THE IMPACT OF PARAFFIN ON GERMINATION OF SELECTED CROP

SEEDS AND ITS POSSIBLE PEST REPELLENT ACTION

by

John Sembeba Kadende

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Supervisor: Dr. P.J. Pieterse

Co-supervisor: Prof G. A. Agenbag

Department of Agronomy

Stellenbosch University

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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General abstract

Abstract

Paraffin, also called kerosene is used by small-scale soya bean farmers in some parts of Africa as a pest repellent. The repellent action is claimed to be effective against parasites during seed germination and development of the seedlings. Seeds are immersed in commercial paraffin for a few seconds and sown in the soil immediately. This method raised some questions about possible negative effects on the seed after the imbibition process but also on humans and animals consuming the plants and seeds. Experiments were designed to investigate whether this practice would have negative effects on seed germination and vigour of the resulting seedlings of seven selected crop species. A trial was also carried out to test the effectiveness of paraffin as a pest repellent on canola in a field situation. The collected data were analyzed using STATISTICA, software version 11. Wherever the experiments showed significant interaction or differences within main factors, the means were separated making use of Fischer's LSD *post-hoc* analysis at p = 0.05.

The first series of experiments was done in the laboratory. It was carried out on seeds of seven crop species: canola (*Brassica napus* L.), common beans (*Phaseolus vulgaris* L.), ground nuts (*Arachis hypogea* L.), maize (*Zea mays* L.), soya bean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) and wheat (*Triticum aestivum* L.). In the germination trial, seeds were subjected to a 7X5X4 factorial design treatment with factors Crop species (CS) (see above), Paraffin concentration (PC) (0, 25, 50, 75 and 100% of commercial paraffin diluted with distilled water) and Time of immersion (TOI) (1, 5, 10, and 30 minutes). Treatments were repeated four times. After immersion seeds were dried with water absorbent paper and immediately germinated in 90 mm diameter petri dishes containing two filter papers and 5 ml of distilled water. Germination tests included 10 seeds per replicate and were incubated at a constant temperature of 20°C under dark conditions in an incubator. Findings showed that canola, sunflower and soya bean are paraffin tolerant (>70 % germination), wheat and groundnuts are less tolerant (30% – 70% germination) and beans and maize are intolerant (< 30 % germination). The paraffin had a negative influence on the rate of germination but there were no statistically significant differences between the 25% to 100% paraffin concentrations.

Measurements of the quantity of water and of paraffin absorbed were done after seeds of the seven crop species were immersed in 0, 25, 50, 75 and 100% paraffin concentrations for 30 minutes. Beans absorbed more water at 100% water and more paraffin at 25% paraffin than the other crop species. The paraffin uptake decreased with the increase of paraffin concentration while water uptake increased with the increase in water percentage. In both cases canola had the lowest uptake. Differential uptake of water and paraffin did not explain the results of the germination test.

Seeds of the seven crop species immersed in different paraffin concentrations (0, 25, 50, 75 and 100%) for thirty minutes were dried and then soaked in distilled water for 20 hours. The electrical conductivity (EC) of the liquid was determined by means of an EC meter after 20 hours of soaking. This was done to investigate whether paraffin treatment influenced leaking of electrolytes, which would indicate damage to the cell membranes in the seed. Results showed that sunflower leaked more electrolytes than any other seed, while wheat and maize had lower electrolyte leakage than the other species. This showed that the negative effect of paraffin on the germination of some crop species was unlikely to be due to membrane damage because sunflower seeds that leaked most electrolytes had a high germination percentage while the maize and wheat seeds that leaked little electrolytes, had poor germination after paraffin treatments.

The second experiment was conducted in a glasshouse. Seeds of the seven crop species were subjected to the same PC and TOI treatments as described in the germination experiment above but instead of being placed in an incubator to germinate, they were planted in 8cm x 8 cm plastic pots (10 seeds in each) in coarse sand in a glasshouse that was running at approximately 20ºC. The establishment of the seedlings was monitored daily in the glasshouse. The final percentage of establishment was calculated. Three weeks after planting, the seedlings were thinned to one plant per pot. The mean root and stem lengths as well as dry mass of the seedlings was recorded when the seedlings were thinned. The one plant per pot that was retained was harvested six weeks after establishment. Root and stem length and dry mass were determined. Establishment percentage and tolerance indices were calculated. Maize and beans showed the lowest establishment percentages and sunflower scored the highest establishment percentage after treatment with paraffin. The root and stem lengths of the crops were generally unaffected by paraffin treatments. In terms of dry mass paraffin had a significant negative effect on groundnut at three weeks but at six weeks no effect of paraffin on any of the vegetative growth parameters could be observed.

The third experiment was run in the microscope laboratory. A test using a confocal and fluorescence microscope was carried out to determine if residues of paraffin could be found in germinating soya bean seeds and seedlings. Specimens collected from the germinating soya bean seed and seedlings were mounted on the fluorescent microscope and stained with a solution of 100 µg.ml^{-1} Nile Red and observed with LD Plan-Neofluar 60X/0.6. Results showed that paraffin did penetrate the soya bean seed and was translocated within the plant system (endodermis) as the plant grows. The concentrations of paraffin in the tissue were however quite low.

The fourth experiment was run on the Langgewens Experimental Farm near Moorreesburg in the Western Cape Province. Forty blocks were spatially grouped into two separate groups. Twenty blocks received the five paraffin treatments replicated four times and the other twenty blocks received the five water treatments also replicated four times. Within each group the treatments were allocated randomly to the plots. The experimental design was a 2X5 Factorial experiment with factors Treatment liquid (distilled water and paraffin) and Time of immersion (0, 1, 5, 10 and 30 minutes)

replicated four times. No pesticides were applied to the canola crop. Stand density, leaf area and dry mass were recorded at the first harvest at 12 weeks, and then dry mass was determined at 21 weeks. Final yield was determined after 27 weeks when the plots were harvested by means of a combine plot harvester. The stand density, leaf area and dry mass were significantly increased by paraffin treatments at the time of the first harvest. After 21 weeks paraffin treatment had no significant effect on the dry mass production of the canola and the same was true of the final seed yield. . Even though there was no serious attack by pests, the little feeding damage that occurred in the water treated plots and not in the paraffin treated plots, indicate that paraffin may have a repellent effect. Paraffin had no negative effects whatsoever on the growth and yield of canola in this experiment.

This study indicates that different crops react differently to seed treatment with paraffin. The results of the fourth experiment indicate that paraffin might be used as pest repellent on certain selected crops but more research is needed on the subject.

Opsomming

Paraffien, ook genoem keroseen, word deur kleinskaalse boere in sekere dele van Afrika gebruik as 'n pesafweermiddel. Dit word beweer dat die afweeraksie suksesvol is teen parasiete tydens saadontkieming en vroeë saailinggroei. Saad word in kommersiële paraffien gedoop vir 'n paar sekondes en dan onmiddelik daarna geplant. Die metode skep vrae oor die moontlike negatiewe gevolge op die saad na die imbiberingsproses maar ook op mense en diere wat die plante en sade benut. Eksperimente is beplan om vas te stel of die praktyk negatiewe gevolge op die saadontkieming en groeikragtigheid van die daaropvolgende saailinge van sewe geselekteerde gewasspesies sal hê. 'n Eksperiment is ook uitgevoer om die effektiwiteit van paraffien as pesafweermiddel op kanola in 'n veldsituasie te toets. Die data wat ingesamel is is ontleed deur gebruik te maak van STATISTICA, sagteware, uitgawe 11. Waar betekenisvolle interaksies of verskille binne hooffaktore voorgekom het, is die gemiddeldes geskei deur middel van Fischer se LSD *post-hoc* ontleding by p = 0.05.

Die eerste reeks eksperimente is uitgevoer in 'n laboratorium. Dit is uitgevoer op sade van sewe gewasspesies naamlik . kanola (*Brassica napus* L.), gewone bone (*Phaseolus vulgaris* L.), grondbone (*Arachis hypogea* L.), mielies (*Zea mays* L.), sojabone (*Glycine max* L.), sonneblom (*Helianthus annuus* L.) en koring (*Triticum aestivum* L.). In die ontkiemingsproef is die sade onderwerp aan 'n 7X5X4 ewekansige blokontwerp wat faktoriaal gerangskik is met faktore Gewasspesies (CS) (sien hierbo), Paraffien konsentrasie (PC) (0, 25, 50, 75 en 100% van kommersiële paraffien verdun met gedistilleerde water) en Tyd van indompeling (TOI) (1, 5, 10, en 30 minute). Behandelings is vier keer herhaal. Na indompeling is die sade met waterabsorberende papier gedroog en onmiddelik daarna in 90 mm deursneë petribakkies wat twee filtreerpapiere en 5 ml gedistilleerde water bevat het, ontkiem. Tien sade per petribakkie is gebruik en die petribakkies is geïnkubeer by 'n konstante temperatuur van 20ºC in die donker in 'n inkubasiekas. Resultate het getoon dat kanola, sonneblom en sojaboon bestand is teen paraffienbehandelings (>70% ontkieming), koring en grondboon is minder bestand (30-70% ontkieming) en mielies en gewone bone is sensitief vir paraffienbehandeling (<30% ontkieming). Die paraffien het oor die algemeen 'n negatiewe effek op ontkiemingstempo gehad maar daar was geen statisties betekenisvolle verskille tussen die 25% en 100% paraffienbehandelings nie.

