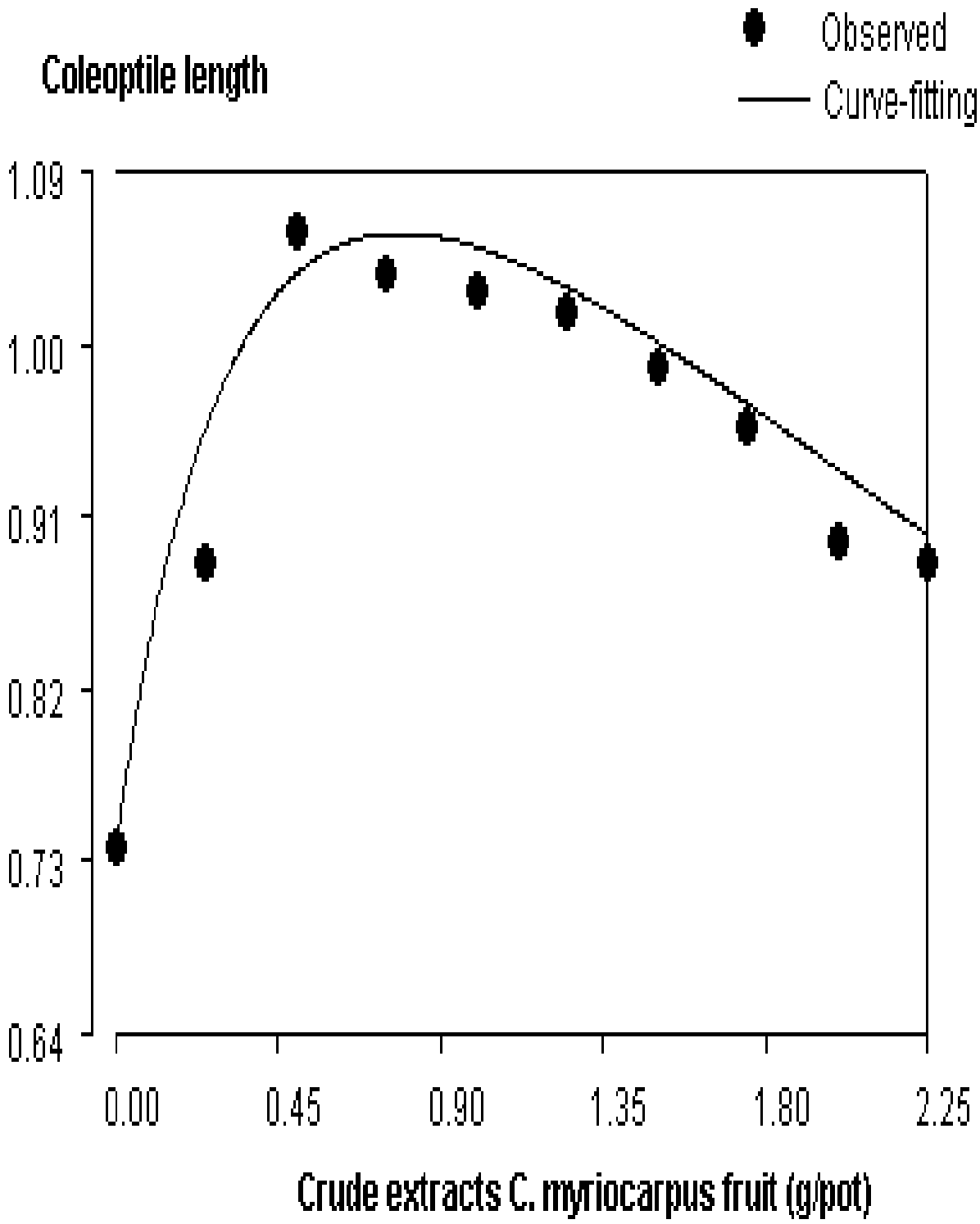

Figure 5.15 Response of coleoptile length of maize seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

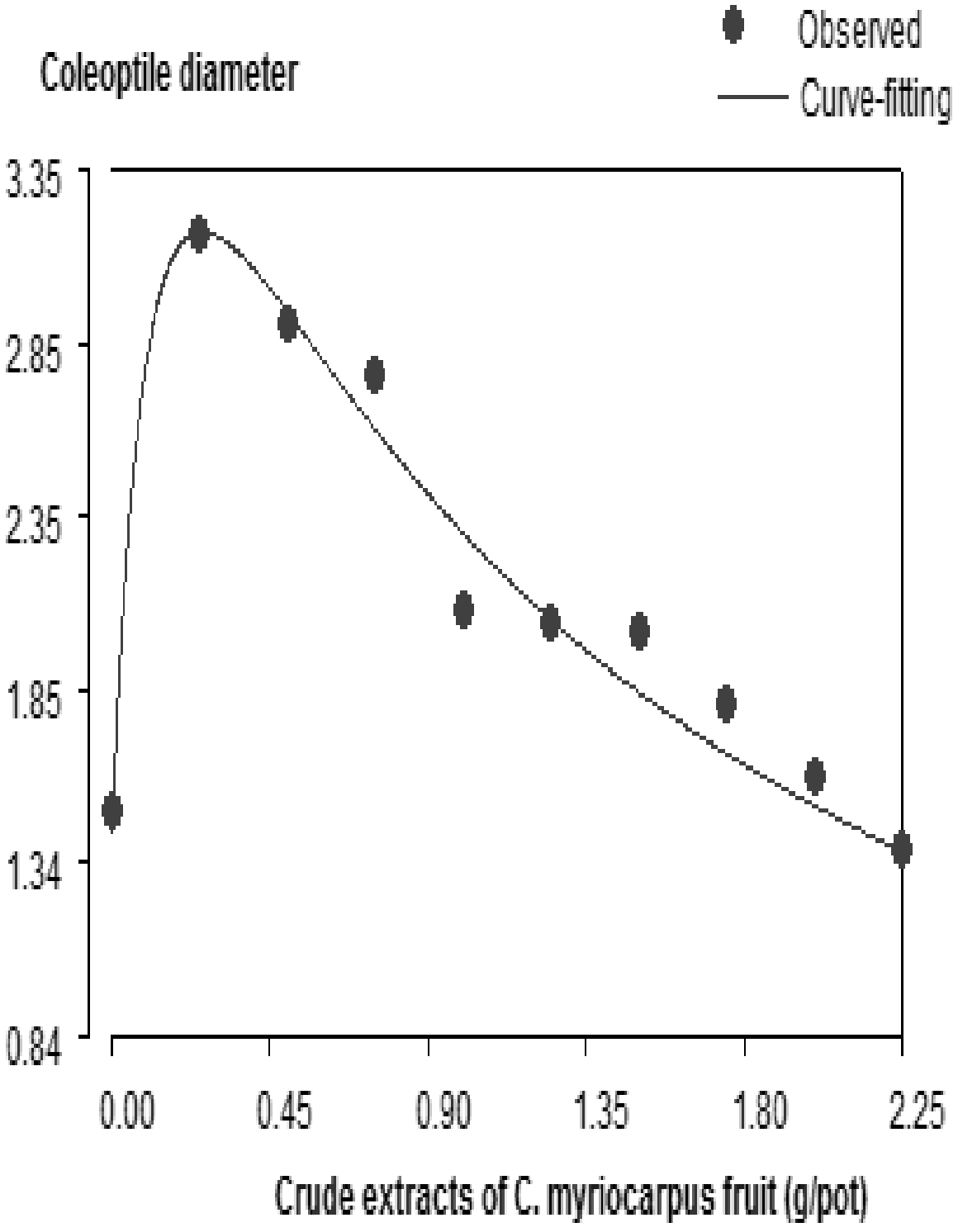

Figure 5.16 Response of coleoptile diameter of maize seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Table 5.9 Responses of four yield components of millet seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.41[Z]	0.20	0.32	0.15	0.27
Saturation point (R_h)	2.12	2.48	0.81	0.34	1.44
0% inhibition (D_0)	1.23	0.90	1.02	0.94	1.02
50% inhibition (D_{50})	1.72	1.41	1.56	1.59	1.57
100% inhibition (D_{100})	2.20	2.20	2.40	2.30	2.28
K	**k = 2**	**k = 6**	**k = 8**	**k = 2**	**4.5**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 18					

[Z] Dosage in grams.

Table 5.10 Quadratic relationships of seedling height, radicle length, coleoptile length and coleoptile diameter of millet at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Seedling height	$y = 14.453x^2 - 24.592x + 3.620$	0.96
Radicle length	$y = 38.778x^2 - 151.571x + 2.875$	0.98
Coleoptile length	$y = 6.121x^2 - 28.924x + 0.251$	0.94
Coleoptile diameter	$y = 1.151x^2 - 2.262x + 0.484$	0.98

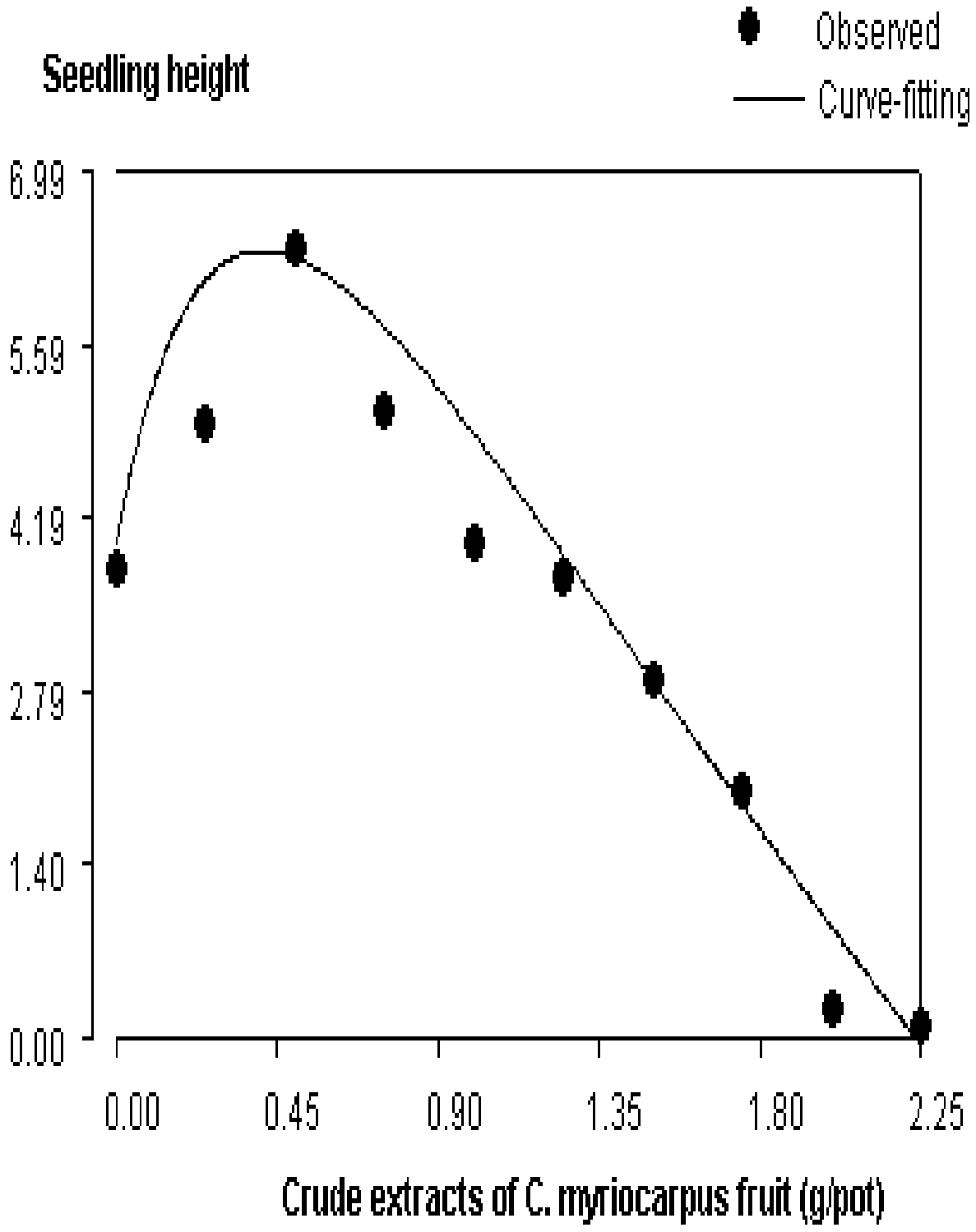

Figure 5.17 Response of seedling height of millet seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

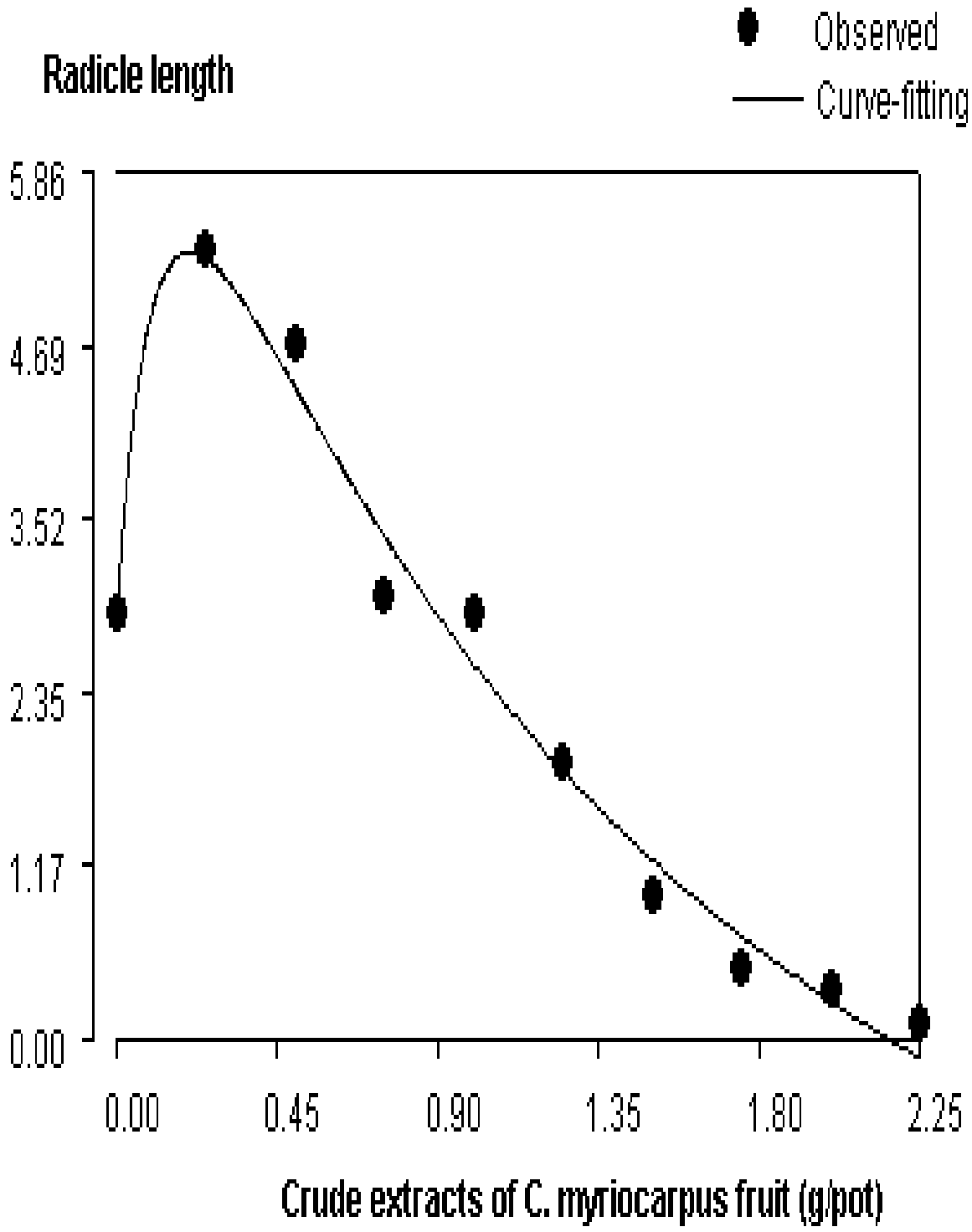

Figure 5.18 Response of radicle length of millet seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