Die hoeveelheid water en paraffien wat opgeneem is deur sade van die sewe gewasspesies nadat dit in paraffienkonsentrasies van 0, 25, 50, 75 en 100% ingedompel is vir 30 minute, is bepaal. Gewone bone het meer water by die 100% water behandeling en meer paraffien by die 25% paraffien behandeling opgeneem as die ander spesies. Die paraffienopname het afgeneem met toename in paraffienkonsentrasie terwyl wateropname toegeneem het met toenemende waterkonsentrasies. Beide in geval van wateropname en paraffienopname het kanola die minste water opgeneem. Differensiële opname van water en paraffien het nie die resultate van die ontkiemingstoets verklaar nie.

Sade van die sewe gewasspesies is in verskillende paraffienkonsentrasies (0, 25, 50, 75 en 100%) gedompel vir 30 minute, gedroog en daarna in gedistilleerde water geweek vir 20 uur. Aan die einde van die 20 uur wekingsperiode is die elektriese konduktiwiteit (EC) van die wekingsvloeistof bepaal deur middel van 'n EC meter. Dit is gedoen om vas te stel of paraffienbehandeling die uitlek van elektroliete vanuit die saad, wat 'n aanduiding van beskadigde selmembrane van die saad kan wees, beïnvloed. Resultate het aangedui dat sonneblom die meeste elektroliete vrygestel het en koring en mielies die minste. Dit dui aan dat die negatiewe invloed van paraffien op sommige gewasspesies waarskynlik nie deur membraanbeskadiging veroorsaak is nie omdat sonneblom, wat die meeste elektroliete vrygestel het, die hoogste ontkiemingspersentasie na behandeling met praffien gehad het terwyl mielies en koring, wat die minste elektroliete vrygestel het, baie swak ontkieming gehad het na paraffienbehandeling.

Die tweede eksperiment is in 'n glashuis uitgevoer. Sade van die sewe gewasspesies is onderwerp aan dieselfde paraffienkonsentrasies en tye van indompeling as in die ontkiemingseksperiment hierbo maar in plaas van om die sade in 'n inkubasiekas te ontkiem, is dit in 8 cm x 8 cm plastiekpotte wat gevul is met growwe sand geplant (10 sade per pot) in 'n glashuis wat by 'n konstante temperatuur van ongeveer 20ºC geloop het. Die vestiging van die saailinge in die glashuis is daagliks gemonitor en die finale persentasie van vestiging is bereken. Drie weke na plant is die saailinge uitgedun sodat een per pot oorgebly het. Die uitgedunde saailinge se gemiddelde wortel- en stamlengtes is bepaal asook die gemiddelde droëmassas. Die een plant wat per pot oorgebly het is na ses weke ge-oes en weer is wortel- en stamlengtes bepaal asook die droëmassas. Vestigingspersentasies en toleransie indekse is bereken. Mielies en gewone bone het die laagste vestigingspersentasies getoon en sonneblom die hoogste nadat die gewasse met paraffien behandel is. Die wortel- en stamlengtes van die gewasse was oor die algemeen nie deur paraffienbehandelings beïnvloed nie. In terme van droëmassa het paraffien 'n negatiewe effek op grondbone gehad drie weke na plant maar na ses weke kon geen invloed van paraffienbehandelings op enige van die vegetatiewe groeiparameters waargeneem word nie.

Die derde eksperiment is in 'n mikroskooplaboratorium uitgevoer. 'n Konfokale en fluoreserende mikroskoop is gebruik om te bepaal of oorblyfsels van paraffien gevind kan word in ontkiemende sojaboonsade en saailinge. Monsters wat geneem is van die ontkiemende sojaboonsade saailinge is gemonteer op die fluoreserende mikroskoop en gekleur met 'n oplossing van 100 µg.ml^{-1} Nile Red oplossing en ge-evalueer met LD Plan-Neofluar 60X/0.6. Resultate het getoon dat paraffien wel die sojaboonsaad kon infiltreer en dat dit ook in die saailinge se endodermis vervoer kon word en opspoorbaar was. Die konsentrasies van paraffien in die weefsel was egter laag.

Die vierde eksperiment is uitgevoer op die Langgewens Proefplaas naby Moorreesburg in die Wes-Kaap Provinsie. Veertig blokke is ruimtelik in twee groepe van twintig elk grangskik. Twintig blokke het die vyf paraffienbehandelings ontvang en twintig die vyf gedistilleerde waterbehandelings. Die behandelings is vier keer herhaal. Binne elke blok is die behandelings ewekansig toegeken aan persele. Die proefontwerp was 'n 2X5 ewekansige geneste blokontwerp (split plot) wat faktoriaal

gerangskik is met faktore Behandelingsvloeistof (gedistilleerde water en paraffien) en indompelingstyd (0, 1, 5, 10 and 30 minute). Geen insekdoders is op die kanola toegedien nie. Plantdigtheid, blaaroppervlakte en droëmassa is bepaal tydens die eerste monsterneming 12 weke na plant en daarna is slegs droëmassa bepaal na 21 weke. Na 27 weke is finale oesopbrengs bepaal deur die persele met 'n perseelstroper te stroop. Plantdigtheid, blaaroppervlakte en droëmassa is betekenisvol verhoog deur paraffienbehandelings na 12 weke. Na 21 weke het die paraffienbehandelings egter geen betekenisvolle invloed op die droëmassa van die plante gehad nie en daar was ook nie verskille ten opsigte van finale oesopbrengs nie. Alhoewel daar nie ernstige insekskade waargeneem is nie, was dit tog duidelik dat die bietjie vreetskade wat in die waterbehandelings voorgekom het, nie in die paraffienbehandelings voorgekom het nie. Dit dui aan dat die paraffien moontlik 'n afwerende invloed gehad het. Paraffien het geen negatiewe invloed enigsins gehad op die groei en produksie van kanola in hierdie eksperiment nie.

Hierdie studie dui aan dat verskillende gewasse verskillend reageer op saadbehandeling met paraffien. Die resultate van die vierde eksperiment dui aan dat paraffien moontlik as 'n pesafweermiddel op sekere geselekteerde gewasse gebruik kan word maar meer navorsing word benodig op die onderwerp.

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Dedication

To my beloved family, I dedicate this thesis. It is not easy to rebuild in the ruins and it is not simple to jump in race competition after more than ten years in a confined space. Only the hand of Elohim can sustain your effort and your zeal to finish the race. The journey has been long. I remember that on every rainy and foggy day, our hope was louder, shouting an assurance of sunshine. While crossing a tunnel, the echo and fear had and still has dominion over us making us feeling powerless, lost, small and forgotten by the normal world. Keep not being worried; Elohim said by Himself that we will not go back to Him before we see what He promised us on earth. Out of love and high esteem, we humbly thank Him (Hebrews 11:1).

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List of Abbreviations

ATSDR   Agency for Toxic Substances and Disease Registry

CR   Conditional Response

CS   Crop Specie (s)

Co S   Conditional Stimuli

EC   Electrical conductivity

ECHCDG   European Commission Health and Consumers Directorate-General

EFSA   European Food Safety Authority

GI   Germination index (indices)

HSD   Honest Significant Difference

ISTA   International Seed Testing Association

ISTA   International Seed Testing Association

OJEU   Official Journal of the European Union

PAH   Polycyclic Aromatic Hydrocarbon (s)

PC   Paraffin concentrations

PC   Paraffin concentrations

ST   Seed type

TOI   Time of immersion

UR   Unconditional Response

USEPA   United States Environmental Protection Agency

Chapter 1

General Introduction

1.1 General

On the farm, crops are exposed to diverse destructive factors (Rahman 2003). In his capacity and knowledge, the farmer tries to stop and eradicate crop predators and the diseases using different methods such as uprooting the attacked plants, applying chemicals and rotating different plants (Panagiotakopulu et al. 1995). The choice of method depends on its efficiency, the crop to be protected and side effects of the chemicals on humans or the environment (Chitwood 2002). Amongst those methods, agrochemicals are the most used. They are selected due to their low cost and the guaranteed action (Isman 2006, Bhattacharyya et al. 2009). Pesticides kill the organisms which destroy crops by feeding on them and acting as a vector of diseases, but pest repellents rather deter those organisms only. Although famers would opt for eradication methods, environmentalists welcome repelling methods and those pesticides intercept organisms because they do not destroy the phytophage (Avery et al. 2001).