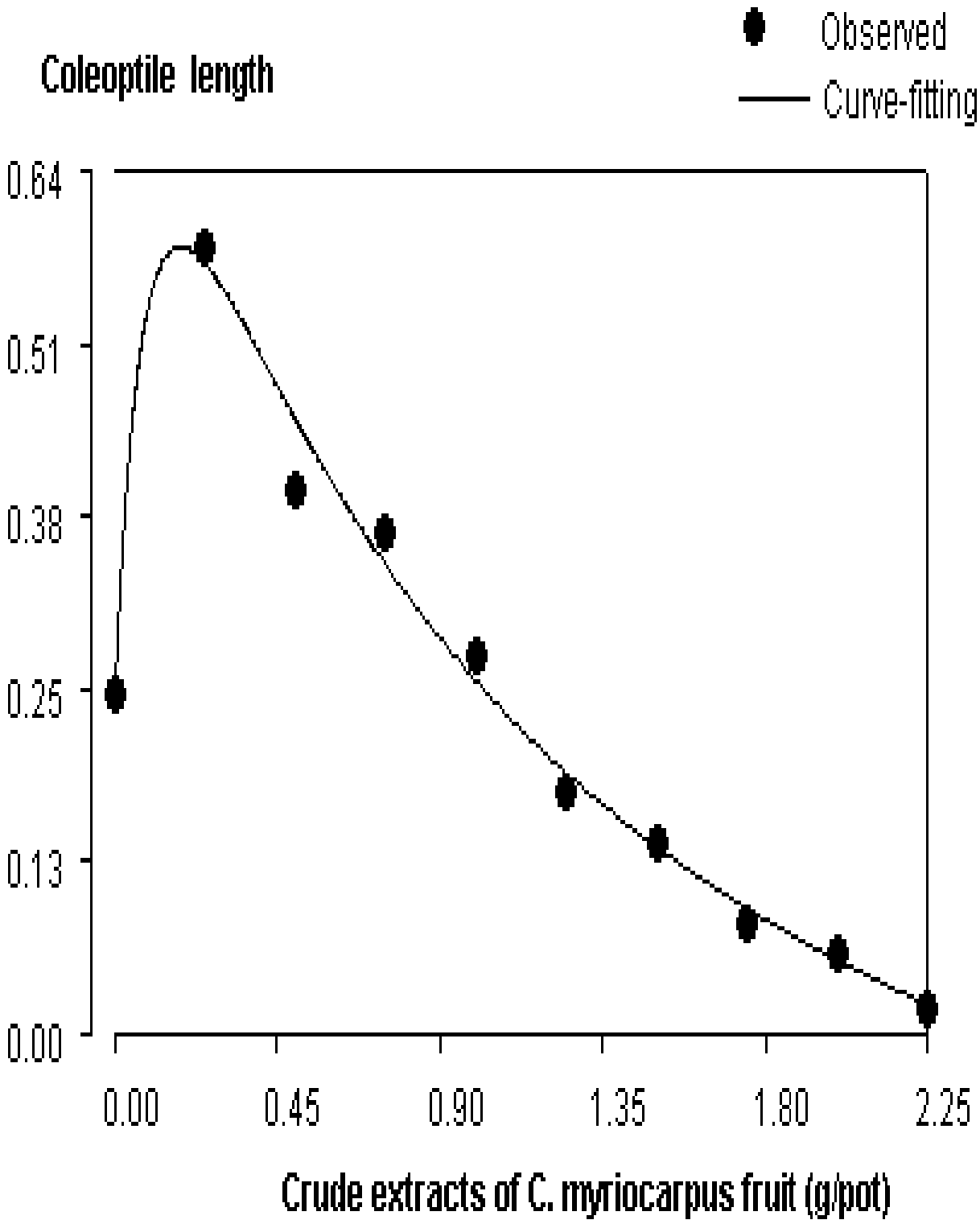

Figure 5.19 Response of coleoptile length of millet seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

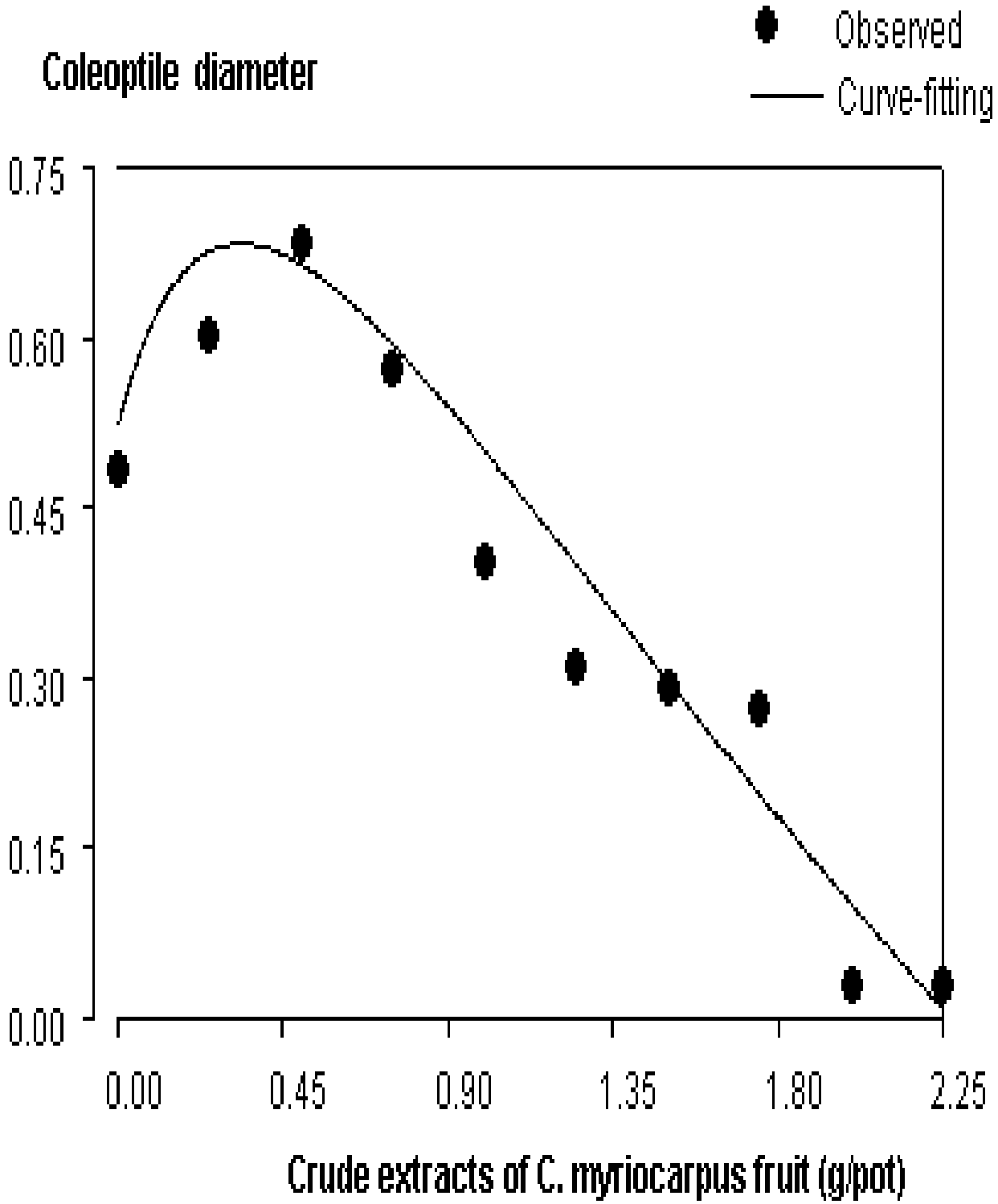

Figure 5.20 Response of coleoptile diameter of millet seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Table 5.11 Responses of four yield components of sorghum seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Seedling height (cm)	Radicle length (cm)	Coleoptile length (cm)	Coleoptile diameter (mm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.35[2]	0.59	0.67	0.14	0.44
Saturation point (R_h)	5.11	0.88	0.88	0.37	1.81
0% inhibition (D_0)	1.64	2.16	1.78	1.06	1.66
50% inhibition (D_{50})	2.23	2.38	2.16	2.29	2.26
100% inhibition (D_{100})	3.00	2.60	2.50	3.80	2.97
K	**k = 4**	**k = 2**	**k = 1**	**k = 2**	**2.25**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 9					

[2] Dosage in grams.

Table 5.12 Quadratic relationships of seedling height, radicle length, coleoptile length and coleoptile diameter of sorghum at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Seedling height	$y = 48.996x^2 - 117.470x + 3.920$	0.98
Radicle length	$y = 46.410x^2 - 60.641x + 2.998$	0.96
Coleoptile length	$y = 3.464x^2 - 3.393x + 1.019$	0.99
Coleoptile diameter	$y = 1.033x^2 - 1.896x + 0.713$	0.98

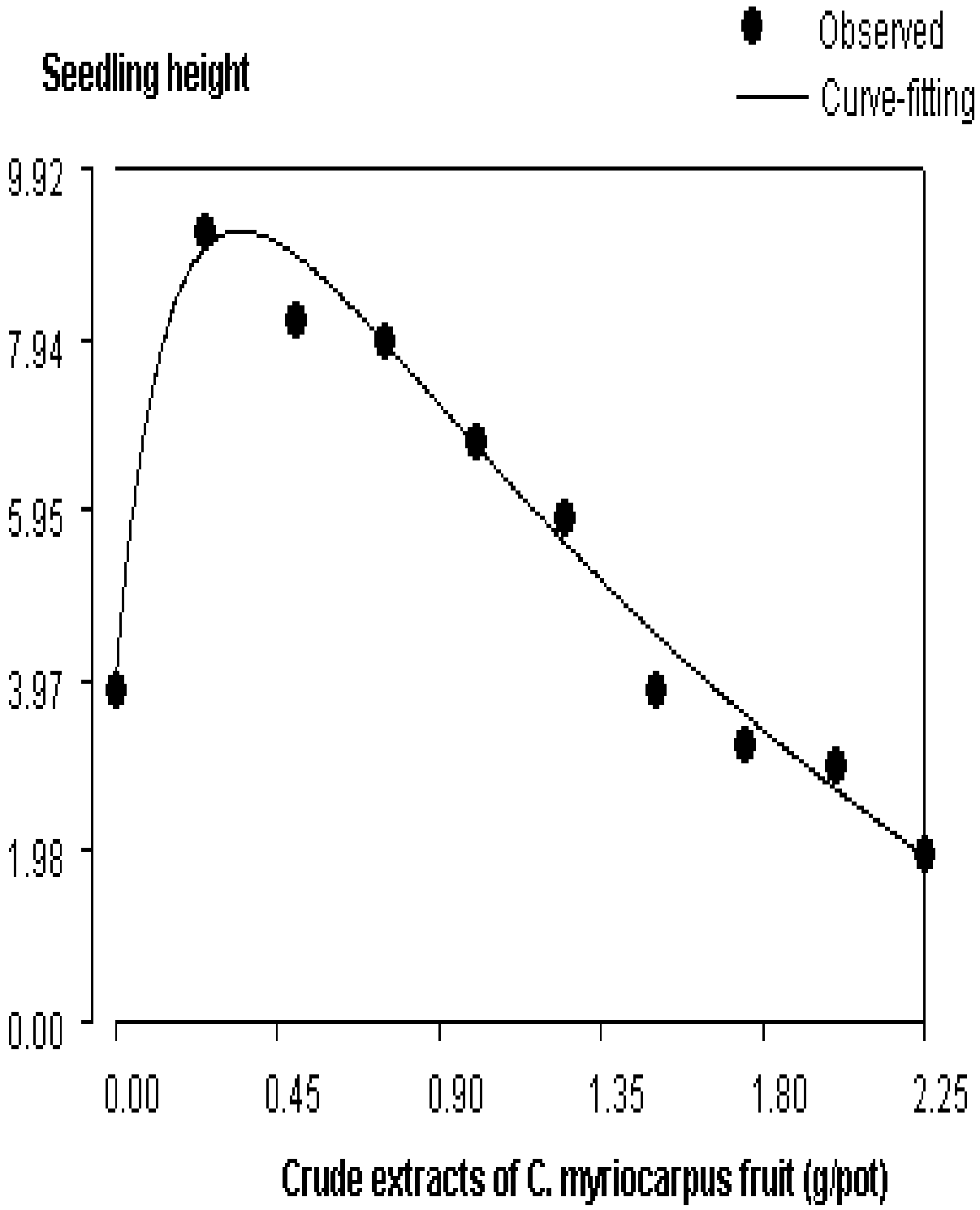

Figure 5.21 Response of seedling height of sorghum seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

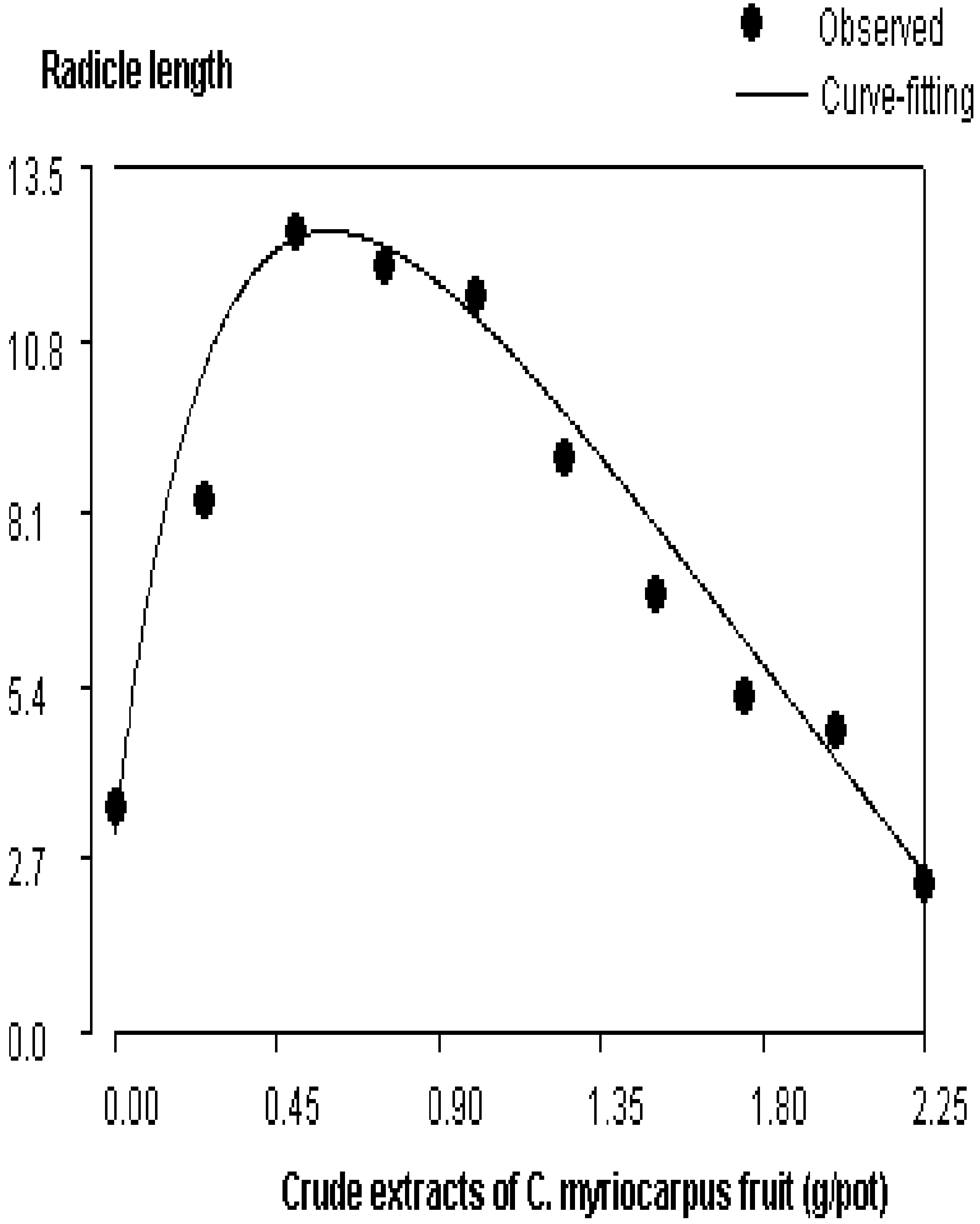

Figure 5.22 Response of radicle length of sorghum seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

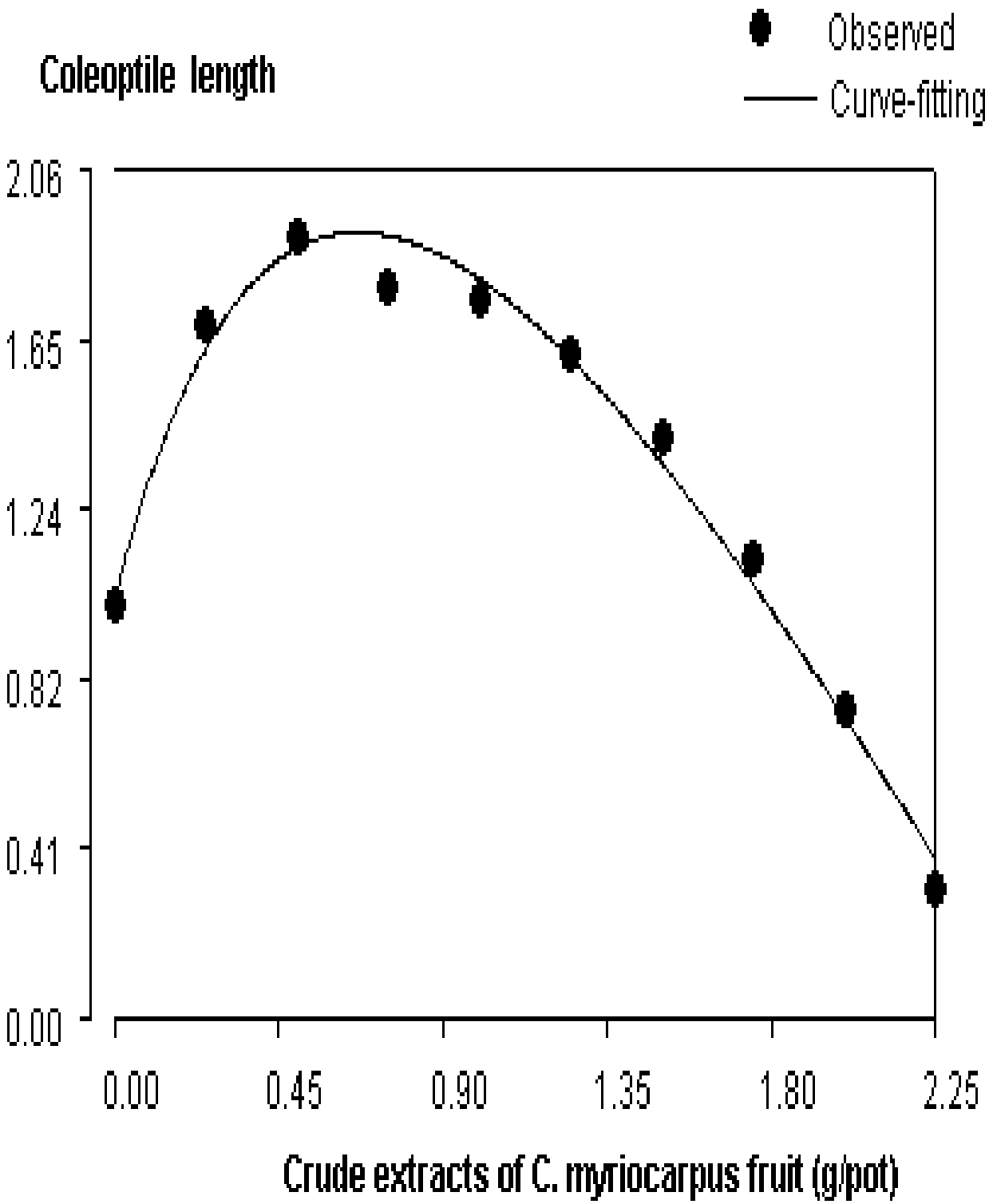

Figure 5.23 Response of coleoptile length of sorghum seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

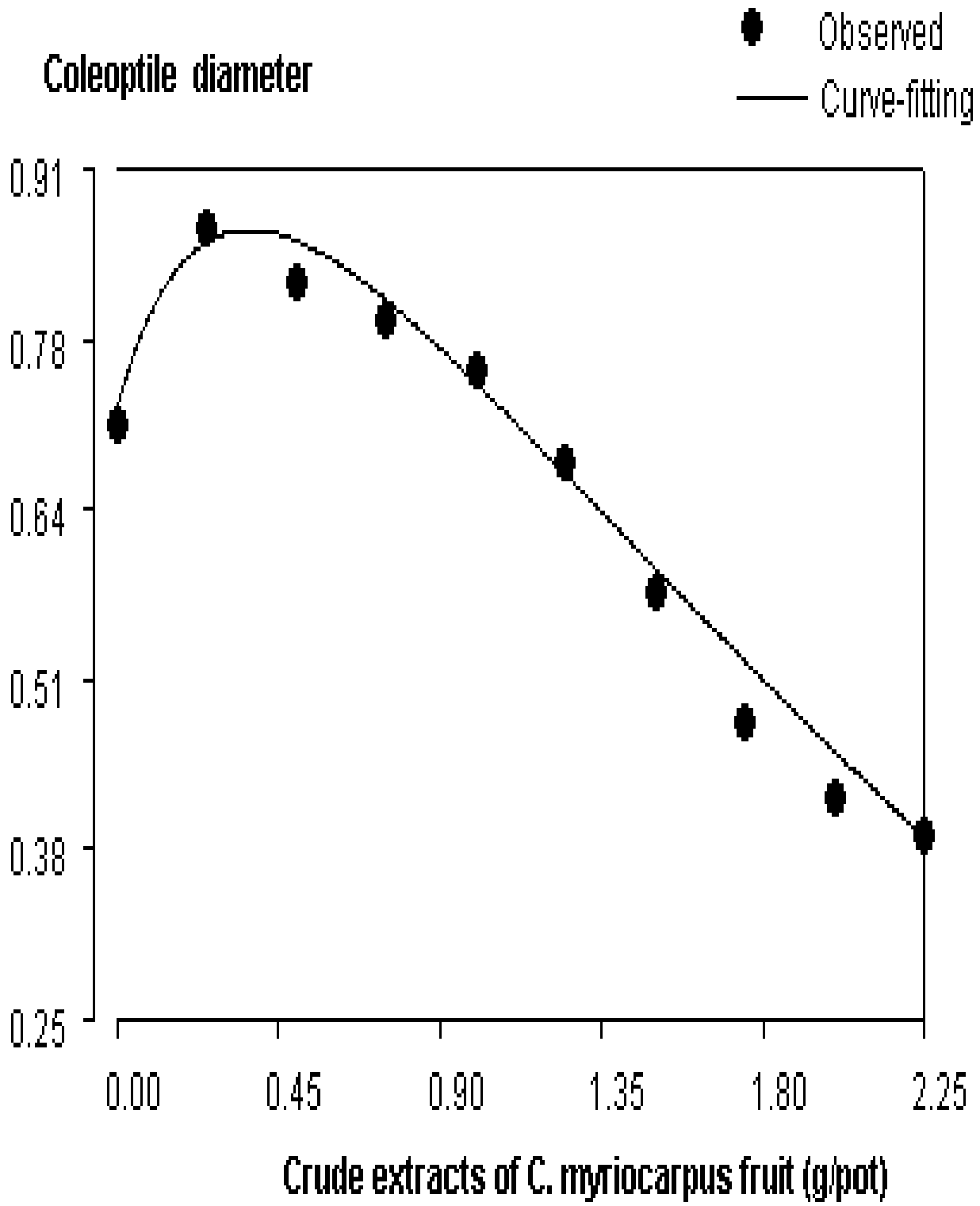

Figure 5.24 Response of coleoptile diameter of sorghum seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

5.3.3 Solanaceae family

In all measured organs, the coefficients of determination (R^2) were averaging 0.97 (range 0.93 - 0.97), suggesting the existence of strong density-dependent interactions between the variables measured in eggplant and test dosages (Table 5.14), pepper (Table 5.16) and tomato (Table 5.18). The relationships of the four variables measured and the dosages of crude extracts of *C. myriocarpus* fruit were graphically summarised for eggplant (Figures 5.25 – 5.28), pepper (Figures 5.29 – 5.32) and tomato (Figures 5.33 – 5.36).

Generally, at low dosages the material stimulated growth of various organs, whereas at high dosages the material inhibited growth. Also, as shown by the k values within organs, the sensitivity of the four measured variables differed from crop to crop. In eggplant (Table 5.13), the transformation levels for hypocotyl diameter increased from k = 0 (R^2 = 0.92) to k = 1 (R^2 = 0.93). Further increases in k values resulted in the decrease of R^2 to 0.70 at k = 5. Consequently, in eggplant the best fit to the data was at k = 1. Similarly, for epicotyl length, hypocotyl length and seedling height in eggplant, the best fits to the data were at k = 5, k = 1 and k = 2, respectively, whereas in pepper best fits were at k = 5, k = 10, k = 8 and k = 9, respectively (Table 5.15), and in tomato at k = 15, k = 20, k = 9 and k = 7, respectively (Table 5.17).

Hypocotyl diameter and hypocotyl length of eggplant both had k = 1 values, in both pepper and tomato no variables had the same k values. Among the crops, pepper and tomato had k = 9 values for seedling height, whereas eggplant and pepper had k = 5 values for epicotyl length and hypocotyl diameter, respectively. In terms of the model, eggplant hypocotyl diameter and hypocotyl length, with k = 1 value were the

most sensitive to crude extracts of *C. myriocarpus* fruit, whereas tomato epicotyl length with k = 20 was the least sensitive to the material. Overall, eggplant with ∑k = 9 was the most sensitive to the material, whereas tomato with ∑k = 51 was the least sensitive to the material.

Table 5.13 Responses of four yield components of eggplant seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Hypocotyl diameter (mm)	Epicotyl length (cm)	Hypocotyl length (cm)	Seedling height (cm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.71[2]	0.29	0.61	0.49	0.52
Saturation point (R_h)	0.73	0.97	0.70	1.47	0.96
0% inhibition (D_0)	1.99	1.56	1.58	1.62	1.68
50% inhibition (D_{50})	2.01	1.99	1.94	2.01	1.98
100% inhibition (D_{100})	2.40	2.05	2.30	2.40	2.28
k	**k = 1**	**k = 5**	**k = 1**	**k = 2**	**2.25**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 9					

[2] Dosage in grams.

Table 5.14 Quadratic relationships of hypocotyl diameter, epicotyl length, hypocotyl length and seedling height of eggplant at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Hypocotyl diameter	$y = 2.602x^2 - 2.376x + 0.386$	0.93
Epicotyl length	$y = 11.323x^2 - 32.892x + 0.501$	0.98
Hypocotyl length	$y = 2.968x^2 - 3.132x + 0.897$	0.97
Seedling height	$y = 8.735x^2 - 12.968x + 1.332$	0.99

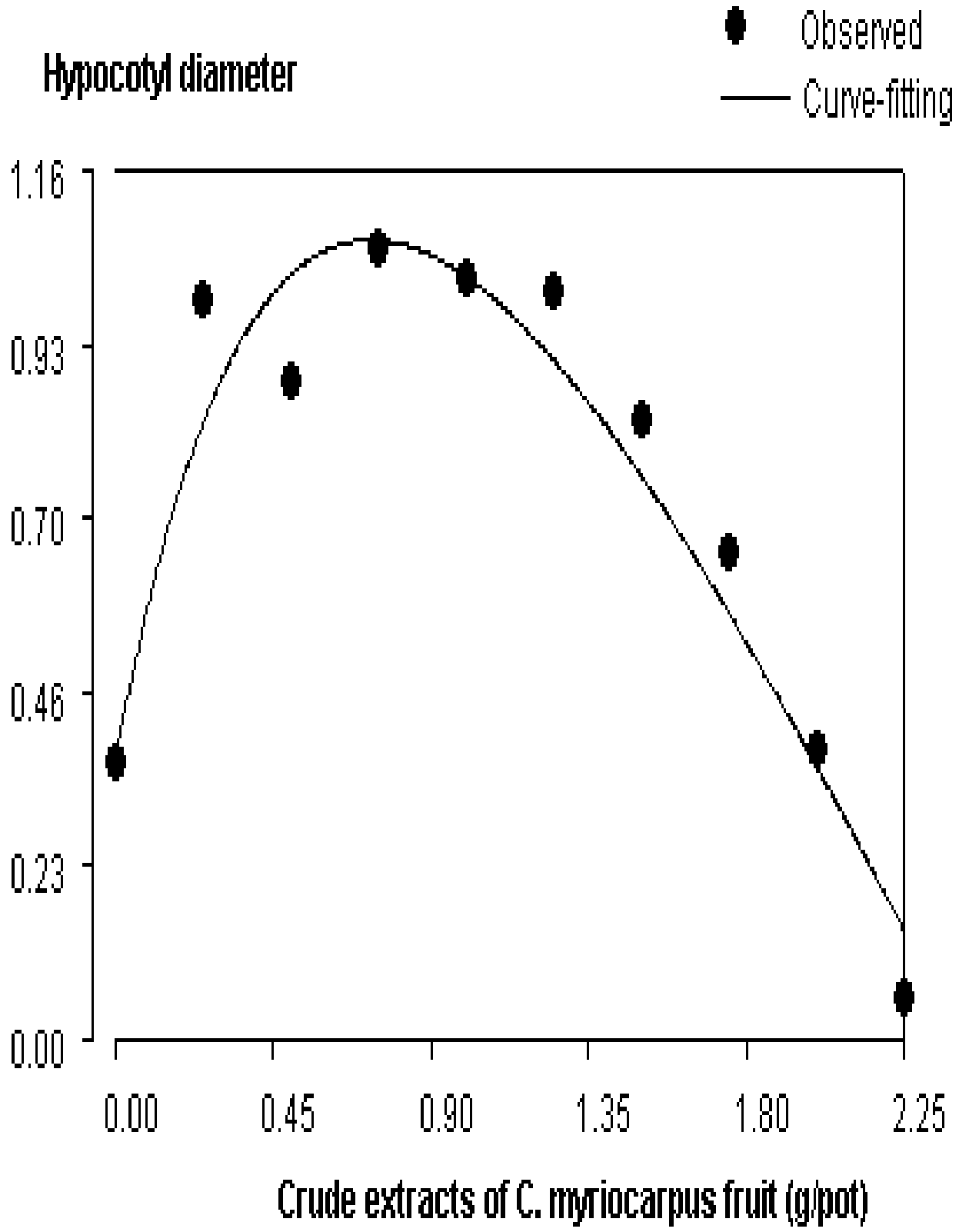

Figure 5.25 Response of hypocotyl diameter of eggplant seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

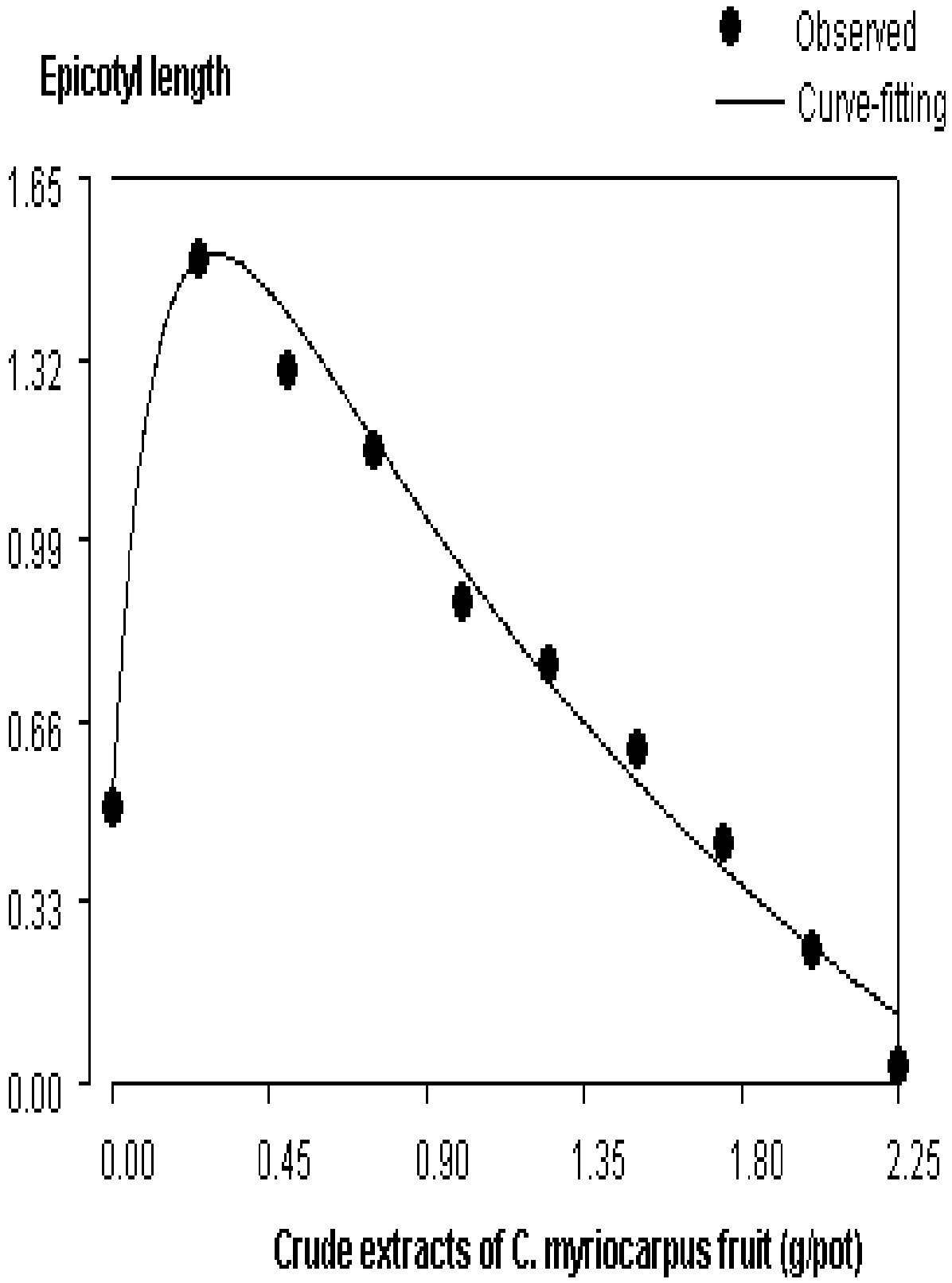

Figure5.26 Response of epicotyl length of eggplant seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50) .