Due to the unavailability of conventional pesticides and repellents in central African regions (Agricultural Development Economics Division 2009), small farmers struggle and resort to household chemicals at hand such as soap, paraffin and chili (Stonehouse et al. 1997, Wale 2003). The application of paraffin used on soya beans as dressing product directly before planting has produced good results in the South West of Rwanda, Democratic Republic of the Congo (DRC) and in Malawi (Personal observation). Slugs, snails, insects or birds did not attack soya bean crops while still in the ground or after germination. Harvested seeds did not smell of paraffin and there was not any distinctive indication or complaints about any paraffin residue detected on the leaves or in the seeds by the consumers or farmers.

However, this practice of coating seeds with paraffin has raised concerns for some because it is applied on the seed and may affect germination and seedling growth which are the most vulnerable stage of plant growth (Sheppard et al. 2011). Chemical composition of paraffin, its general properties, and its behavior in contact with a plant have been described (Irwin et al. 1997, Tidd 2011), as well as the interactions between paraffin and plants in the establishment and the early growth stages (Irwin et al. 1997, Vandevivere and Vertstraede 2001, Wyszkowska and Kucharski 2000, Robson 2003). This study was therefore initiated to

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investigate the effect of paraffin on seed germination and early seedling vigour of several crop plants.

1.2 Objectives

The main objective of this study was to investigate the effect of paraffin on seed germination and seedling vigour in seven crop species. The species investigated were canola (*Brassica napus* L.), common beans (*Phaseolus vulgaris* L.), ground nuts (*Arachis hypogea* L.), maize (*Zea mays* L.), soya beans (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L.). The secondary objective of the study was to investigate the efficiency of paraffin as phytophage repellent on crops that show good germination and seedling vigour after treatment with paraffin.

1.3 Thesis outline

The format of this thesis follows the editing instructions of the South African Journal of Plant and Soil. It is composed of six chapters. Chapter 1 introduces the thesis and highlights the main objectives of the research conducted. The second chapter consists of the literature review. The four following chapters focused on the research results. Chapter 3 investigates the germination of crop seeds treated with paraffin in an incubator. Chapter 4 focuses on the germination and development of seedlings of different crops after treated with paraffin in the glasshouse and Chapter 5 describes the attempt to find paraffin residues within the seedlings grown out of the seed imbibed with paraffin. Chapter 6 investigates the establishment and growth of canola under field conditions after treated with paraffin and Chapter 7 summarizes the main findings and draws conclusions on the study.

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Chapter 2

Literature Review

2.1 History of Paraffin

A Persian scholar named Rāzi (or Rhazes), in the 9th century, attempted an elementary distillation from coal tar, which generated the concoction. He named it white naphtha (Tidd 2011). His work was published in *Kitab al-Asrar (Book of Secrets)*. His archaic method used clay or ammonium chloride accompanied by a repeated distillation process to purify the paraffin. Many experimental trials for proper paraffin isolation continued and in 1854, Abraham Gesner succeeded to distil paraffin from bituminous coal and oil shale on experimental basis (Chilcott 2007).

Commercial production of paraffin commenced after 1854 in Charlottetown in Canada to replace whale oil, which was used as lamp oil. Gesner named it Kerosene a contraction of *keroselaion* "wax oil" (Ripley and Dana 2009). In the same period, the Scot James Young conducted a distillation from the seep of coal and extracted a mixture of resinous liquids having oil lubricating and illuminating properties (Chilcott 2007). He called the mixture "paraffin oil" because it tended to congeal into a substance resembling the then known paraffin wax at low temperatures. From two different geographical areas, Young and Gesner had isolated the same product. Paraffin oil or kerosene started to be sold into the market to the public and to companies, and its properties leading to various uses increased research interest in paraffin (Tidd 2011). Names like Kerosene or simply Kero, Paraffin oil, Paraffin, Petroleum based oil, Pyrethrum extract solvent, deodorized base oil, Range oil and Kerosine was used but the most preferred names are kerosene and paraffin (Ripley and Dana 2009).

2.2 Properties

Paraffin is one of the products obtained by fractional distillation of petroleum or crude oil between 150 and 275^0C (Tidd 2011). It is a mixture of hydrocarbons where each molecule contains between 10 and 16 carbons. The major components are alkyl benzenes, naphthalene and *n*-dodecane, with their derivatives. Many authors agree on different components of paraffin as a mixture but disagree on the percentage composition. Irwin et al. (1997)

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proportioned alkanes and cycloalkanes at 68.6%, benzene with substituted benzene at 13.7%, naphthalene and other aromatics at 17.7%.

At normal temperatures, paraffin is liquid with a clear pale yellowish colour. Its volume mass of 0.78- 0.81 g.cm^{-3} with its non-polarity makes it immiscible with water (Leifer 2006). Paraffin and diesel have the same components but only differ in terms of proportions of concentrations of the components. Diesel fuel is a mixture of 64% aliphatic hydrocarbons in the C_{10} to C_{20}, 1 to 2 % olefinic hydrocarbons and 35% aromatic hydrocarbons (Irwin et al. 1997, Leifer 2006).

2.3 Paraffin and health

Paraffin was later replaced by medicine with less negative side effects, but it continued to be used as vermifuge in the early period of its discovery (Wilson 2011). Today it is one of the raw ingredients used to make some pharmaceutical chemicals (Wilson 2011). Many people in rural areas affirmed that paraffin is good medicine to use as therapy for pain relief. It is also used for the treatment of hemorrhoids, ailments, as an anti-constipation, as a vermifuge and also to combat toe and nail fungi (Sharif et al. 2001). Paraffin has been a regular household and industrial product being used to heat houses in winter, for lighting and food preparation. However, little is known about the negative impact on human health, plants and animals. There is no evidence of any cancer resulting from paraffin usage (EFSA 2012). Greece was commissioned by the European Union to investigate the detrimental effect of paraffin as a chemical used in the agro industry in 2005 (ECHCDG 2011). Findings were as follows: "For paraffin oil of high purity no toxicological concern is raised as regards to exposure to humans" (OJEU 2009). However, the commission regulated that the maximum concentration of 5.2- 16 litres mixed in 800 to 1000 litres of water be sprayed on one hectare of crops with minimum application interval of 15 days (OJEU 2009, Vassiliou 2009, EFSA 2012).

The Safety data sheet report issued by Nynäs (Swedish oil company), the largest Swedish Petroleum firm, highlights that paraffin does not show any effect of mutagenicity and teratogenicity or any toxicity on fertility. There is no critical hazard or known significant damage caused by paraffin once technical rules of transportation, storage and disposal are observed (Wyszkowska et al. 2002, NYNÄS 2011). Paraffin is also a stain remover and rural families use it to take out stains from fridge linings or sinks (Wilson 2011).

Nonetheless, paraffin has occasionally caused various types of damage because it was carelessly handled. In some parts of Asia, particularly in India, and in Africa, investigations

and surveys proved that houses caught fire due to flames ignited by spilled paraffin. Some children have experienced lung injuries caused by negligence by adult guardians who did not dispose of paraffin or did not keep it out of reach of children (Abed et al. 1998). Prolonged skin contact with paraffin may cause a kind of dermatitis but this is reversible once paraffin exposure stops (Leifer 2006). When used as jet fuel, it can cause carbon monoxide poisoning resulting from incomplete combustion (Leifer 2006).

2.4 Paraffin and the environment

The natural ecosystem is threatened by industrial chemicals, which are partially or not completely biodegradable (Maila and Cloete 2002, Kathi and Khan 2011). The biosphere and the atmosphere are at high risk, because of harmful xenobiotic substances added (Wyszkowska et al. 2002, Angela et al. 2011), disrupting the natural ecological equilibrium (Baek et al. 2004, Wiens et al. 2010). Plants and animals are killed, some to the point of extinction. Humans are the cause of this disorder (Agarry et al. 2010). In their investigation, Vandevivere and Vertstraede (2001) found that at least 35 000 sites in Western Europe are contaminated where petroleum based hydrocarbons compose the major part of the contaminants (Troquet et al. 2003). Petroleum derivatives such as engine oil, paraffin and plastics are amongst the main pollutants of the soil. Currently, measures to reverse the process are underway (Baek et al. 2004).

Bioremediation and/or phytoremediation are the main rehabilitation techniques of disturbed systems. The damage amplifies with wrong or improper handling of the substances (Asli and Houshmandfar 2011). This investigation focuses on paraffin, how it affects water but mainly how it impacts the soil where it spills or is improperly disposed of. Paraffin as liquid in normal conditions is obtained by fractional distillation of petroleum. Paraffin does not possess hydrophilic sensitive groups which would facilitate hydrolysis in the environment.