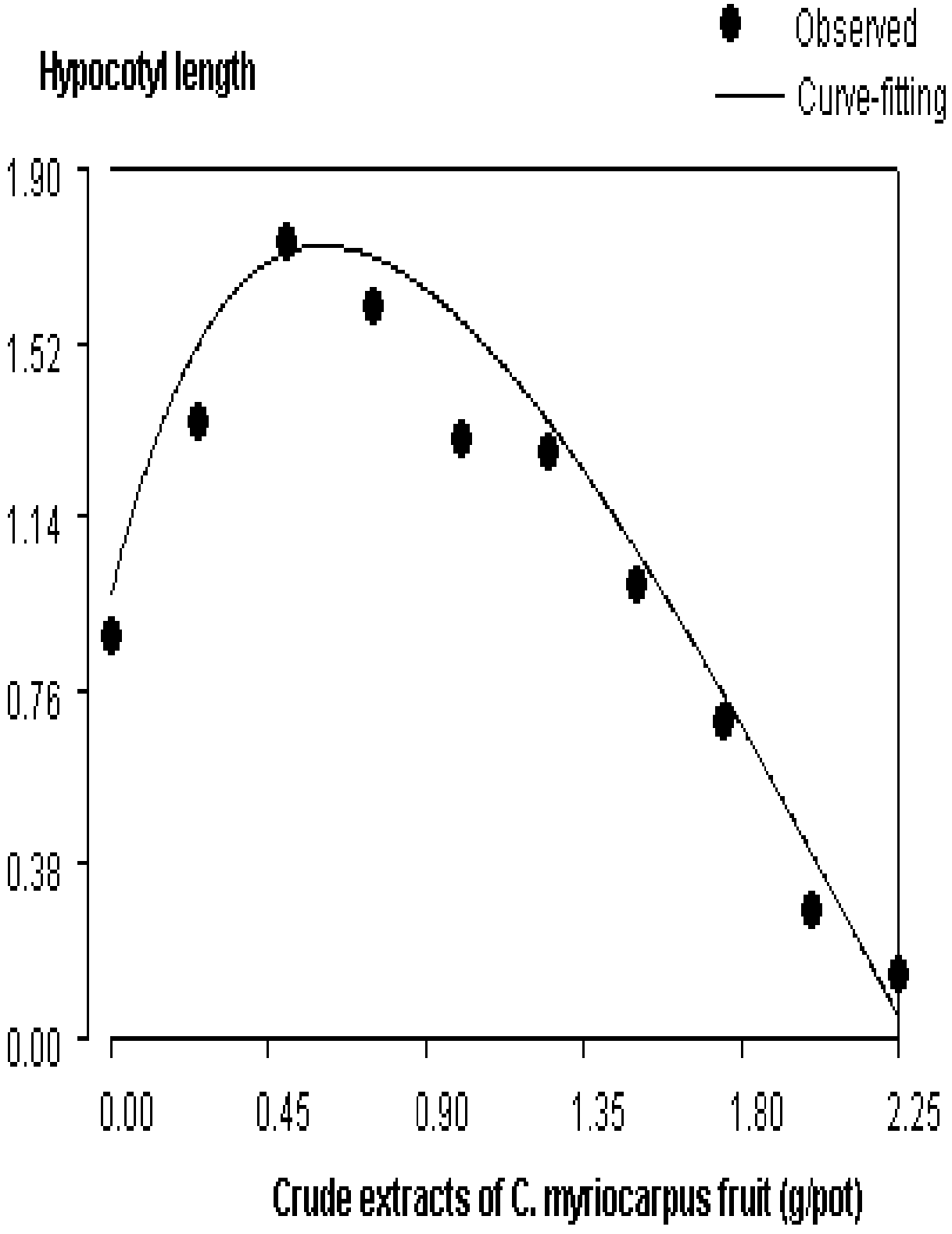

Figure 5.27 Response of hypocotyl length of eggplant seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

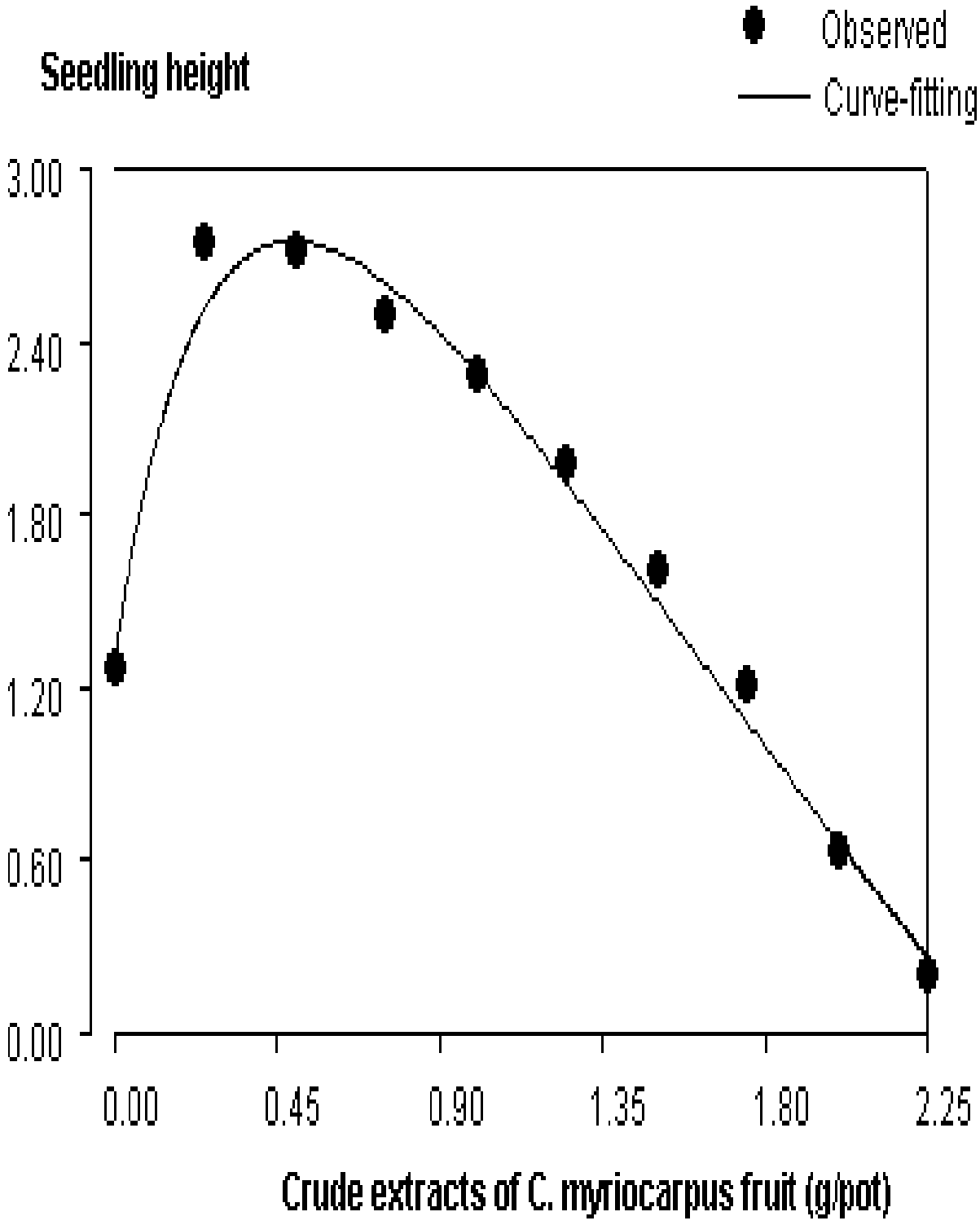

Figure 5.28 Response of seedling height of eggplant seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Table 5.15 Responses of four yield components of pepper seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Hypocotyl diameter (mm)	Epicotyl length (cm)	Hypocotyl length (cm)	Seedling height (cm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.09[Z]	0.10	0.11	0.10	0.10
Saturation point (R_h)	0.08	0.85	1.11	1.98	0.1.01
0% inhibition (D_0)	0.23	0.41	0.40	0.37	0.35
50% inhibition (D_{50})	0.91	0.87	0.93	0.86	0.89
100% inhibition (D_{100})	2.20	2.00	2.11	2.00	2.08
k	**k = 5**	**k = 10**	**k = 8**	**k = 9**	**8**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking : ∑k = 32					

[Z] Dosage in grams.

Table 5.16 Quadratic relationships of hypocotyl diameter, epicotyl length, hypocotyl length and seedling height of pepper at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Hypocotyl diameter	$y = 2.245x^2 - 15.396x + 1.314$	0.95
Epicotyl length	$y = 24.818x^2 - 180.567x + 1.992$	0.98
Hypocotyl length	$y = 24.189x^2 - 179.617x + 3.704$	0.98
Seedling height	$y = 56.098x^2 - 396.760x + 6.336$	0.97

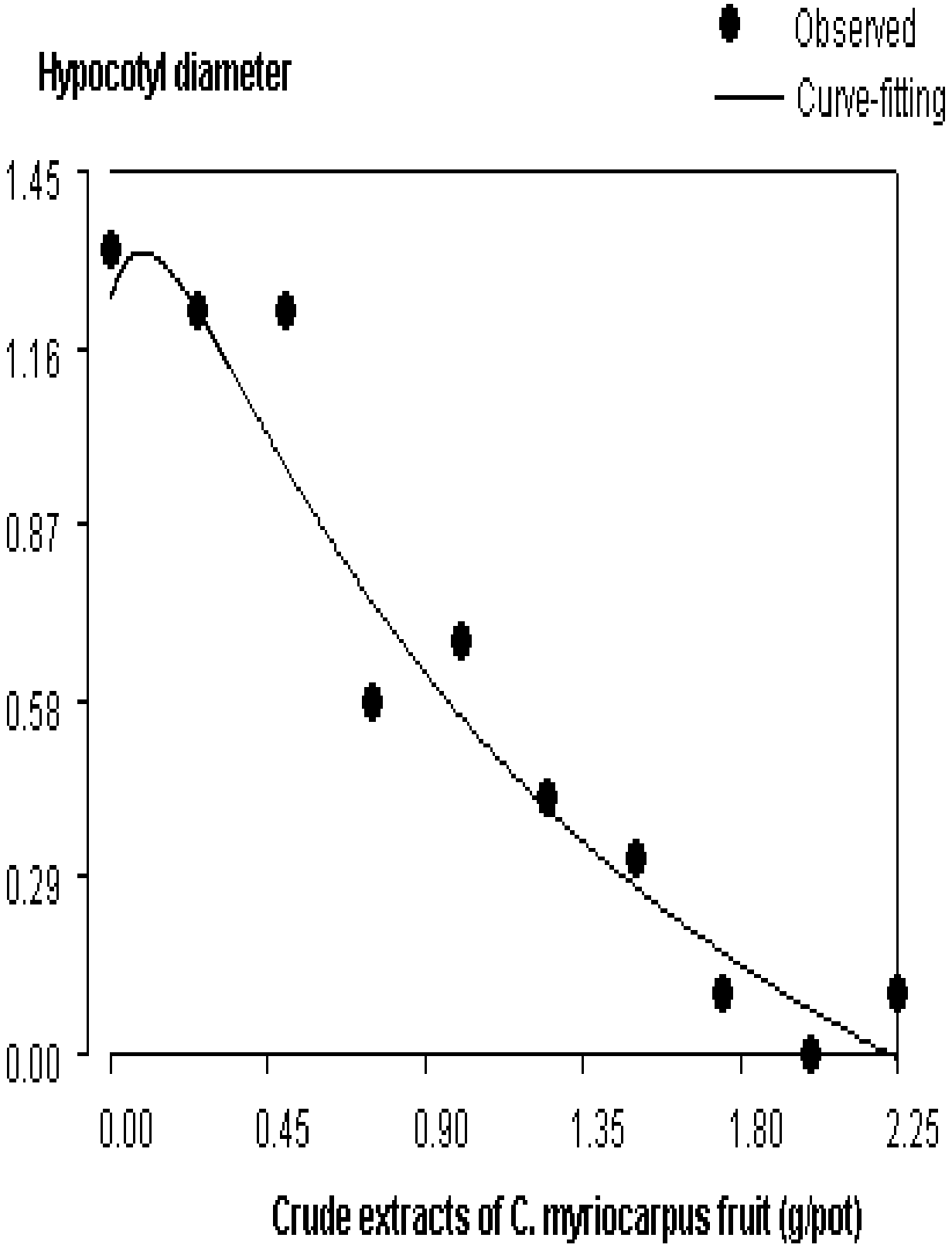

Figure 5.29 Response of hypocotyl diameter of pepper seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

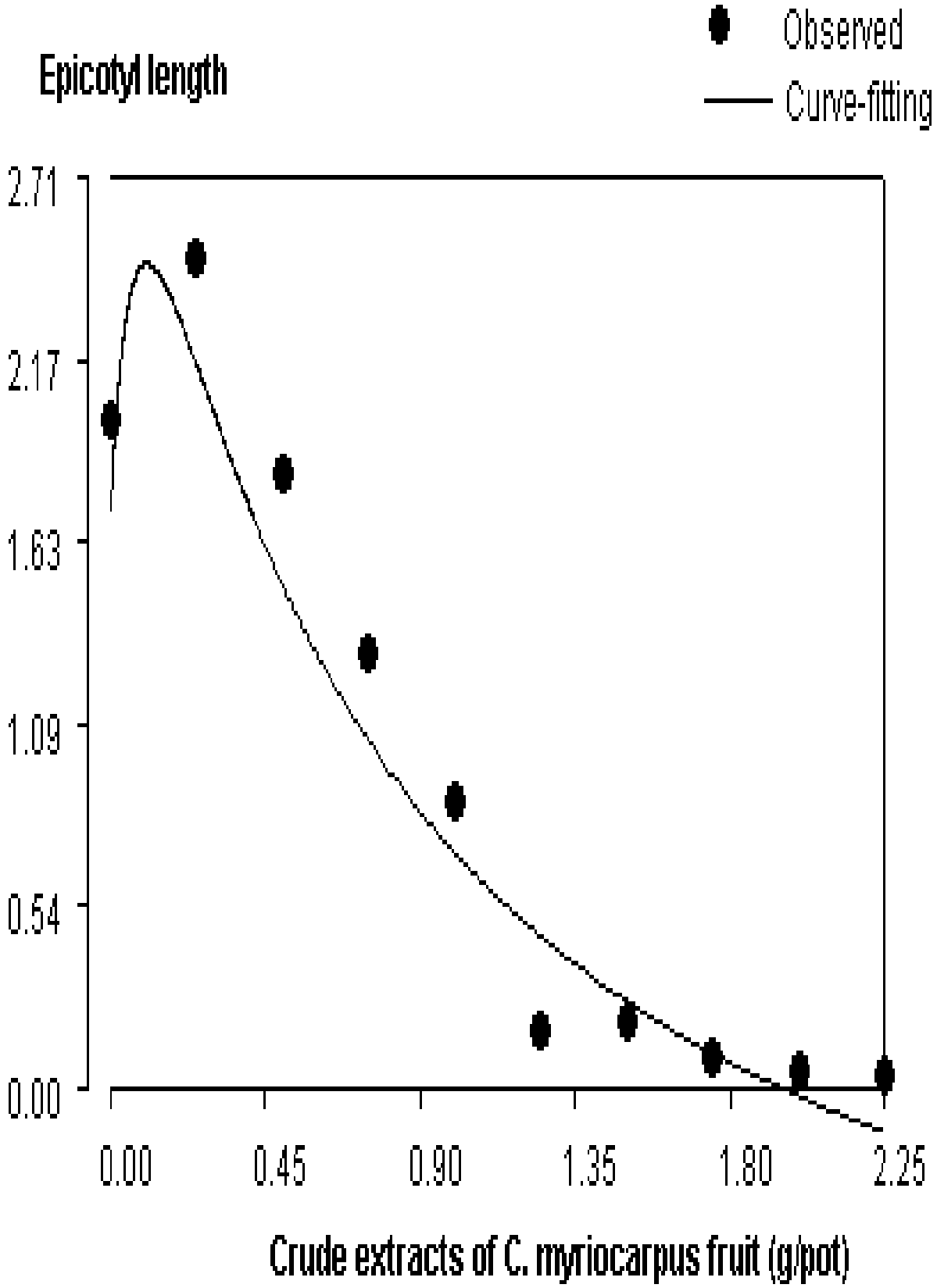

Figure 5.30 Response of epicotyl length of pepper seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

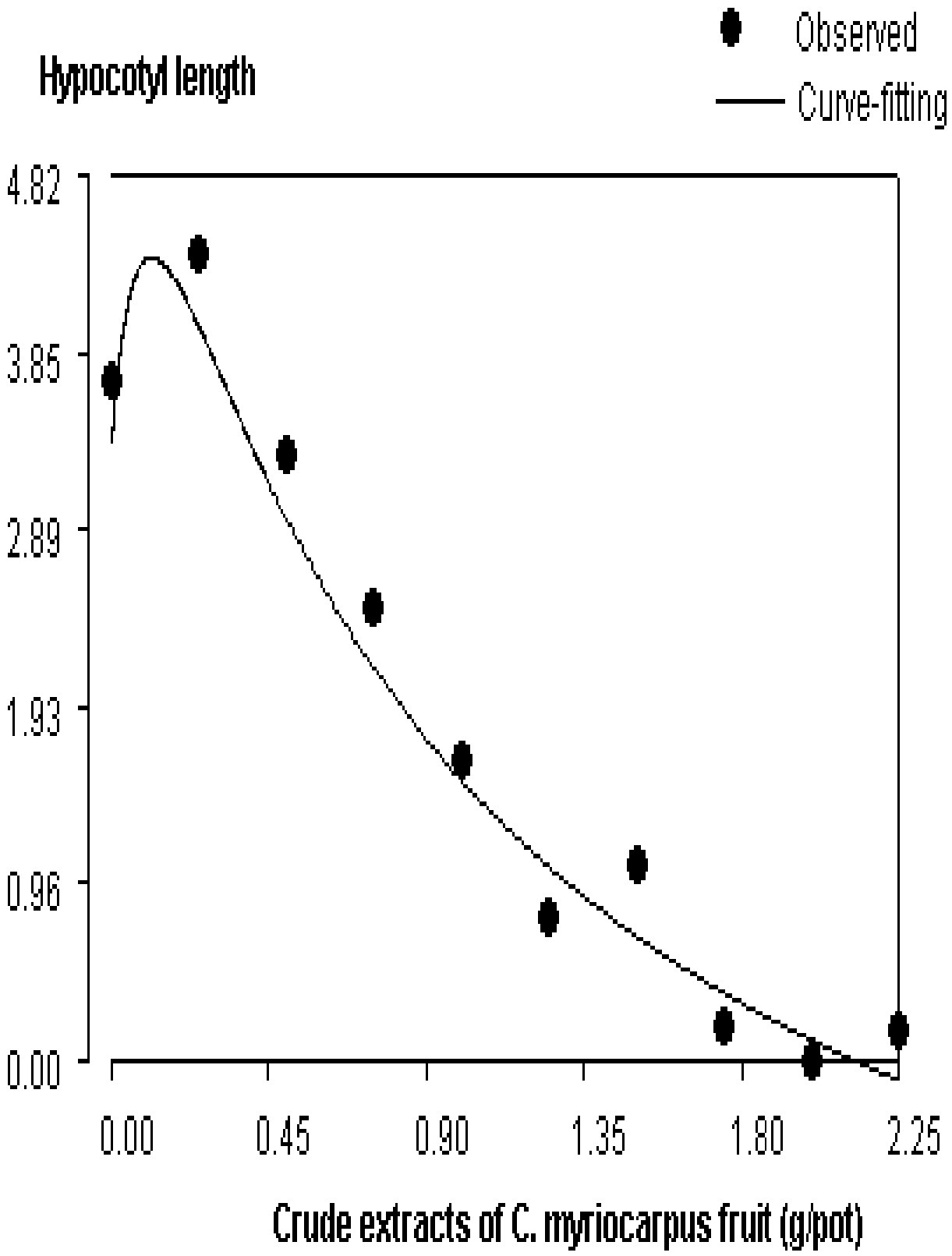

Figure 5.31 Response of hypocotyl length of pepper seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

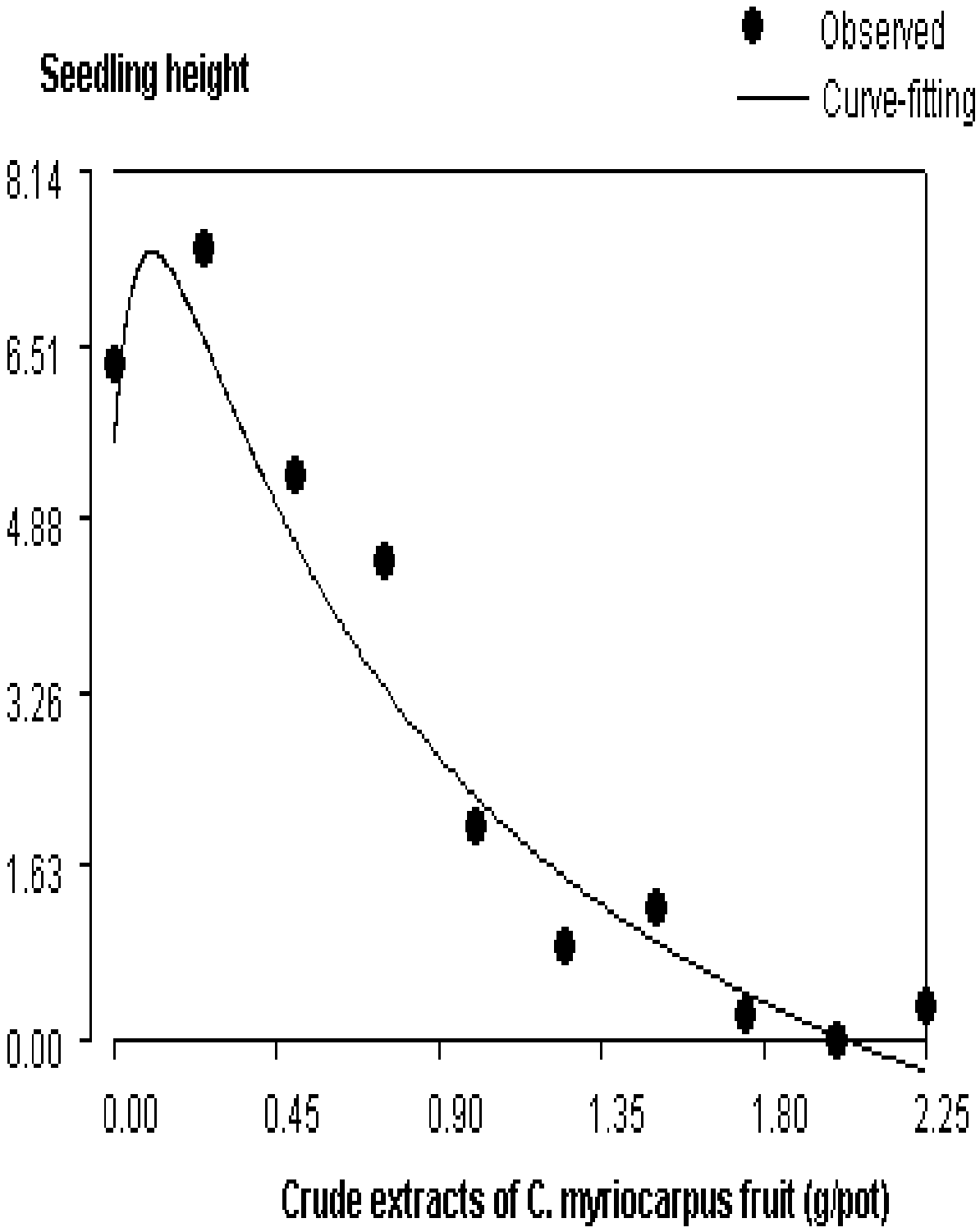

Figure 5.32 Response of seedling height of pepper seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Table 5.17 Responses of four yield components of tomato seedlings to dosages from crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

Model variables	Hypocotyl diameter (mm)	Epicotyl length (cm)	Hypocotyl length (cm)	Seedling height (cm)	Mean
	Dosage of crude extracts of *C. myriocarpus* fruit (g)				
Threshold stimulation (D_m)	0.09[2]	0.06	0.11	0.14	0.10
Saturation point (R_h)	0.64	0.71	0.99	1.53	0.97
0% inhibition (D_0)	0.46	0.33	0.42	0.54	0.44
50% inhibition (D_{50})	1.08	2.17	1.04	1.27	1.39
100% inhibition (D_{100})	3.90	0.00	2.80	3.00	2.43
k	**k = 15**	**k = 20**	**k = 7**	**k = 9**	**12.75**
P-value	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	
Sensitivity ranking: ∑k = 51					

[2] Dosage in grams.

Table 5.18 Quadratic relationships of hypocotyl diameter, epicotyl length, hypocotyl length and seedling height of tomato at 18 days after planting (n = 50).

Variable	Quadratic relationship	R^2
Hypocotyl diameter	$y = 24.314x^2 - 229.764x + 1.022$	0.95
Epicotyl length	$y = 36.410x^2 - 467.692x + 2.104$	0.97
Hypocotyl length	$y = 26.650x^2 - 178.929x + 3.012$	0.97
Seedling height	$y = 31.717x^2 - 164.666x + 4533$	0.96

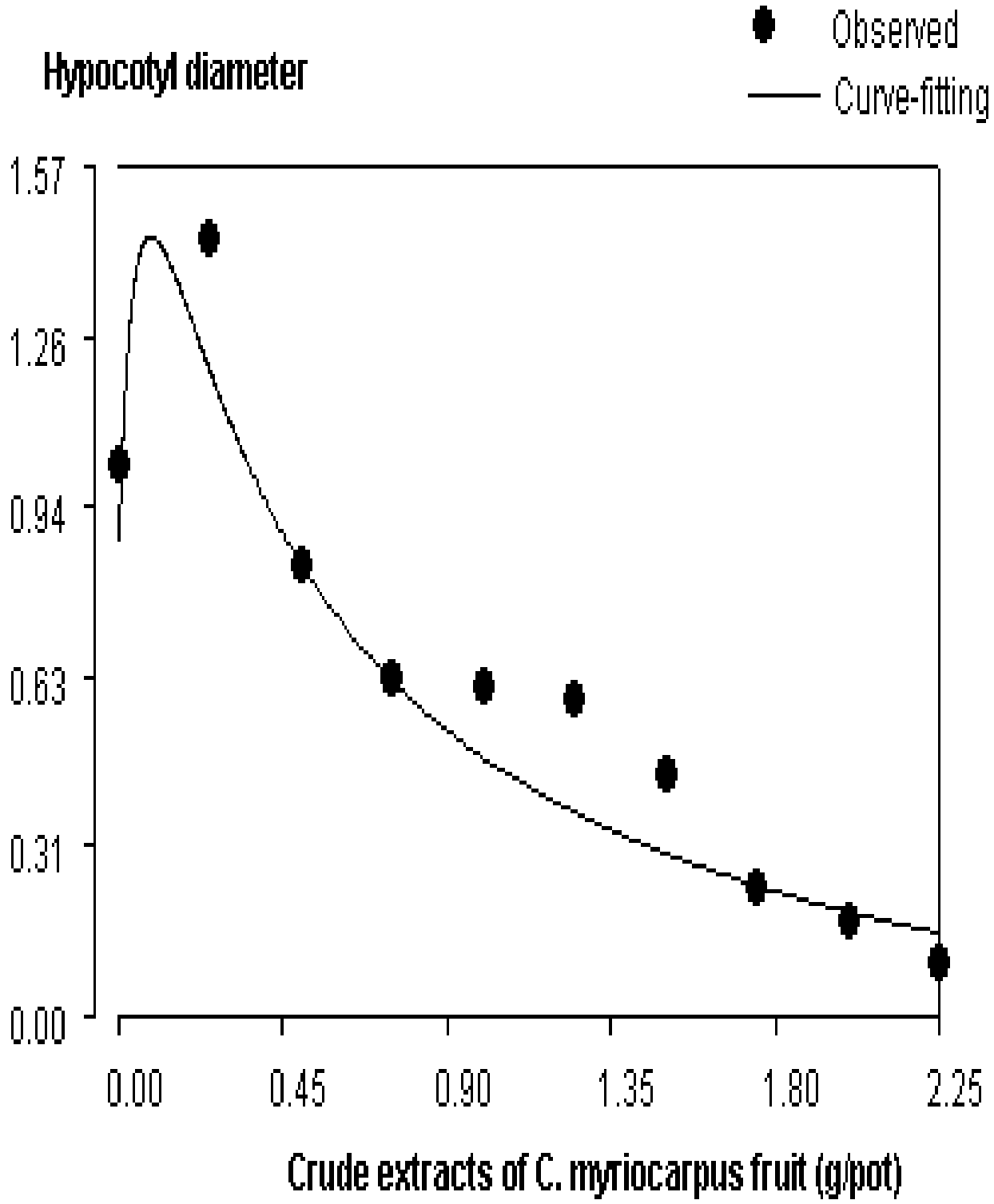

Figure 5.33 Response of hypocotyl diameter of tomato seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

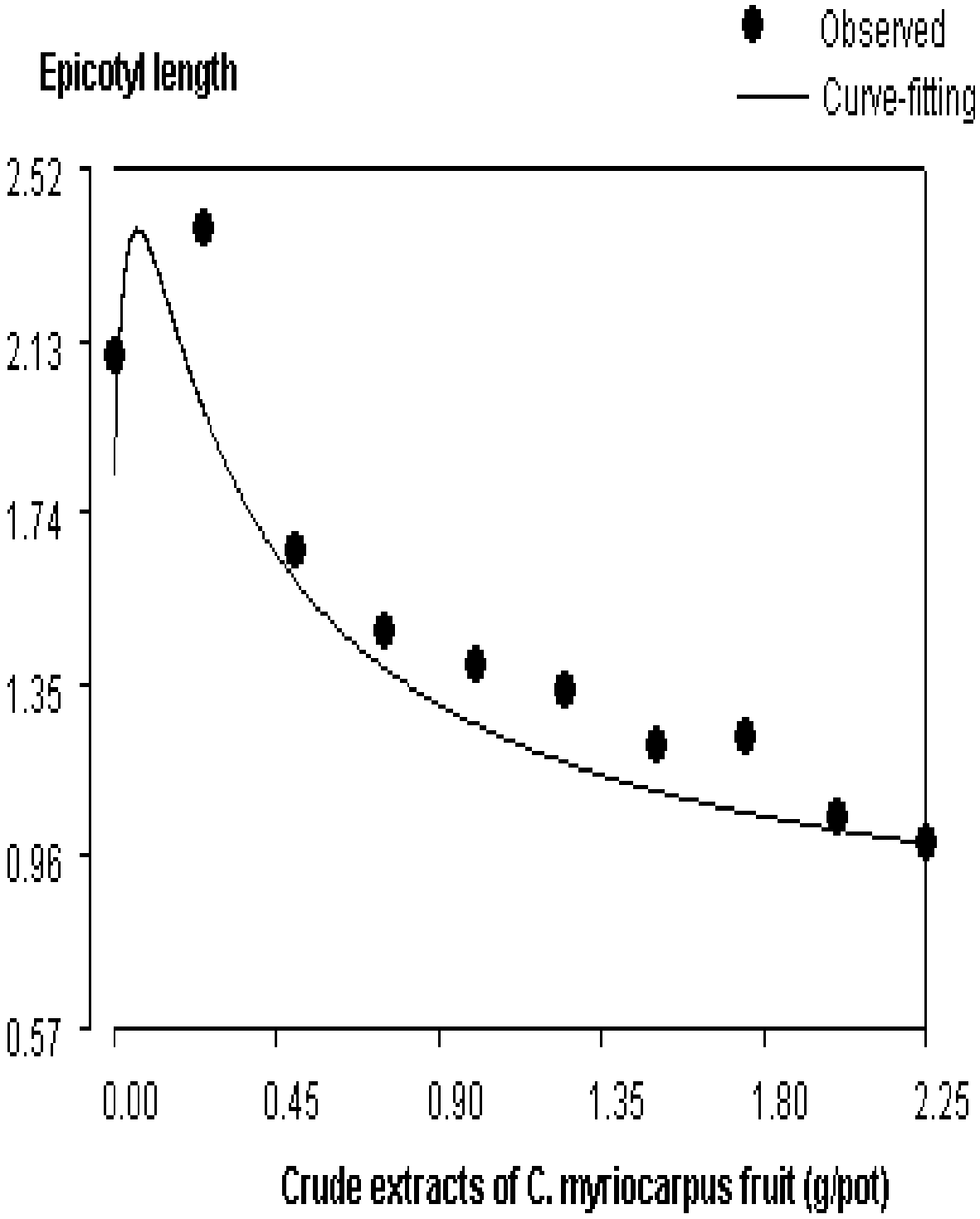

Figure 5.34 Response of epicotyl length of tomato seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