2.4.1 Paraffin in water

Immiscible in water with a density of $0.78 – 0.81$g cm^{-3}, paraffin floats on top of water (Tidd 2011). Paraffin is released in water from leaked transport facilities, runoff from factories and workshops where it is used as solvent or fuel, and from intentional disposal in water drains (ATSDR 1995). Only a continuous, non-centred agitation and vigourous movement can produce a suspension which will distinctly separate in less than two minutes once the agitation stops. Experience showed that paraffin spilled in ponds spreads over it, resulting in

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a micro layer with a thickness that is proportional to the quantity of paraffin and the size of the pond. This layer prevents atmospheric oxygen from mixing with water (Risher and Rhodes 1995). Water disturbance by the wind remains a vital phenomenon in providing oxygen needed by aquatic life. Thus a paraffin layer on water renders life almost impossible by causing asphyxia (ATSDR 1995).

Wrongly disposed paraffin may, in the long term, become a threat by contamination to ground water, marine environment, plankton sediments and fish (Sengupta et al. 1993). Irwin et al. (1997) suspected that some light water soluble compounds in the mixture such as benzene and xylenes are the most detrimental. Some volatile components might be found in precipitations causing air pollution, which may be detrimental in the long term (Irwin et al. 1997, Petukphov et al. 2000). Also a long exposure to paraffin spills in water could result in oil coating of sea birds, fish and sea otters, causing acute toxicity (Irwin et al. 1997). Fortunately, Buckley et al. (1976) found that amongst microorganisms in water, 63% of bacteria and 71% of fungi were able to degrade paraffin using it as sole carbon source. This vital process of biodegradation starts with short carbon chained components and is enhanced by water temperature increases.

2.4.2 Paraffin in the soil

Soil condition was in the past neglected by mining industries, manufacturing firms and even by those who work in diverse public works. Ignoring that the soil sustains life on earth was historically a great mistake that was pointed out by researchers and environmentalists. Such environmentalists are in a permanent "watchdog state" against the offenders who seem to ignore the long term impact of their actions (Shabir et al. 2008). This section will investigate the behaviour of paraffin in the soil and its effect on the fauna and flora.

Paraffin is found in the soil mainly because it has been wrongly handled, spilled accidentally or due to escape from leaking tanks above- or underground (Risher and Rhodes 1995), recklessly disposed of in open areas or in water drainages. The general properties of the soil such as texture, porosity, permeability, percentage of moisture, and the chemical composition of the soil influence its reaction to paraffin as a pollutant (Risher and Rhodes 1995). In return paraffin, as a water insoluble product, changes the original soil properties and thus impacts on aerobic organisms and plants roots in the soil (Guiteras et al. 1998).

Soil texture and structure are altered proportionally to the quantity of oil spills and frequency of deposition in that soil. Life in such altered soil is also disturbed to the extent of coming to a complete halt (Sharonova and Breus 2012). Some species of microorganisms,

plants and insects disappear and seeds of planted crops or weeds fail to germinate. Such a site is declared dead till a natural or semi natural ecological colonisation and system reconstruction take place (Wyszkowska and Kucharski 2000). This natural recovery system takes too long and sometimes is interrupted by repetitive spills. Intensive research aiming to recover the equilibrium of arable soil using selected plants capable to resist and degrade long hydrocarbons into small chains is in place and is called phytoremediation (Wyszkowska and Kucharski 2001).

Investigations were carried out to determine the dissipation and the redistribution of petroleum pollutants in the soil and the findings of Rubin and Narkis (2001) on the Mediterranean coastal side of Israel showed that paraffin and its residues migrated in depth from the surface where it was spilled and that migration was activated by irrigation or rain. The investigation showed that on some sites the paraffin concentration was 2300 to 4500 μg per gram of soil for the first 40 cm from the surface. Under irrigation of 1000 mm and within 180 days, the chemical analysis found 500 μg of hydrocarbons per gram of soil on the surface of the soil and 150 μg paraffin per gram of soil in the deeper layers from 40 cm downwards (Rubin and Narkis 2001). Conclusively the amount of 400-3850 μg of paraffin was dissipated in the soil. This redistribution and dissipation is enhanced by multi-process integration approaches (Kathi and Khan 2011) mainly biological, activated by physical and chemical factors of the soil (Rubin and Narkis 2001).

Barasubramaniyam (2012) explained that some paraffin components are bound or sorbed to different soil components such as iron oxides, clays and organic matters. The increase of organic matter remains one of the major factors allowing the soil to break down paraffin as an intrusive chemical in different short chained chemical such as CO_2 and H_2O normally available in the ecosystem (Smith et al. 2006). Once deposition is not repeated, the soil is reconstructed and the soil-plant equilibrium is re-established. Nevertheless, continuous addition of paraffin pollutants in the soil ends up prevailing over the influence of above-mentioned factors changing the soil into a dumping site of paraffin or its derivatives.

2.4.3 Paraffin and its biodegradability in the environment.

The majority of hydrocarbon or petroleum derivatives are environmentally recalcitrant and may be found in a place of dumping after years causing deleterious consequences to the environment (Agarry et al. 2010). This recalcitrant behavior increases with the length of molecular chains and is due to the absence of natural organisms to break polycarbonic chains. It is known that fungi and bacteria constitute the initial base of the degradation chain.

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However studies concluded that some specific fungi and bacteria developed associations with angiosperms to accelerate hydrocarbon degradation components (Sharonova and Breus 2012). These angiosperms secrete chemicals that stimulate microbial activities and facilitate the degradation of hydrocarbons (Robson 2003). Some plants are capable of absorbing and metabolizing specific hydrocarbon products, including paraffin components (Adekunle and Adebambo 2007, Sharonova and Breus 2011) at different rates. Robson (2003) identified sunflower (*Helianthus annus*), and saskaoon berry (*Amelanchier alnifolia* Nutt.) and Jonescou (1979) mentioned slender wheatgrass (*Artemisia frigid* Willd.), wild barley, yellow sweet clover *(Melilotus officinalis* (Pursh) Dunal)), and many asteraceae species as plants likely to grow on abandoned coal mines in the South-East of Canada where residues of hydrocarbons are still present.

Bacteria play an important role in degrading complex organic molecules into simple ones, thus helping in diverse important biological cycles (Ogbo et al. 2010). They are diversely adapted to aerobic and anaerobic media. Within distinctive genera, some species specialize in colonizing paraffin as a feeding medium. Major Paraffinophile groups and their species are *Acinetobacter calcoaceticus, Pseudomonas aeroginosa, Pseudomonas balearica, Rhodococcus* (Sheppard et al. 2011) and *Bacillus* species *such as B. subtilis, B. amyloliquefaciens, B. pumilus, B. megaterium, B. agri, B. sphaericus, B. pabuli* and *B. polymyxa* (Van Gestel et al. 2003). Pioneering for bioremediation, Rynearson and Peterson (1965) isolated 13 species of *Aspergillus,* five species of *Chaetomium,* two strains *Penicillium, Trichoderma* and *Stemphylium,* with single species of *Cunninghamella, Hormiscium* and *Syncephalastrum* capable to grow on paraffinic media. Table 2.1 gives a list of bacteria and fungi that can degrade hydrocarbons.

Table 2.1: Species of soil microorganisms capable of degrading hydrocarbons (Adapted from Robson 2003)

Bacteria		Fungi		
Achromobacter	*Nocardia*	*Acremonium*	*Fusarium*	*Polyporus*
Acinetobacter	*Proteus*	*Aspergillus*	*Geotricum*	*Rhodotorula*
Alcaligens	*Pseudomonas*	*Aureobacidium*	*Gliocladium*	*Saccharomyces*
Bacillus	*Rhodococcus*	*Beauveria*	*Graphium*	*Sclerobasidium*
Brevibacterium	*Sarcina*	*Botrytis*	*Humicola*	*Porobomyces*
Chromobacterium	*Serratia*	*Candida*	*Monilia*	*Spicaria*
Corynebacterium	*Spirillum*	*Chrysosporum*	*Motierella*	*Sporotrichum*
Cytophaga	*Streptomyces*	*Cladosporium*	*Paecilomyces*	*Syncephalastrum*
Erwinia	*Vibrio*	*Cochliobolus*	*Penicilillium*	*Tolypocladium*
Flavobacterium	*Xanthomonas*	*Cunninghamella*	*Phoma*	*Torulopsis*
Micrococcus		*Cylindrocarpon*	*Phanerochaete*	*Trichoderma*
Mycobacterium		*Debaryomyces*	*Plerotus*	*Verticillum*

The degree of degradation is proportional to the speed of multiplication of these paraffin degrading microorganisms and the multiplication depends on how they persist in the environment. Two specific strains of fungi *Aspergillus sulphurous* and *Aspergillus ustus* are called paraffinolitic fungi as they are capable of growing solely on paraffin, using it as the exclusive carbon source (Rynearson and Peterson 1965). Paraffin spilled in limited non-repetitive quantities is not regarded as a polluting threat to the environment due to it being recycled by the microorganisms available in the soil (Barasubramaniyam 2012).