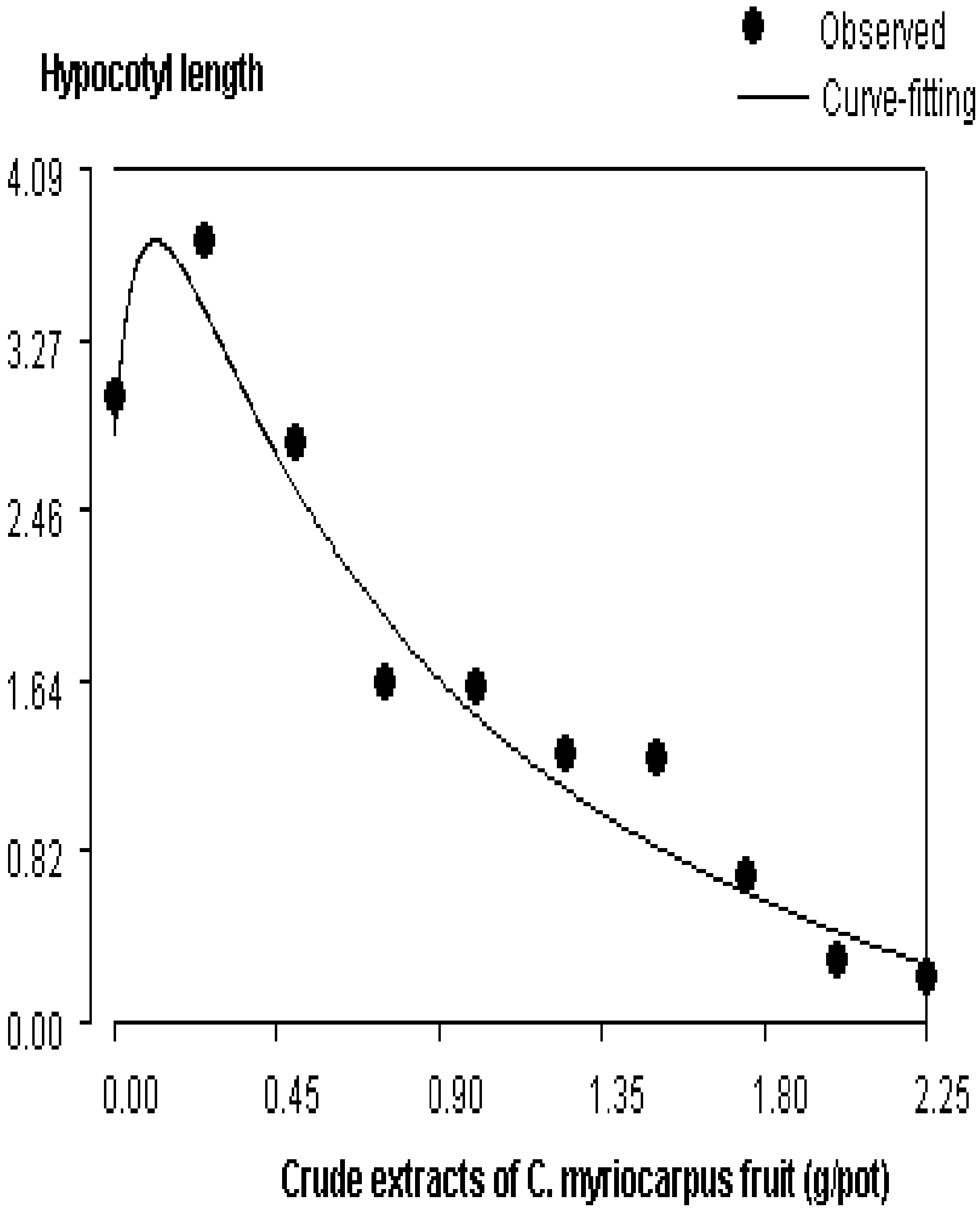

Figure 5.35 Response of hypocotyl length of tomato seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

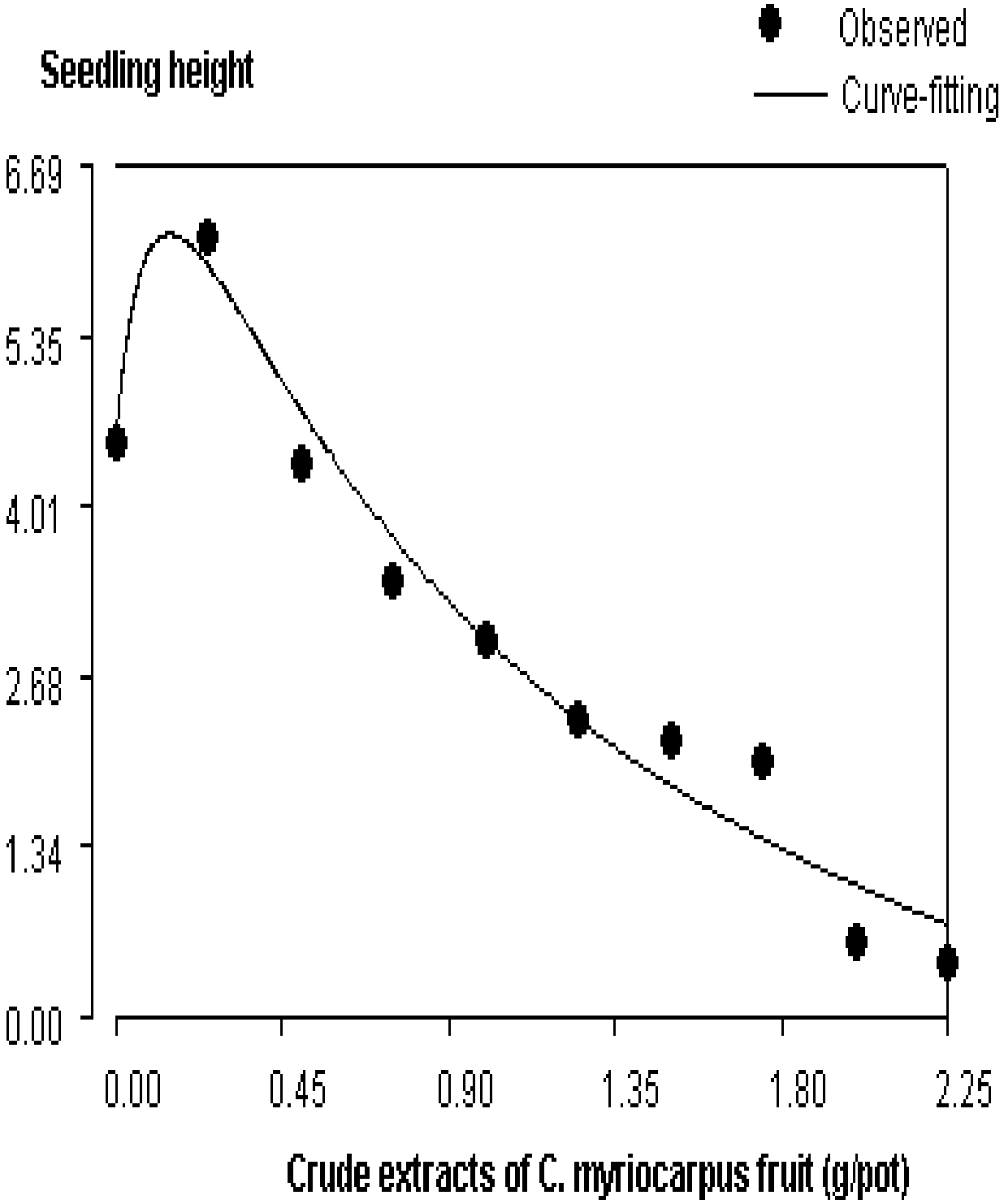

Figure 5.36 Response of seedling height of tomato seedlings to dosages of crude extracts of *Cucumis myriocarpus* fruit at 18 days after planting (n = 50).

5.4 Mean dosage stimulation response

Mean dosage stimulation response [(MDSR) = (D_m + R_h)/2] for crude extracts of *C. myriocarpus* fruit, as a pre-emergent bio-nematicide in the family Alliaceae ranged from 0.45 to 1.19 g, in the family Gramineae from 0.86 to 1.13 g and in the family Solanaceae from 0.53 to 1.11 g (Table 5.19).

Table 5.19 Mean dosage stimulation response (MDSR) for using crude extracts of *Cucumis myriocarpus* fruit as a pre-emergent bio-nematicide for selected crops.

Family	Crop	Integrated sensitivity (∑k)[z]	Sensitivity ∑k ranking	MDSR	Mean
Alliaceae	Chive	24	Moderate	1.19	
	Leek	49	Low	0.68	0.77
	Onion	22	High	0.45	
Gramineae	Maize	11	Moderate	1.13	
	Millet	18	Low	0.86	1.04
	Sorghum	9	High	1.12	
Solanaceae	Eggplant	9	High	0.74	
	Pepper	32	Moderate	1.11	0.79
	Tomato	51	Low	0.53	

[z] The higher the ∑k value, the lower the sensitivity, *vice versa*.

5.5 Validation of estimated dosages

Three test crops for validation were selected on the basis of their integrated sensitivity (∑k) to crude extracts of *C. myriocarpus* fruit (Table 5.19). According to integrated sensitivity ranking, onion was the highly sensitive crop to crude extracts of *C. myriocarpus* fruit in the family Alliaceae, while millet and tomato were the least sensitive in the families Gramineae and Solanaceae, respectively. Methodology for validation trials was as explained in this chapter; except that the treatments included control and validation dosage each with 1 000 J2s of *M. incognita* race 2. Validation dosages for onion, millet and tomato were 0.45, 0.86 and 0.53 g crude extracts of *C. myriocarpus* fruit, respectively. Nematode inoculum was prepared and applied as described elsewhere (Pofu *et al*., 2010a). Validation results showed that the material reduced Pi and had no effect on emergence of millet and tomato, but reduced emergence of onion seedlings by 15% (Table 5.20).

Table 5.20 Seedling emergence and final nematode numbers of *Meloidogyne incognita* race 2 at 18 days after treatment with mean dosage stimulation response (MDSR) of crude extracts of *Cucumis myriocarpus* fruit as pre-emergent bio-nematicide (n = 12).

Treatment	Onion		Millet		Tomato	
	Emergence (%)	Nematode (Pf)	Emergence (%)	Nematode (Pf)	Emergence (%)	Nematode (Pf)
Nematode alone	100	576	100	613	100	498
Nematode + MDSR	85	5	100	4	100	5
[z] Relative reduction (%)	15%**	99%**	0^{ns}	99%**	0^{ns}	99%**

[z] Relative reduction % = (1 - treatment/control) x 100
** Significant at P < 0.05 level, ns = not significant at P < 0.05 level.

5.6 Discussion

At low dosages, crude extracts of *C. myriocarpus* fruit consistently stimulated growth of various organs in the nine test plants, whereas at high dosages the material invariably inhibited growth. Stimulation and inhibition responses observed in this study agreed with the major characteristics of density-dependent growth patterns in biological systems (Liu *et al*., 2003). Results of CARD computer model provided an explanation as to why at low levels, crude extracts of *C. myriocarpus* fruit and other materials in the ground leaching technology (GLT) system had a fertiliser effect on tomato plants (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2008; Mashela *et al*., 2010).

Relationships generated by CARD computer model are, generally, dependent on k, which is the number of In(D+1) transformations, that serve as a biological indicator for the degree of sensitivity to an extrinsic or intrinsic factor to the variable measured (Liu *et al*., 2003). The lower the integrated sensitivity (∑k) value, the higher the sensitivity of the plant to the test material and *vice versa.* Generally, in the model, as k values increased, R^2 values also increased to a peak, where k = i and then started to decrease from i + 1 transformations until the model ceased to run (Liu *et al*., 2003). In this and other studies (Kato-Noguchi, 2003), the model provided dosages beyond the saturation point, where inhibition of plant growth invariably sets in.

Integrated sensitivities (∑k) per crop of measured variables in the three Alliaceae test crops differed, with the increasing order of sensitivity to crude extracts of *C. myriocarpus* fruit being onion > chive > leek. In the family Gramineae, the integrated sensitivities of the measured variables in the three test crops were in the increasing

order of sorghum > maize > millet, while in the family Solanaceae, the integrated sensitivity was the highest in eggplant, followed by chili, pepper and then tomato. The lowest sensitivities of tomato to crude extracts of *C. myriocarpus* fruit may help to explain the successful use of this material in GLT system on tomato plants under various conditions (Mashela, 2002; Mashela *et al*., 2008).

In this study, CARD computerised model was useful since it indicated the degree of sensitivity of various organs within the test plant, whereas conventional methods are limited to absolute integrated suppression of growth (Djurdjevic *et al.,* 2004; Xuan *et al*., 2004). Among the three families tested using CARD computer model, in the family Alliaceae, seedling height in onion was the most sensitive to allelopathic chemicals from crude extracts of *C. myriocarpus* fruit, while in leek, radicle length was the least sensitive. In the family Gramineae, the radicle length in maize was the most sensitive, while coleoptile length in millet was the least sensitive. Similarly, in the family Solanaceae, hypocotyl diameter and hypocotyl length in eggplant were the most sensitive, while epicotyl length in tomato was the least sensitive. Observations in this study demonstrated for the first time that different organs within the same plant species have different sensitivities to crude extracts of *C. myriocarpus* fruit, which may be extended to other extrinsic factors.

In all three families tested, the overall family MDSRs were at 0.77, 1.04 and 0.79 g crude extracts of *C. myriocarpus* fruit for the crops in the families Alliaceae, Gramineae and Solanaceae, respectively. However, in this study, three crops, each from one of the three families, was validated using its own MDSR value. Validation results suggested that MDSR concept holds for two of the three selected crops (*viz*.

millet and tomato), while it did not hold for onion, which has been empirically shown to be highly sensitive to the material. In all three crops, suppression of Pi was almost 100%, suggesting that on onion the dosage of the material could still be reduced to ameliorate phytotoxicity to seedlings. Results of validation, therefore, demonstrated that MDSR, as proposed in this study, was an appropriate yardstick in determining bio-pesticide dosages from botanicals.

The MDSR quantities intended for use as pre-emergent bio-nematicide for individual crops or for the family were below the amount of 2 g crude extracts of *C. myriocarpus* fruit used at transplanting as post-emergent bio-nematicide. The different quantities took into account the assertion that the degree of sensitivity of plants to allelopathy, in addition to being related to the quantity of the material, was also related to the age of the receptor plant (Rice, 1984; Einhellig and Leather, 1988). Also, the dosage integrated the sensitivities of all organs in the emerging seedlings.

5.7 Conclusions

The CARD model demonstrated that the response of three crops each from the families Alliaceae, Gramineae and Solanaceae, when regressed to a series of crude extracts of *C. myriocarpus* fruit, exhibited the density-dependent growth pattern, characterised by stimulation, saturation and inhibition responses. Using the integration of the responses of various organs, crude extracts of *C. myriocarpus* fruit within the plant in the region between the start and the end points of stimulation, the quantity of the material which could be used as pre-emergent bio-nematicide was

estimated and referred to as mean dosage stimulation response (MDSR) either for the crop or for the family.

CHAPTER 6
SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH AND CONCLUSIONS

6.1 Summary

Use of conventional methods in establishing the relationship between seedling emergence and seed germination of dicotyledonous and monocotyledonous crops with a series of crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit demonstrated the existence of the density-dependent growth pattern, which is characterised by (i) stimulation, (ii) saturation and (iii) inhibition. The Curve-Fitting Allelochemical Response Data (CARD) model was used to quantify the dosage for stimulation, saturation and inhibition ranges (Liu *et al*., 2003). The dosage range for stimulation (D_m + R_h), was used to compute the mean dosage stimulation response (MDSR) as (D_m + R_h)/2, which could be used as the dosage for applying crude extracts of *C. myriocarpus* fruit as a pre-emergent bio-nematicide without negatively affecting seed germination and seedling emergence. The differences in the MDSR for different crops are in agreement with the observation that the degree of sensitivity to allelochemicals differs with the plant species (Rice, 1984; Einhellig and Leather, 1988).

6.2 Significance of findings

The results from CARD computerised model demonstrated for the first time why at low levels, crude extracts of *C. myriocarpus* fruit and other materials in the ground leaching technology (GLT) system on tomato plants had a fertiliser effect. Furthermore, this study provided the MDSR for nine crops within the families Alliaceae, Gramineae and Solanaceae. Within each family, the MDSR or individual dosage per crop could be used. The significance of the CARD and MDSR is that this

concept can be expanded to other botanicals in the assessment of the materials in plant protection and perhaps also in human medicine.

6.3 Recommended future research

- Evaluating the influence of soil type on the efficacy of the recommended MDSR values on both crop sensitivities and efficacy of reducing nematode numbers.
- Evaluating the environmental impact of the MDSR quantities.
- Generally, GLT systems are labour-intensive and would be costly in commercial cropping systems. Attempts should be made to use MDSR concepts to develop formulations which can be used through irrigation systems for the management of plant-parasitic nematodes.

6.4 Conclusions

Crude extracts of *C. myriocarpus* fruit consistently suppressed numbers of the southern root-knot nematode (*Meloidogyne incognita*) when used as a pre-emergent bio-nematicide. The study used empirical and computer modelling to provide quantities of crude extracts of *C. myriocarpus* fruit for use as a pre-emergent bio-nematicide, where the material would stimulate seed germination and/or seedling emergence. Established MDSR concepts can also be used in other botanicals in plant protection.

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APPENDICES

Appendix 3.1 Samples of experimental layout for seedling emergence experiments, **A**. Bean, **B**. Chili, **C**. Cucumber, **D**. Eggplant, **E**. Pepper and **F**. Lettuce.

Appendix 3.2 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on bean seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	2.77	1.00	0.42
Treatment	6	4.971	80.56	19.33	0.00
Error	24	1.028	16.67		
Total	34	6.171			

Appendix 3.3 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on chili seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	2.77	1.00	0.42
Treatment	6	4.971	80.56	19.33	0.00
Error	24	1.028	16.67		
Total	34	6.171			

Appendix 3.4 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on cucumber seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.114	1.71	1.00	0.42
Treatment	6	5.885	88.03	34.33	0.00
Error	24	0.685	10.26		
Total	34	6.685			

Appendix 3.5 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on eggplant seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.114	1.71	0.36	0.83
Treatment	6	4.685	70.09	9.94	0.00
Error	24	1.885	28.20		
Total	34	6.685			

Appendix 3.6 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on lettuce seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.400	5.98	0.86	0.50
Treatment	6	3.485	52.14	4.98	0.01
Error	24	2.800	41.88		
Total	34	6.685			

Appendix 3.7 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on pea seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	2.78	1.00	0.42
Treatment	6	4.971	80.56	19.33	0.00
Error	24	1.028	16.66		
Total	34	6.171			

Appendix 3.8 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on pepper seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.114	1.71	1.00	0.42
Treatment	6	5.885	88.03	34.33	0.00
Error	24	0.685	10.26		
Total	34	6.685			

Appendix 3.9 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on sunflower seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	3.06	1.00	0.42
Treatment	6	4.400	78.57	17.11	0.00
Error	24	1.028	18.37		
Total	34	5.600			

Appendix 3.10 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on tomato seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	2.17	0.30	0.87
Treatment	6	4.285	54.35	5.00	0.01
Error	24	3.428	43.48		
Total	34	7.885			

Appendix 3.11 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on watermelon seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	3.06	0.56	0.69
Treatment	6	3.600	78.57	7.88	0.01
Error	24	1.828	18.37		
Total	34	5.600			

Appendix 3.12 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on chive seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	2.77	4.44	0.04
Treatment	6	4.971	80.56	19.33	0.00
Error	24	1.028	16.67		
Total	34	6.171			

Appendix 3.13 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on leek seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.114	2.30	1.00	0.42
Treatment	6	4.171	83.91	24.33	0.00
Error	24	0.685	13.79		
Total	34	4.971			

Appendix 3.14 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on maize seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.571	6.67	1.30	0.29
Treatment	6	5.371	62.67	8.71	0.01
Error	24	2.628	30.66		
Total	34	8.571			

Appendix 3.15 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on millet seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	3.06	1.29	0.02
Treatment	6	4.400	78.57	17.11	0.00
Error	24	1.028	18.37		
Total	34	5.600			

Appendix 3.16 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on onion seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.171	3.06	0.72	0.58
Treatment	6	4.400	78.57	11.20	0.00
Error	24	1.028	18.37		
Total	34	5.600			

Appendix 3.17 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on rye seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.285	3.99	1.30	0.29
Treatment	6	5.542	77.60	16.87	0.00
Error	24	1.314	18.41		
Total	34	7.142			

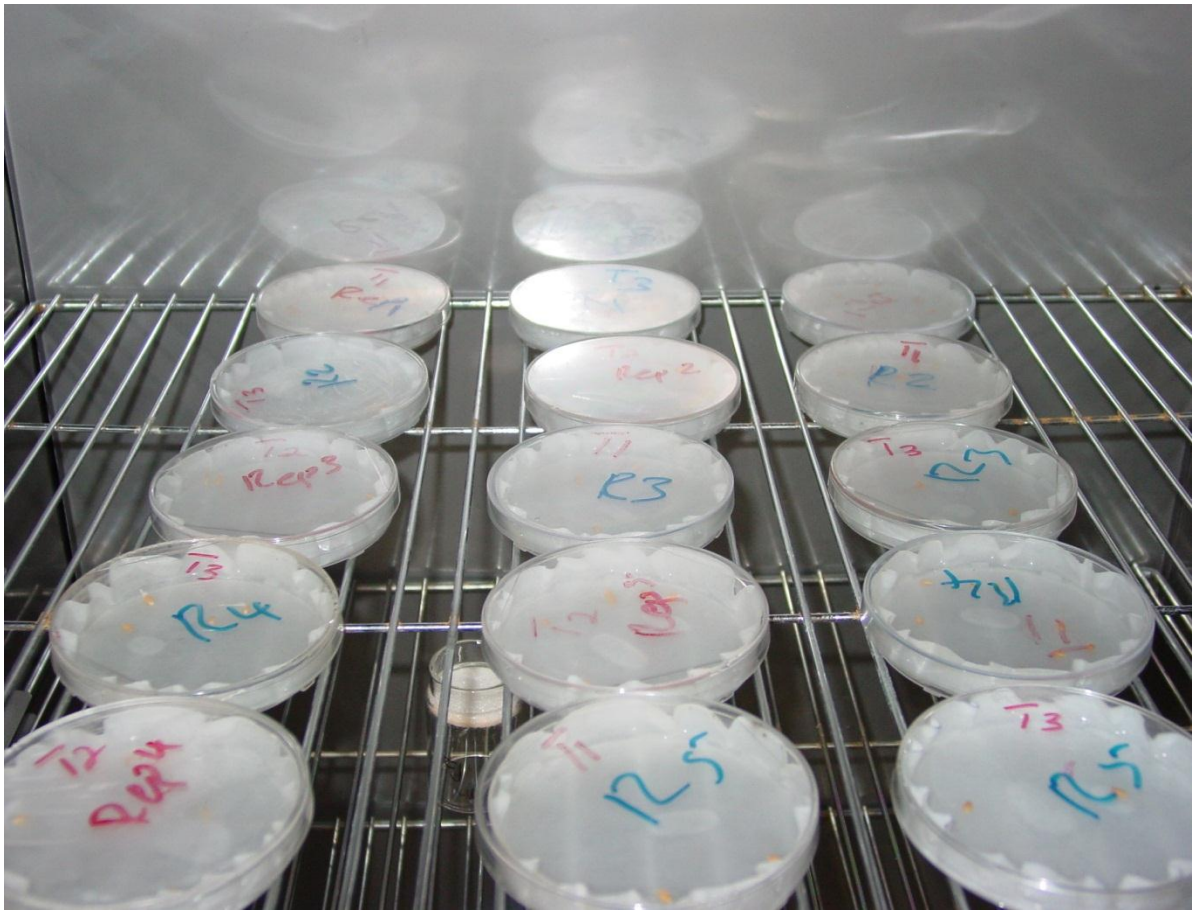
Appendix 3.18 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on sorghum seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P≤
Replication	4	0.400	8.05	1.50	0.23
Treatment	6	2.971	59.77	7.43	0.01
Error	24	1.600	32.18		
Total	34	4.971			

Appendix 3.19 Analysis of variance (ANOVA) of seven different concentrations of crude extracts of C*ucumis myriocarpus* fruit on wheat seedling emergence (n = 35).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.114	2.30	0.03	0.87
Treatment	6	4.171	83.91	24.33	0.00
Error	24	0.685	13.79		
Total	34	4.971			

Appendix 4.1 Sample of experimental layout for all seed germination experiments with petri dishes placed in the growth chamber.



Appendix 4.2 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on bean (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	172.714	74.78	10.38	0.00
Error	21	58.250	25.22		
Total	27	230.964			

Appendix 4.3 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on butternut squash (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	292.214	85.70	20.98	0.00
Error	21	48.750	14.30		
Total	27	340.964			

Appendix 4.4 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on chili (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	153.214	98.87	306.43	0.00
Error	21	1.750	1.13		
Total	27	154.964			

Appendix 4.5 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on eggplant (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	176.929	96.85	107.70	0.00
Error	21	5.750	3.15		
Total	27	182.679			

Appendix 4.6 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on lettuce (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	346.429	97.13	118.29	0.00
Error	21	10.250	2.87		
Total	27	356.679			

Appendix 4.7 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on pea (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	222.429	90.17	32.10	0.00
Error	21	24.250	9.83		
Total	27	246.679			

Appendix 4.8 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on pepper (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	158.500	94.76	63.40	0.00
Error	21	8.750	5.24		
Total	27	167.250			

Appendix 4.9 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on sunflower (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	317.500	92.84	45.36	0.00
Error	21	24.500	7.16		
Total	27	342.000			

Appendix 4.10 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on tomato (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	245.500	99.29	491.00	0.00
Error	21	1.750	0.71		
Total	27	247.250			

Appendix 4.11 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on watermelon (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	337.357	96.91	109.84	0.00
Error	21	10.750	3.09		
Total	27	348.107			

Appendix 4.12 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on chive (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	165.857	85.45	20.55	0.00
Error	21	28.250	14.55		
Total	27	194.107			

Appendix 4.13 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on leek (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	187.357	93.63	51.43	0.00
Error	21	12.750	6.37		
Total	27	200.107			

Appendix 4.14 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on maize (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	276.929	89.71	30.53	0.00
Error	21	31.750	10.29		
Total	27	308.679			

Appendix 4.15 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on millet (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	279.429	97.47	134.90	0.00
Error	21	7.250	2.53		
Total	27	286.679			

Appendix 4.16 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on onion (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	256.857	95.18	69.15	0.00
Error	21	13.000	4.82		
Total	27	269.857			

Appendix 4.17 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on rye (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	285.714	97.53	137.93	0.00
Error	21	7.250	2.47		
Total	27	292.964			

Appendix 4.18 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on sorghum (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	133.929	73.01	9.47	0.00
Error	21	49.500	26.99		
Total	27	183.429			

Appendix 4.19 Analysis of variance (ANOVA) of seven different levels of aqueous extracts of C*ucumis myriocarpus* fruit on wheat (n = 28).

SOURCE	Df	SS	Percent	F	P ≤
Treatment	6	278.429	97.12	118.12	0.00
Error	21	8.250	2.88		
Total	27	286.679			

Appendix 5.1 Sample of Curve-fitting Allelochemical Dosage Response (CARD) computer model output summary for eggplant epicotyl length.

OBSERVATIONS/CARD MODEL INPUT

0.00	0.34
0.25	0.62
0.50	0.72
0.75	0.71
1.00	0.64
1.25	0.43
1.50	0.31
1.75	0.18
2.00	0.05
2.25	0.06

SUMMARY OF RESULTS

Number of ln(D+1) transformations: 2

Coefficient of determination (R^2): 0.967

Maximum value of stimulation (R_h): 0.391

Dose for the highest stimulation (D_m): 0.474

Dose for 0% reduction (D_0): 1.527

Dose for 50% reduction (D_{50}): 1.864

Dose for 100% reduction (D_{100}): 2.2

The fitted equation:

$R = .329 + 2.382\ g(D) - 3.63\ [g(D)]^2$

Where g(D) =ln(ln(D+1)+1) ,R is the response; D is the allelochemical dose.

No-Ln	R^2	F-test	RPERCENTE	ME
0.0	0.8209	16.0386	0.1040	0.8209
1.0	0.9369	51.9354	0.0617	0.9369
2.0	0.9673	103.6374	0.0444	0.9673
3.0	0.9519	69.2292	0.0539	0.9519
4.0	0.9184	39.4139	0.0701	0.9184
5.0	0.8803	25.7362	0.0850	0.8803
6.0	0.8430	18.7926	0.0973	0.8430

MODEL PREDICTION

Crude extract of C*ucumis myriocarpus* fruit (g/pot) Epicotyl length

Dosage	Response
0.00000	0.32866
0.00237	0.33427
0.00474	0.33982
0.00711	0.34530
0.00949	0.35072
0.01186	0.35607
0.01423	0.36136
0.01660	0.36658
0.01897	0.37175
0.02134	0.37685
0.02372	0.38189
0.02609	0.38687
0.02846	0.39179
0.03083	0.39665
0.03320	0.40145
0.03557	0.40620
0.03795	0.41089
0.04032	0.41552
0.04269	0.42010
0.04506	0.42463
0.04743	0.42909
0.04980	0.43351
0.05218	0.43787
0.05455	0.44218
0.05692	0.44644
0.05929	0.45065
0.06166	0.45480

0.06403	0.45891
0.06641	0.46296
0.06878	0.46697
0.07115	0.47093
0.07352	0.47484
0.07589	0.47870
0.07826	0.48252
0.08064	0.48629
0.08301	0.49001
0.08538	0.49369
0.08775	0.49732
0.09012	0.50091
0.09249	0.50445
0.09487	0.50795
0.09724	0.51141
0.09961	0.51483
0.10198	0.51820
0.10435	0.52153
0.10672	0.52482
0.10910	0.52807
0.11147	0.53128
0.11384	0.53445
0.11621	0.53757
0.11858	0.54066
0.12095	0.54372
0.12333	0.54673
0.12570	0.54970
0.12807	0.55264
0.13044	0.55554
0.13281	0.55840
0.13518	0.56123
0.13755	0.56402
0.13993	0.56678
0.14230	0.56950
0.14467	0.57218
0.14704	0.57483
0.14941	0.57745
0.15178	0.58003
0.15416	0.58258
0.15653	0.58509
0.15890	0.58757
0.16127	0.59002
0.16364	0.59244
0.16601	0.59483
0.16839	0.59718
0.17076	0.59951
0.17313	0.60180
0.17550	0.60406
0.17787	0.60629
0.18024	0.60849

0.18262	0.61066
0.18499	0.61280
0.18736	0.61492
0.18973	0.61700
0.19210	0.61906
0.19447	0.62108
0.19685	0.62308
0.19922	0.62505
0.20159	0.62700
0.20396	0.62891
0.20633	0.63080
0.20870	0.63267
0.21108	0.63450
0.21345	0.63631
0.21582	0.63810
0.21819	0.63986
0.22056	0.64159
0.22293	0.64330
0.22531	0.64498
0.22768	0.64664
0.23005	0.64827
0.23242	0.64988
0.23479	0.65147
0.23716	0.65303
0.23954	0.65457
0.24191	0.65608
0.24428	0.65757
0.24665	0.65904
0.24902	0.66049
0.25139	0.66191
0.25376	0.66332
0.25614	0.66469
0.25851	0.66605
0.26088	0.66739
0.26325	0.66870
0.26562	0.67000
0.26799	0.67127
0.27037	0.67252
0.27274	0.67375
0.27511	0.67496
0.27748	0.67615
0.27985	0.67732
0.28222	0.67847
0.28460	0.67960
0.28697	0.68071
0.28934	0.68180
0.29171	0.68287
0.29408	0.68393
0.29645	0.68496
0.29883	0.68598

0.30120	0.68697
0.30357	0.68795
0.30594	0.68891
0.30831	0.68986
0.31068	0.69078
0.31306	0.69169
0.31543	0.69258
0.31780	0.69345
0.32017	0.69430
0.32254	0.69514
0.32491	0.69596
0.32729	0.69677
0.32966	0.69755
0.33203	0.69832
0.33440	0.69908
0.33677	0.69982
0.33914	0.70054
0.34152	0.70125
0.34389	0.70194
0.34626	0.70261
0.34863	0.70327
0.35100	0.70392
0.35337	0.70455
0.35575	0.70516
0.35812	0.70576
0.36049	0.70634
0.36286	0.70691
0.36523	0.70747
0.36760	0.70801
0.36997	0.70854
0.37235	0.70905
0.37472	0.70955
0.37709	0.71003
0.37946	0.71050
0.38183	0.71096
0.38420	0.71140
0.38658	0.71183
0.38895	0.71225
0.39132	0.71265
0.39369	0.71304
0.39606	0.71342
0.39843	0.71378
0.40081	0.71414
0.40318	0.71447
0.40555	0.71480
0.40792	0.71511
0.41029	0.71542
0.41266	0.71571
0.41504	0.71598
0.41741	0.71625

0.41978	0.71650
0.42215	0.71674
0.42452	0.71697
0.42689	0.71719
0.42927	0.71740
0.43164	0.71759
0.43401	0.71778
0.43638	0.71795
0.43875	0.71811
0.44112	0.71826
0.44350	0.71840
0.44587	0.71853
0.44824	0.71864
0.45061	0.71875
0.45298	0.71885
0.45535	0.71893
0.45773	0.71901
0.46010	0.71907
0.46247	0.71913
0.46484	0.71917
0.46721	0.71921
0.46958	0.71923
0.47196	0.71925
0.47433	0.71925
0.47670	0.71925
0.48381	0.71918
0.49093	0.71902
0.49804	0.71878
0.50516	0.71846
0.51227	0.71806
0.51939	0.71758
0.52650	0.71703
0.53362	0.71641
0.54073	0.71571
0.54785	0.71494
0.55496	0.71411
0.56208	0.71321
0.56919	0.71224
0.57631	0.71121
0.58342	0.71012
0.59054	0.70897
0.59765	0.70776
0.60477	0.70650
0.61188	0.70517
0.61900	0.70379
0.62611	0.70236
0.63323	0.70088
0.64034	0.69934
0.64746	0.69776
0.65457	0.69613

0.66169	0.69444
0.66880	0.69272
0.67592	0.69094
0.68303	0.68913
0.69015	0.68727
0.69726	0.68536
0.70438	0.68342
0.71149	0.68144
0.71861	0.67941
0.72572	0.67735
0.73283	0.67525
0.73995	0.67312
0.74706	0.67094
0.75418	0.66874
0.76129	0.66650
0.76841	0.66422
0.77552	0.66192
0.78264	0.65958
0.78975	0.65721
0.79687	0.65481
0.80398	0.65238
0.81110	0.64992
0.81821	0.64743
0.82533	0.64492
0.83244	0.64238
0.83956	0.63981
0.84667	0.63721
0.85379	0.63459
0.86090	0.63195
0.86802	0.62928
0.87513	0.62659
0.88225	0.62388
0.88936	0.62114
0.89648	0.61838
0.90359	0.61561
0.91071	0.61281
0.91782	0.60998
0.92494	0.60714
0.93205	0.60429
0.93917	0.60141
0.94628	0.59851
0.95340	0.59560
0.96051	0.59266
0.96763	0.58971
0.97474	0.58675
0.98186	0.58377
0.98897	0.58077
0.99609	0.57775
1.00320	0.57473
1.01032	0.57168