2.5 Dynamics of seed germination

The seed in a silo is in a state of dormancy (Roberts 1986). The seed undergoes dormancy if it has attained its physiological maturity characterized by maximum dry weight and maximum vigour (Mejia 1985). With such an ultimate physiological stage of seed development, a series of metabolic processes such as maturation of the embryo to the

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capacity of germinability, cessation of metabolic dependence from the mother plant (Hilhorst and Toorop 1997) and cessation of synthesis of reserves (Harris 1987, Kelly et al. 1992) take place. It is linked to the conditions of the environment. Due to water loss, the seed volume reduces, the seed coat hardens and internal seed substances become more concentrated (Harris 1987). Such a seed is being prepared for future independent life (Hilhorst and Toorop 1997) leading to a new plant by germination. Germination is thus defined as the total of preceding processes that reactivate the matured seed and cause the embryo to start developing till its radicle protrudes and becomes visible. The seed's environment plays a crucial role since the factors which trigger changes of transformation towards germination come from that environment (Rolston 1978).

2.5.1 The seed coat and its properties.

A normal seed which achieved its maturity has an outer covering called the seed coat (Dübbern de Souza and Marcos-Filho 2001) also referred to as the testa (Chrispeels and Sadava 2003). The seed coat protects the embryonic root called the radicle, the plumule and the food reserve tissues named endosperm which are general essential parts in the grain. The seed coat contains the hilum, a scar like specialized area by which the seed was attached to the ovary and micropyle, a tiny hole in the testa near the hilum through which water reaches the embryo and where the radicle will grow out during germination (Roberts 1986). Figure 2.1 shows a longitudinal cut slice of the common bean the position of the embryo and some of its sites susceptible to facilitating water penetration into the seed.
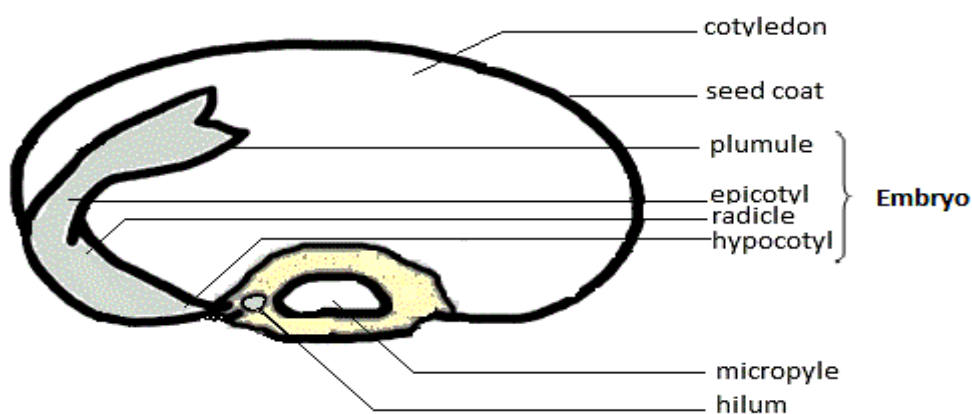

Figure 2.1 Seed structure of the common bean dissected symmetrically, showing the embryo and its components adapted from Műller et al. (2006).

All interactions between the seed and the environment are done via the seed coat (Dübbern de Souza and Marcos-Filho 2001). From seed to seed, from one individual of the same variety to another, seed coats differ in constitution, hardness, thickness, size, form and colour (Mohamed -Yasseen et al. 1994, Büyükkartal et al. 2013). The structural analysis shows that it is composed of four layers as it is shown in Figure 2.2. The waxy cuticle layer, light line or lumen palisade air space or intercellular space and parenchyma are adjacent to and in contact with the endosperm and constitute the set of barriers that play a selectivity role to allow any exchange with the seed environment (Dübbern de Souza and Marcos-Filho 2001).

The properties of the seed coat are derived from its physical and chemical composition (Mohamed-Yasseen et al. 1994). Some seeds are considered to be soft-seeded while others are hard-seeded. Büyükkartal et al. (2013) studying *Pisum sativum* concluded that the mechanical strength of the seed coat is based on macrosclereid and osteosclereid layers and its hardness is determined by the thickness of the cuticle, the size of the macrosclereids and the development of the outer wall of the light line. Seed coat permeability is independent of the hardness of the seed in the same species, but it is rather dependent on the chemico-structural differences between seeds (Büyükkartal et al. 2013). The seed coat must be semi-permeable allowing respiration during the seed's quiescent life (Sousa et al. 2012) even when it is considered impermeable. However Kelly et al. (1992) wrote that the seed coat may impose dormancy on the seed by mechanical prevention of the radicle extension, the presence of inhibitory substances and its resistance to water and gas permeability.
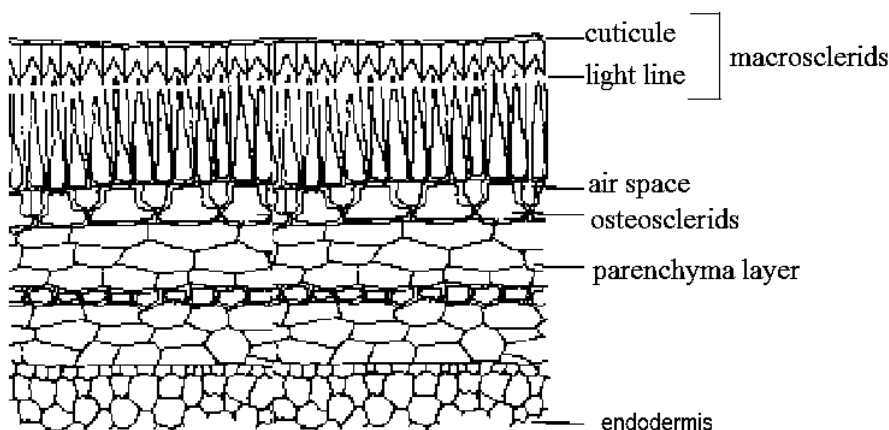

Figure 2.2 Longitudinal section of the seed coat of sweet clover from Martin and Watt (1944) cited by Rolston (1978).

The environment harbours adverse destructive factors like bacteria, fungi, viruses, chemicals and micro-climate (Mohamed-Yasseen et al. 1994, Mattews and Powell 2006). The seed coat is a physical and chemical barrier against exterior harmful factors protecting the seed as a whole in general and the embryo in particular. Once damaged, the seed coat is no longer able to resist fungal or bacterial infections, which reduce the viability of the seed (Mohamed-Yasseen et al. 1994).

2.5.2 Seed germination

Naturally, the specific role of the seed is perpetuating life in time and space by germination. Therefore a good quality of seed pertain a high yield (Miloševic et al. 2010). It marks a start of modern plant production, either in field or in vegetable crops. During the germination process, seeds abandon the dormancy state to become active (Hilhorst and Toorop 1997) and to develop into future plants. Sousa et al. (2012) wrote that water is the most important factor influencing germination and proceeding with the remaining metabolic processes. Water decisively causes biochemical reactions such as hydrolytic digestion of reserve tissues, solubilisation of metabolites, their distribution and an increase of respiration ratio within the seed (Beeckman et al. 2000). Empirical observations by Mei and Song (2008) show an increase of the respiration rate up to 40 times at 30^0 C for the first thirty hours of maize seed imbibition in comparison to dry seeds (Koorneef et al. 2002). Every type of seed has a minimum of water uptake required to activate metabolites responsible for the germination process (Sousa et al. 2012).

The Gibberellin hormone levels in the seed increases thus ending dormancy by triggering genes responsible for the growth of the embryo (Roberts 1986, Hilhorst and Toorop 1997). The embryo, having absorbed enough water, manifests two major changes: elongation of the radicle and initialization of plumule development. Many authors affirm that the seed coat tearing by the radicle and its protrusion marks the end of germination giving place to the establishment of the seedling (Koorneef et al. 2002, Sousa et al. 2012). Nevertheless farmers keep defining germination as the number of sprouts appearing from the surface of the seedbed (Chrispeels and Sadava 2003). The biochemical reactions occurring during seed germination and seedling development require specific conditions: optimum temperatures, quantity of water, presence or absence of light and air for respiration (Luhach and Chaudry 2012) and seed vitality (Black et al. 2006).

Seed science embarked on minimizing any seed related negative factors tending to impact on the yield. Determination of seed quality for the market requires rigorous tests as stipulated

in the rules of the International Seed Testing Association (ISTA) to ensure a disease free and satisfactory production (Taylor 2009). According to McDonald (2000) these tests may be grouped into three categories: physical (size and mass), physiological (using germination and growth parameters) and biochemical (Tetrazolium test, conductometric mesurements, enzyme and respiration activities). As this chapter introduces experiments investigating paraffin action on crop seed germination, a brief comprehension of some of these tests relating to seed coat such as germination tests and conductometric measurements is pertinent. By comparing the total percentage of germination and other parameters such as speed of germination, length of radicles, length of stems and roots, leaf surface areas and dry masses, it can be determined where the process of germination is handicapped or what are best conditions for germination.

2.5.3 Electrical conductivity

It was mentioned that damaged seeds become vulnerable. Damage may be the result of injuries caused by the farmer during different mechanical manipulations. Some injuries may be caused by different chemicals used to protect the seed from different pests (Milosevic et al. 2010). Damage may be occasioned by age which physiologically destroys or weakens the electrolytes retention capacity of the seed (Tajbakhsh 1990). All these damage mechanisms destroy intrinsic properties of the seed coat which is normally the mechanical and biochemical barrier of the seed. Electrical conductivity (E.C.) is one of the reliable methods available to test the viability of the seed and also the soundness of the seed testa.

To determine E.C., accurate data are recorded after about 16 to 24 hours of imbibition, on seeds that originally had less than 20 % moisture content (Sørensen et al. 1996). The containers must be glass or plastic, washed and rinsed with de-ionized or distilled water, covered with plastic films or lids to avoid dust or evaporation. Electrical conductivity is calculated in micro-Siemens per centimetre per gram ($\mu S \ cm^{-1} \ g^{-1}$). The difference of readings below 30 $\mu S \ cm^{-1} \ g^{-1}$ between two replicate must not exceed 4 $\mu S \ cm^{-1} g^{-1}$ and above 30 $\mu S.cm^{-1} \ g^{-1}$ the difference should not exceed 5 $\mu S \ cm^{-1} g^{-1}$ (Sørensen 1995). The water used in the experiment must be de-ionized or distilled water having been exposed in the room of analysis less than 20 ºC for 24 hours with an E.C. reading of less than 5 $\mu S \ cm^{-1} g^{-1}$. Generally, dry seeds immersed in pure water simultaneously experience a rapid water uptake and a leak of organic and inorganic solutes from inside the seed. Tests showed that the inorganic solutes leaked are phosphate salt based and organic solutes are made of organic acids, amino acids, lipids and sugars (Tajbakhsh 1990). The E.C. observed during the

imbibition period originates from those solutes which act as electrolytes (Bewley and Black 1994). As the imbibition time continues, solute leakage is minimized by the re-establishment of the membrane. That membrane re-establishment is assumed to be a bio-physiological phenomenon because it was proven that more viable, young and good quality seeds leaked less solute than bad quality, aged or dead seeds (Mattews and Powell 2006, Miloševic et al. 2010, Salinas 1996).

2.6 Seed germination in a paraffin contaminated medium

The germination process and its conditioning factors have been intensively researched (Angela et al. 2011, Sheppard et al. 2011). Any variation of these determining factors causes a major impact on the percentage of germination, the degree of establishment, vigour of the seedling and consequently the growth of the new plant and production in general. Besides natural germination factors, oil spills as contaminants of have effects both on the soil and on the seed (Barasubramaniam 2012).

Though oil spills are of various categories, this study focuses on investigating the effect of paraffin on seeds. Unfortunately the effect of paraffin on plants is not as well documented as other related petroleum products such as diesel (Van Gestel et al. 2003). The behaviour of paraffin contamination in the soil is presumably the same as diesel, since the only difference between both products is the concentration of mixture components. As diesel toxicity on plants is extensively researched, it is expected that paraffin would be less detrimental compared to diesel because it contains less naphtalenic products, which are the most important seed damagers (Robson 2003).

In general when a seed is washed with paraffin, a film of molecules of the components of paraffin is formed on the surface of the seed, resulting in a physical barrier for water and oxygen uptake (Luhach and Chaudhry 2012, Sousa et al. 2012), which lowers osmotic potential, thus inhibiting or slowing down germination activity. Water soluble components of the paraffin mixture are able to pass through the seed coat and may become toxic to the seed embryo (Henner et al. 1999) inhibiting amylase activity and starch phosphorylase (Achuba 2006), two of the major biochemical activities responsible for providing energy during germination. Some species manage to resist the influence of paraffin and germinate. Germination on paraffin contaminated medium, either in clean sand in a glass house or distilled water in the petri dish proves to be slower than in non-contaminated media (Barasubramaniam 2012).

It is difficult to compare the seeds planted in a paraffin polluted soil and the seeds washed in paraffin before they are planted in a pollution free soil. In both cases the seed has to resist the paraffin influence to germinate and grow but understandably the polluted soil would cause more damage to plants due to the regular exposure of the roots to paraffin. The residue of paraffin stuck on the seed surface during seed imbibition is quantitatively insignificant for continuous impact on growth. Radicles and roots are the most affected parts of the plant as they are in permanent contact with the contaminant (Baud-Grasset et al. 1993). The above ground plant parts, which are dependent on the root system suffers consequences such as chlorophyll degradation (Malallah et al. 1998) and disturbance of stomatal mechanism, leading to reduction of photosynthesis in its different stages and respiration (Baker 1970). The plants used in phytoremediation have a capacity to develop some resistance allowing them to metabolize foreign chemicals in their system. Some of those plants have developed stress related phytohormones, toxic sequestering systems and secreting of chemicals to invigourate remedying microorganisms in the soil (Petukphov et al. 2000). Research on cowpeas sown in paraffin contaminated soil showed a reduction in germination percentage, the leaf surface and the height of the stem. This is due to a shortage of available nitrogen for plants in the soil (Agbogidi et al. 2006) and a disruption of nutrient absorption capacity by the roots caused by paraffin (Wyszkowska and Kucharski 2000). The reaction of degradation of paraffin disrupts the water balance in the soil and results in depletion of phosphorus (Baran et al. 2002)

Species resist pollutants differently but generally high concentrations of petroleum derivatives; paraffin in this case, have negative impacts on the germination of seed and development of new plants (Luhach and Chaudhry, 2012). Depending on how seeds and seedlings can resist contaminants in the media, a scale can be established. That scale could help not only to collect species, varieties or cultivars which could be used in bioremediation, but also to evaluate the degree of toxicity of pollutants in the soil. Sharonova and Breus (2012) rated the plants to be more tolerant when seeds germinated in a polluted soil are above 70%, less tolerant at 30-70% and intolerant under 30%. Soil pollution with paraffin is considered low at less than 2% paraffin concentration; average at 3-10%, high at 10-15% and very high over at 15% (Sharonova and Breus 2012). The extent of toxicity varies with the variation of pollutants in the environment and increases when more hydrocarbon chemicals are dumped in the environment. A decrease in toxicity may occur when the pollutant is reduced either by being removed, by vertical erosion taking it into deep strata on the site or

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simply by an intensive bioremediation action converting it into site usable simple chained chemicals (ATSDR 1995).

2.7 Paraffin as a repellent

Crops constantly exposed to different types of weather conditions suffer invasion from an array of crop pests and farmers would use anything to save their crops (Martin 2012). Birds, mammals, insects, nematodes, mollusks, viruses, bacteria and fungi alternate to try to feed on crops and some would persistently remain on the farm for years till thorough methods of eradication are applied (Riedle-Bauer et al. 2011). Chemicals used as pesticides proved the most efficient means of eradication in recent years, (Bhattacharyya et al. 2009). However, environmentalists and nature conservation specialists advocate for preservation of crop parasites and urge farmers to use product repellents (Whitford et al. 2003).

Welch (1967) defines a chemical repellent as any material which will reduce or eliminate predation through odour, or possibly irritation, when applied to seeds, plants or other materials being damaged by animals. This definition is supported by the fact that some animals are repelled by light with specific characteristics or some devices emitting waves or sound, proven to be irritable to predators or parasites. Paraffin is one of the household commodity chemicals that have been used as a pesticide to repel parasites, to kill insects but mainly as carrier for other chemical insecticides since its discovery (Holmes 1992, Isman 2006). The next few pages elucidate areas of efficacy of paraffin as a chemical that is used or can be used on the farm, due to its repellent properties.