1.01743	0.56863
1.02455	0.56555
1.03166	0.56247
1.03878	0.55937
1.04589	0.55626
1.05301	0.55313
1.06012	0.55000
1.06724	0.54685
1.07435	0.54369
1.08147	0.54051
1.08858	0.53733
1.09570	0.53414
1.10281	0.53093
1.10993	0.52772
1.11704	0.52449
1.12415	0.52126
1.13127	0.51801
1.13838	0.51476
1.14550	0.51150
1.15261	0.50823
1.15973	0.50495
1.16684	0.50166
1.17396	0.49837
1.18107	0.49506
1.18819	0.49175
1.19530	0.48843
1.20242	0.48511
1.20953	0.48178
1.21665	0.47844
1.22376	0.47509
1.23088	0.47174
1.23799	0.46838
1.24511	0.46502
1.25222	0.46165
1.25934	0.45828
1.26645	0.45490
1.27357	0.45151
1.28068	0.44812
1.28780	0.44472
1.29491	0.44132
1.30203	0.43792
1.30914	0.43451
1.31626	0.43110
1.32337	0.42768
1.33049	0.42426
1.33760	0.42084
1.34472	0.41741
1.35183	0.41398
1.35895	0.41054
1.36606	0.40710

1.37318	0.40366
1.38029	0.40022
1.38741	0.39677
1.39452	0.39332
1.40164	0.38987
1.40875	0.38641
1.41587	0.38296
1.42298	0.37950
1.43010	0.37604
1.43721	0.37257
1.44433	0.36911
1.45144	0.36564
1.45856	0.36217
1.46567	0.35870
1.47279	0.35523
1.47990	0.35176
1.48701	0.34828
1.49413	0.34481
1.50124	0.34133
1.50836	0.33786
1.51547	0.33438
1.52259	0.33090
1.52970	0.32742
1.53682	0.32394
1.54393	0.32046
1.55105	0.31698
1.55816	0.31350
1.56528	0.31002
1.57239	0.30654
1.57951	0.30305
1.58662	0.29957
1.59374	0.29609
1.60085	0.29261
1.60797	0.28913
1.61508	0.28565
1.62220	0.28217
1.62931	0.27869
1.63643	0.27521
1.64354	0.27173
1.65066	0.26825
1.65777	0.26477
1.66489	0.26129
1.67200	0.25782
1.67912	0.25434
1.68623	0.25086
1.69335	0.24739
1.70046	0.24392
1.70758	0.24045
1.71469	0.23697
1.72181	0.23351

1.72892	0.23004
1.73604	0.22657
1.74315	0.22310
1.75027	0.21964
1.75738	0.21618
1.76450	0.21271
1.77161	0.20925
1.77873	0.20580
1.78584	0.20234
1.79296	0.19888
1.80007	0.19543
1.80719	0.19198
1.81430	0.18853
1.82142	0.18508
1.82853	0.18163
1.83565	0.17819
1.84276	0.17474
1.84987	0.17130
1.85699	0.16786
1.86410	0.16443
1.87122	0.16099
1.87833	0.15756
1.88545	0.15413
1.89256	0.15070
1.89968	0.14727
1.90144	0.14643
1.90321	0.14558
1.90497	0.14473
1.90673	0.14388
1.90850	0.14303
1.91026	0.14218
1.91202	0.14134
1.91379	0.14049
1.91555	0.13964
1.91731	0.13879
1.91908	0.13794
1.92084	0.13710
1.92260	0.13625
1.92437	0.13540
1.92613	0.13456
1.92789	0.13371
1.92966	0.13286
1.93142	0.13202
1.93319	0.13117
1.93495	0.13032
1.93671	0.12948
1.93848	0.12863
1.94024	0.12779
1.94200	0.12694
1.94377	0.12609

1.94553	0.12525
1.94729	0.12440
1.94906	0.12356
1.95082	0.12271
1.95258	0.12187
1.95435	0.12103
1.95611	0.12018
1.95787	0.11934
1.95964	0.11849
1.96140	0.11765
1.96316	0.11680
1.96493	0.11596
1.96669	0.11512
1.96845	0.11427
1.97022	0.11343
1.97198	0.11259
1.97374	0.11175
1.97551	0.11090
1.97727	0.11006
1.97904	0.10922
1.98080	0.10837
1.98256	0.10753
1.98433	0.10669
1.98609	0.10585
1.98785	0.10501
1.98962	0.10417
1.99138	0.10332
1.99314	0.10248
1.99491	0.10164
1.99667	0.10080
1.99843	0.09996
2.00020	0.09912
2.00196	0.09828
2.00372	0.09744
2.00549	0.09660
2.00725	0.09576
2.00901	0.09492
2.01078	0.09408
2.01254	0.09324
2.01430	0.09240
2.01607	0.09156
2.01783	0.09072
2.01959	0.08988
2.02136	0.08905
2.02312	0.08821
2.02489	0.08737
2.02665	0.08653
2.02841	0.08569
2.03018	0.08486
2.03194	0.08402

2.03370	0.08318
2.03547	0.08234
2.03723	0.08151
2.03899	0.08067
2.04076	0.07983
2.04252	0.07900
2.04428	0.07816
2.04605	0.07732
2.04781	0.07649
2.04957	0.07565
2.05134	0.07482
2.05310	0.07398
2.05486	0.07314
2.05663	0.07231
2.05839	0.07147
2.06015	0.07064
2.06192	0.06980
2.06368	0.06897
2.06544	0.06813
2.06721	0.06730
2.06897	0.06647
2.07074	0.06563
2.07250	0.06480
2.07426	0.06397
2.07603	0.06313
2.07779	0.06230
2.07955	0.06147
2.08132	0.06063
2.08308	0.05980
2.08484	0.05897
2.08661	0.05814
2.08837	0.05730
2.09013	0.05647
2.09190	0.05564
2.09366	0.05481
2.09542	0.05398
2.09719	0.05315
2.09895	0.05231
2.10071	0.05148
2.10248	0.05065
2.10424	0.04982
2.10600	0.04899
2.10777	0.04816
2.10953	0.04733
2.11130	0.04650
2.11306	0.04567
2.11482	0.04484
2.11659	0.04401
2.11835	0.04318
2.12011	0.04235

2.12188	0.04153
2.12364	0.04070
2.12540	0.03987
2.12717	0.03904
2.12893	0.03821
2.13069	0.03739
2.13246	0.03656
2.13422	0.03573
2.13598	0.03490
2.13775	0.03408
2.13951	0.03325
2.14127	0.03242
2.14304	0.03159
2.14480	0.03077
2.14656	0.02994
2.14833	0.02912
2.15009	0.02829
2.15185	0.02746
2.15362	0.02664
2.15538	0.02581
2.15715	0.02499
2.15891	0.02416
2.16067	0.02334
2.16244	0.02251
2.16420	0.02169
2.16596	0.02087
2.16773	0.02004
2.16949	0.01922
2.17125	0.01839
2.17302	0.01757
2.17478	0.01675
2.17654	0.01592
2.17831	0.01510
2.18007	0.01428
2.18183	0.01346
2.18360	0.01263
2.18536	0.01181
2.18712	0.01099
2.18889	0.01017
2.19065	0.00935
2.19241	0.00853
2.19418	0.00770
2.19594	0.00688
2.19770	0.00606
2.19947	0.00524
2.20123	0.00442
2.20300	0.00360
2.20476	0.00278
2.20652	0.00196
2.20829	0.00114

2.21005	0.00032
2.21181	-0.0005
2.21358	-0.00132
2.21534	-0.00214
2.21710	-0.00295
2.21887	-0.00377
2.22063	-0.00459
2.22239	-0.00541
2.22416	-0.00623
2.22592	-0.00704
2.22768	-0.00786
2.22945	-0.00868
2.23121	-0.0095
2.23297	-0.01031
2.23474	-0.01113
2.23650	-0.01195
2.23826	-0.01276
2.24003	-0.01358
2.24179	-0.0144
2.24355	-0.01521
2.24532	-0.01603
2.24708	-0.01684
2.24885	-0.01766
2.25061	-0.01847

Appendix 5.2 Analysis of variance (ANOVA) for chive seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	23.042	4.40	0.94	0.45
Treatment	9	278.849	53.30	5.04	0.00
Error	36	221.294	42.30		
Total	49	523.185			

Appendix 5.3 Analysis of variance (ANOVA) for chive radicle length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	52.801	2.64	0.49	0.74
Treatment	9	975.591	48.72	3.99	0.01
Error	36	973.888	48.64		
Total	49	2002.280			

Appendix 5.4 Analysis of variance (ANOVA) for chive coleoptile length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.496	1.03	0.22	0.92
Treatment	9	26.737	55.79	5.17	0.02
Error	36	20.692	43.18		
Total	49	47.925			

Appendix 5.5 Analysis of variance (ANOVA) for chive coleoptile diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.546	4.39	0.91	0.46
Treatment	9	6.508	52.20	4.81	0.03
Error	36	5.413	43.41		
Total	49	12.468			

Appendix 5.6 Analysis of variance (ANOVA) for leek seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	14.654	3.31	0.65	0.62
Treatment	9	226.048	51.06	4.48	0.00
Error	36	201.998	45.63		
Total	49	442.700			

Appendix 5.7 Analysis of variance (ANOVA) for leek radicle length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	76.725	4.03	0.71	0.58
Treatment	9	968.491	50.87	3.55	0.00
Error	36	858.632	45.10		
Total	49	1903.850			

Appendix 5.8 Analysis of variance (ANOVA) for leek coleoptile length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.537	1.26	0.25	0.90
Treatment	9	22.727	53.17	4.67	0.00
Error	36	19.478	45.57		
Total	49	42.743			

Appendix 5.9 Analysis of variance (ANOVA) for leek coleoptile diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.266	2.21	0.43	0.78
Treatment	9	6.220	51.43	4.44	0.00
Error	36	5.609	46.36		
Total	49	12.096			

Appendix 5.10 Analysis of variance (ANOVA) for onion seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	49.984	9.74	1.87	0.13
Treatment	9	240.231	46.82	3.72	0.00
Error	36	223.133	43.44		
Total	49	513.349			

Appendix 5.11 Analysis of variance (ANOVA) for onion radicle length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	66.540	3.37	0.55	0.70
Treatment	9	1097.770	55.52	2.96	0.00
Error	36	812.925	41.11		
Total	49	1977.240			

Appendix 5.12 Analysis of variance (ANOVA) for onion coleoptile length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	2.554	5.43	0.99	0.42
Treatment	9	23.261	49.46	3.65	0.02
Error	36	21.217	45.11		
Total	49	47.033			

Appendix 5.13 Analysis of variance (ANOVA) for onion coleoptile diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	2.777	22.33	5.25	0.02
Treatment	9	4.896	39.37	4.11	0.01
Error	36	4.762	38.30		
Total	49	12.436			

Appendix 5.14 Analysis of variance (ANOVA) for maize seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	76.587	12.09	1.95	0.12
Treatment	9	353.653	55.87	2.29	0.03
Error	36	202.804	32.04		
Total	49	633.044			

Appendix 5.15 Analysis of variance (ANOVA) for maize radicle length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	132.603	4.72	0.74	0.56
Treatment	9	1602.000	56.98	2.69	0.01
Error	36	1076.970	38.30		
Total	49	2811.570			

Appendix 5.16 Analysis of variance (ANOVA) for maize coleoptile length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.346	6.92	1.22	0.32
Treatment	9	2.565	51.17	3.28	0.00
Error	36	2.100	41.91		
Total	49	5.012			

Appendix 5.17 Analysis of variance (ANOVA) for maize coleoptile diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	2.280	6.57	1.90	0.13
Treatment	9	21.617	62.28	8.00	0.00
Error	36	10.811	31.150		
Total	49	34.709			

Appendix 5.18 Analysis of variance (ANOVA) for millet seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	24.210	5.37	1.33	0.27
Treatment	9	262.878	58.30	6.42	0.00
Error	36	163.801	36.33		
Total	49	450.890			

Appendix 5. 19 Analysis of variance (ANOVA) for millet radicle length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	8.927	2.59	0.59	0.67
Treatment	9	198.491	57.58	5.78	0.00
Error	36	137.333	39.83		
Total	49	344.751			

Appendix 5.20 Analysis of variance (ANOVA) for millet coleoptile length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.153	4.90	2.01	0.11
Treatment	9	2.284	73.11	13.30	0.00
Error	36	0.686	21.99		
Total	49	3.124			

Appendix 5.21 Analysis of variance (ANOVA) for millet coleoptile diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.258	7.95	1.49	0.22
Treatment	9	3.256	64.15	8.34	0.00
Error	36	1.561	27.90		
Total	49	5.076			

Appendix 5.22 Analysis of variance (ANOVA) for sorghum seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	150.255	19.94	2.97	0.03
Treatment	9	454.789	51.30	2.48	0.02
Error	36	281.476	28.76		
Total	49	886.520			

Appendix 5.23 Analysis of variance (ANOVA) for sorghum radicle length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	140.169	8.84	1.49	0.22
Treatment	9	845.039	53.26	2.85	0.01
Error	36	601.300	37.90		
Total	49	1586.510			

Appendix 5.24 Analysis of variance (ANOVA) for sorghum coleoptile length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	4.185	8.32	1.44	0.24
Treatment	9	26.226	52.12	3.04	0.08
Error	36	19.904	39.56		
Total	49	50.316			

Appendix 5.25 Analysis of variance (ANOVA) for sorghum coleoptile diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	0.763	8.07	1.29	0.29
Treatment	9	5.332	56.39	2.52	0.02
Error	36	3.360	35.54		
Total	49	9.456			

Appendix 5.26 Analysis of variance (ANOVA) for eggplant seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	15.968	9.30	1.62	0.19
Treatment	9	88.699	51.64	3.03	0.00
Error	36	67.088	39.06		
Total	49	171.757			

Appendix 5.27 Analysis of variance (ANOVA) for eggplant epicotyl length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	3.004	8.90	1.36	0.26
Treatment	9	19.940	59.09	2.17	0.04
Error	36	10.801	32.01		
Total	49	33.745			

Appendix 5.28 Analysis of variance (ANOVA) for eggplant hypocotyl length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	6.282	9.74	1.73	0.16
Treatment	9	32.665	50.66	3.13	0.00
Error	36	25.529	39.60		
Total	49	64.477			

Appendix 5.29 Analysis of variance (ANOVA) for eggplant hypocotyl diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	12.082	8.55	1.06	0.39
Treatment	9	102.709	72.72	1.03	0.43
Error	36	26.445	18.73		
Total	49	141.238			

Appendix 5.30 Analysis of variance (ANOVA) for pepper seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	7.783	1.74	0.70	0.59
Treatment	9	340.768	75.97	13.69	0.00
Error	36	99.560	22.29		
Total	49	448.112			

Appendix 5.31 Analysis of variance (ANOVA) for pepper epicotyl length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P≤
Replication	4	3.470	2.96	0.57	0.68
Treatment	9	58.637	49.99	4.25	0.08
Error	36	55.217	47.05		
Total	49	117.326			

Appendix 5.32 Analysis of variance (ANOVA) for pepper hypocotyl length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P≤
Replication	4	5.302	3.65	1.92	0.12
Treatment	9	115.225	79.22	18.50	0.00
Error	36	24.913	17.13		
Total	49	145.441			

Appendix 5.33 Analysis of variance (ANOVA) for pepper hypocotyl diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	1.029	5.73	1.65	0.18
Treatment	9	11.323	63.05	8.08	0.00
Error	36	5.606	31.22		
Total	49	17.959			

Appendix 5.34 Analysis of variance (ANOVA) for tomato seedling height to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	29.474	6.05	0.97	0.43
Treatment	9	274.677	56.40	2.66	0.01
Error	36	182.870	37.55		
Total	49	487.022			

Appendix 5.35 Analysis of variance (ANOVA) for tomato epicotyl length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	7.229	4.68	0.55	0.70
Treatment	9	118.799	76.86	0.96	0.48
Error	36	28.543	18.46		
Total	49	154.571			

Appendix 5.36 Analysis of variance (ANOVA) for tomato hypocotyl length to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	9.629	5.99	1.29	0.29
Treatment	9	83.640	52.09	4.97	0.00
Error	36	67.302	41.99		
Total	49	160.57			

Appendix 5.37 Analysis of variance (ANOVA) for tomato hypocotyl diameter to dosages of crude extracts of C*ucumis myriocarpus* fruit (n = 50).

SOURCE	Df	SS	Percent	F	P ≤
Replication	4	1.018	6.04	1.17	0.34
Treatment	9	7.846	46.57	4.08	0.00
Error	36	0.001	47.39		
Total	49	16.846			

Appendix 5.38 Validation of estimated mean dosage stimulation response experiments on nematode suppression and seedling emergence for **A**. Tomato, **B**. Millet, **C**. Onion.