2.7.1 How does a repellent work in general?

The action of a repellent is mainly based on the experience acquired by an animal which would define its ecological behaviour (Werner and Clark 2003). In nature, the grazing springbok develops some preferences for species of grazing grass. When the preferred species are not available, it would live on less preferable ones but there are some species instinctively considered unpalatable containing chemicals which may be poisonous to springbok or disturb its physiological system (Avery et al. 2001). A repulsive force acquired in the nervous system of the springbok directs that selection when it is grazing. The interactive action of senses (e.g. sight, touch, taste or smell) works in a synchronized manner to incite a feeding habit of the phytophage (Rogers 1974, Riedle-Bauer et al. 2011).

Researchers distinguish primary and secondary repellents defined according to the mode of reaction of the animal towards the repellent.

Inherent properties of primary repellents such as taste, irritation or intrinsic odour, provoke a kind of withdrawal reflex or escape behaviour within the parasite. In this case, the parasite may not have learned target-oriented avoidance (Werner and Clark 2003). If there is no evolved association with the repellent and escape or withdrawal actions, the parasite would continue its cycle of destructive sampling behaviour. Such a discontinued consumption of food is characterised by repetitive avoidance associated to an aversive stimulus without any total and complete avoidance (Clark 1998).

This type of repellent does not protect crops because parasites do not accumulate or interpret negative experience encountered but always attempt to taste, to the extent of building resistance and adapting to negative stimulus (Willis and Wilkie 1999). Figure 2.3 shows that the parasite instinctively reacts to the external effect by a responsive action but does not show any learned avoidance. It simply means that the parasite will repeat its action without any remembrance of the negative experience.

Secondary repellents are characterised by four critical elements and a learned avoidance behaviour as it is highlighted in Figure 2.3 (Werner and Clark 2003). The first element which is the beginning of the whole repelling system is the active factor which deters the parasite. It is considered as unconditional stimulus (US) eliciting an unpleasing experience, which triggers the second element named unconditional response (UR) involuntarily within the parasite. The parasite associates the UR with sensory cues or conditional stimuli in space and time (CoS) highlighted as the third element leading to formation of conditional response (CR), the result of a learned avoidance. With this learned avoidance, the parasite would always associate any similar stimulus to the experienced malaise conditioning a permanent habit of avoidance response (Garcia 1989). Secondary repellents constitute efficient products worth pursuing for application in crop protection provided parasites find alternative food to divert their attention (Avery et al. 2001, Ikeula et al. 2011).

In repellents, touch and chemical senses, mean olfactory, gustatory and chemesthetic systems (irritation and/or pain) are the key paired points of parasites to be stimulated particularly in systemic repellency where the active ingredient of the repellent is mixed with the material to protect. Parasites have developed specialized neurons named nociceptors capable to provide translated information about noxious chemicals (Werner and Clark 2003). Nociceptors (specialized neurons that provide animals with information about the

noxiousness of chemical, mechanical, and thermal stimuli) provide consequential information of potential tissue damage, threat damage etc. as precursors of adaptive function within the parasite physiology (Clark 1998).
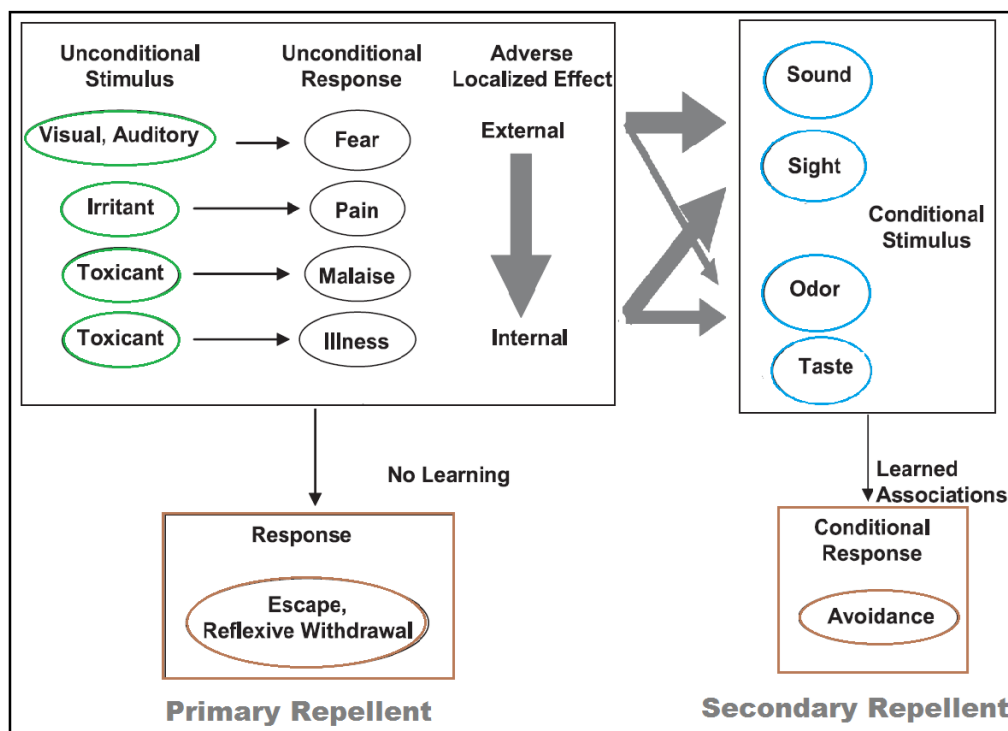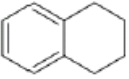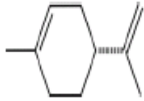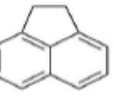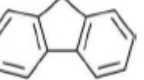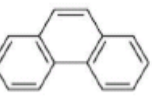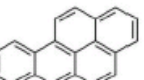

Figure 2.3: Conceptual model for repellency. Primary repellents are compounds that evoke reflexive withdrawal or escape behavior immediately after exposure. Secondary repellents are avoided because an animal associates an aversive experience (e.g. illness, pain) with a sensory stimulus. Arrow width represents relative likelihood of response stimulus association amongst species repelled. (Adapted from Werner and Clark 2003).

From different chemical structures known to repel birds, Clark (1998) developed an established modelling approach, even though repellents vary from one to another. Aromatic heterocyclic molecules containing simple acetophenone structures and nitrogens are strong avian repellents. Terpene compounds used as plant insect chemicals are good avian repellents. Chemicals containing benzene rings are potential repellents provided there is no acidic substitution on the ring. Aromatic ringed molecules contain repelling properties in general and a reaction causing the delocalization of one pair of electrons reduces the repellent effect (Schafer et al. 1983, Clark 1998). Table 2.2 shows some aromatic components of paraffin used as pesticides.

Table 2.2 Some aromatic components of paraffin used as pesticides. The aromatic structure is the source pesticide or repellency properties. (Adapted from Nabih and Metri 1973, ATSDR 1995).

NAMES		CHEMICAL FORMULA		USE	COMMENTS
Common name	IUPAC name	Molecular formula	Spatial		
Tetralin	1,2,3,4-tetrahydro naphthalene	$C_{10}H_{12}$		molluscicides	Released by paraffin heaters and automobiles. Easily metabolized by organisms
limonene	4-methyl-1-(1-ethyl ethyl)-1,3-cyclohexa diene	$C_{10}H_{16}$		d- Isomer used as insecticide/ acaricide in or on food commodities	Synthesized or extracted from *Chenopodium ambrosioides* , main component of essential oils of lemons
Acenaphy-lene	Cyclopenta[de] naphthalene	$C_{12}H_8$		pesticides dyes, and plastics	Ho harm on human, but avoid long exposure to skin and inhalation
Acenaph-thene	1,8-Ethylene na phthalene	$C_{12}H_{10}$		pesticides dyes, and plastics	Ho harm on human, but avoid long exposure to skin and inhalation
Fluorene	9*H*-Fluorene	$C_{13}H_{10}$		pesticides dyes, and plastics	Ho harm on human, but avoid long exposure to skin and inhalation
Phenanth-rene	Phenanthrene	$C_{14}H_{10}$		dyes, plastics and pesticides, explosives and drugs.	Continuous exposure is harmful to health
Pyrene	benzo[def]phe nanthrene	$C16H10$		pesticides dyes, and plastics	Ho harm on human, but avoid long exposure to skin and inhalation

2.7.2 Examples where paraffin is used in agriculture as repellent

Paraffin was experimentally used on the experimental farm of Bahir Dar in Ethiopia to control pea aphid *Asyrthosiphon pisum* (Harris) which would damage crops of grass pea *Lathyrus sativus* (Wale 2003). In Ghana, peasants use paraffin to preserve their yield of maize and cowpeas against Coleoptera seed borers as a conservation preservative, stored for consumption or seed for the next season (Osekele and Opuku-Agyeman 2005). In 2009 paraffin was counted amongst fifty prime pesticides used on nectarines as insecticide in California. While paraffin is used to repel mosquitoes at night, traditional local farmers used it as an insecticide mainly in orchards (Beattie et al. 1989).

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2. 8 Paraffin as pesticide in general

Due to varied and specific pesticides available on the market, paraffin used to be non-specific in its pesticide or repellency properties and is less exploited in this modern era. Small farmers in third world countries who practice intercropping still need to kill insects, slugs and spiders. A pollution information website, trading under the name Scorecard (2011), published a list of over one hundred registered pesticidal chemicals containing various percentages of paraffin. Najar-Rodríguez et al. (2008) researched on the mode of action of petroleum products (including paraffin (Powell 1992)) used as pest control in the farm. The emphasis was on nC_{24} hydrocarbon products. The lipophilic properties of the hydrocarbon caused its rapid penetration in the insect cuticle and its accumulation in the lipid-containing tissues which may even reach the nerve cells. Experimentally in vitro, the penetration of the petroleum product in the cytoplasm of the cell caused the death of the cell within two minutes. The advanced electrophysiological studies proved the presence of petroleum derived products in the ganglia (insect and arachnids) or in the neuromuscular junction (vertebrates). The paraffinic oil which kills the parasite within ten minutes does not exclusively affect the trachea but kill by anoxia causing unusual abdominal contraction, loss of ability to move and dehydration and necrosis. This was applied on *Spodoptera litura, Aphis gossypii , Aphis citricola, Myzus malisuctus* (Kraiss and Cullen 2008). This proves that properties of paraffin caused a shift in its role in the pesticide formulation, as emulsifier, stabilizer, dispersant and surface active ingredients rather than pesticide (Fishel 2013) or pest repellent.

2. 9 Conclusion

A good number of authors have researched the behavior of seed in a soil or in the environment permanently having a high percentage of paraffin with the attempt to find plants to be used in bioremediation (Achuba 2006, Sharonova and Breus 2012). The literature shows that paraffin has pest repellent properties (Wale 2003). As different types of parasites attack the seed and seedlings, an attempt to use paraffin could be easy to apply and try. However, it is very important to first establish whether paraffin does not cause death or injury of seeds and seedlings and eventually causing more damage than benefits due to its repellency (Robson 2003, Sharonova and Breus 2012). Furthermore, little is known on difference in sensitivity between crops and efficiency as a repellent for different pests. Thus a study of the reaction of seeds treated with paraffin and the consequences of such treatment with its possible repellency is the motivation for this research.

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Chapter 3

Effects of paraffin on water imbibition and seed germination of seven crop species

Abstract

Seed germination is a fragile stage of the life cycle of a crop plant and requires special care to protect it against predation, mainly through the use of pesticides. Many pesticides however have detrimental side effects on the germinating seed as well as on the environment. Paraffin is claimed to have repellent properties but research is needed to ascertain if it does not impact negatively on the germination and early growth of crop plants. Different crop species (CS) (canola (*Brassica napus* L.), common bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogea* L.), maize (*Zea mays* L.), soya bean (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L.)) were germinated in petri-dishes in an incubator at a constant temperature of 20ºC after being immersed in different paraffin concentrations (PC) of 0, 25, 50, 75 and 100 % paraffin for different periods of time (TOI) (1, 5, 10 and 30 minutes). Percentage germination and germination rate were calculated. In a separate experiment seeds of the same crop species were immersed in the different paraffin concentrations for 30 minutes and the amount of water and paraffin imbibed were measured.

The seeds were then subjected to an electrical conductivity (EC) test to investigate possible damage to the seeds. In terms of germination, there was a significant three-way TOI, PC and CS interaction with poor germination of beans and maize and moderate germination of groundnuts and wheat. Higher germination percentages (generally >80%) were recorded on paraffin treated canola, soya bean and sunflower. Groundnut and wheat were more negatively influenced by paraffin treatment with germination percentages below 70% and maize and common beans were severely influenced by paraffin with germination percentages generally lower than 40%. There was a two-way interaction between PC and CS on the EC with, sunflower scoring the highest mean values and wheat and maize the lowest values. Water uptake increased with a decrease in PC but paraffin uptake was only significantly reduced at the 100% PC. It therefore appears as if the different crop species were differently

affected by immersion in paraffin and for those not negatively affected, paraffin may have some potential as a pest repellent.

3.1 Introduction

Seed and germination thereof is an important stage of life for plants multiplying by sexual reproduction (Wilson et al. 1971) and its fragility requires thorough treatment and care during its dormancy or germination. In attempts to protect it, various chemicals are used. Many proved to be protective but with harsh detrimental side effects (Irwin et al. 1997). Small scale farmers find it difficult to protect seed by the conventional pesticides due to their unavailability and high prices and hence they try traditional or any household product in order to protect seeds and seedlings on the farm (Wale 2004).

The first documented investigation on the effect of paraffin on seed germination was conducted on maize in 1934 in New Zealand (Irwin et al. 1997). Subsequently such investigations were abandoned due to the perception that paraffin toxicity killed the seed. This quick rejection was later refuted as many species had to be investigated in order to determine which species could be used in remediating sites polluted by hydrocarbon products (Agarry et al. 2010). Amongst those hydrocarbon products, paraffin was one of the most common products in regular use, likely to be found on sites where farming of different plants is done.

Paraffin was experimentally used on the experimental farm of Bahir Dar in Ethiopia to control pea aphid *Asyrthosiphon pisum* (Harris) that would damage crops of grass pea *Lathyrus sativus* (Wale 2003). In Ghana, peasants use paraffin to protect their yield of maize and cowpeas against Coleoptera seed borers when stored for consumption or seed for the next season (Osekele and Opuku-Agyeman 2005).

Paraffin is a mixture of different hydrocarbon products with between 10 and 16 carbons in the chain (Tidd 2011). Molecules of the components do not have any water sensitivity and this causes paraffin to be a hydrophobic product immiscible with water. As water is one of the determining factors of seed germination, the question arises whether paraffin properties may not interrupt water uptake by the seed or disturb other important biochemical reactions taking place during the germination or growth process. Sharonova and Breus (2012) found that the effect of paraffin on plant growth was not as detrimental as was claimed by Luhach and Chaudhry (2012).

In order to investigate the feasibility of treating crop seeds with paraffin as a pest repellent, the effect of paraffin on seed germination was investigated. The main objective of the study was to determine if paraffin has a negative effect on germination of seeds of several crop species. The secondary objective was to investigate reasons for expected differences in response of the different crop species to paraffin treatment.

3.2 Materials and methods

3.2.1 The effect of paraffin treatments on seed germination

Seeds of seven crops were carefully selected to avoid deformed or injured seed. The crop species used were canola (*Brassica napus* L.), common bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogea* L.), maize (*Zea mays* L.), soya bean (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L.). Paraffin (commercially available) concentrations of 0, 25, 50, 75 and 100 % were created by diluting pure paraffin (100%) with distilled water to obtain the desired concentrations. The seeds were immersed in the different concentrations for one, five, ten or thirty minutes. The paraffin/water mixtures had to be agitated continuously by means of a shaker with minimum frequency of 80 shakes per minute to form an emulsion into which the seeds were immersed. Every treatment was repeated four times.

After immersion seeds were dried with water absorbent paper and immediately used for the different experiments. Twenty treated seeds (ten for large seeds such as common beans, maize and groundnuts) were scattered in a 90 mm petri dish containing a pair of Whatman filter papers moistened with 5 ml of distilled water. Petri dishes were closed and sealed in a polyethylene bag to reduce water loss and placed in an incubator adjusted to a constant temperature of 20 ^{0}C in the dark. Petri dishes were inspected daily and every seed displaying a radicle >1 mm were considered germinated and recorded as such. The experiment was terminated after ten days when the un-germinated seeds started decaying. At termination of the experiment percentage germination was calculated by making use of the formula:

Percentage germination = (Total number of germinated seeds at termination of experiment/Total number of seeds put in petri dish) x 100

The germination rate was calculated as follows (Heydecker 1973):

$$\text{Rate of germination} = \sum_{i=1}^{k} n_i / D_i n_i \cdot 100$$

Where   k = final day

         D_i = day of recording

n_i = number of seeds germinated on day Di

i = day 1 to day k

3.2.2 Electrical conductivity test

Electrical conductivity (EC) is a method developed to test the vigour of the seed. It consists of measuring the amount of electrolytes found in the imbibition solution having leaked from the seed (Tajbakhsh 2000) under controlled temperature conditions of 20°C. It is expressed in micro-Siemens per centimeter per gram ($\mu S\ cm^{-1}\ g^{-1}$).

In order to see if the paraffin treatments damaged the seeds of the different crop species an electrical conductivity test were carried out. Though many factors influence germination, this investigation focussed on water movement through the seed coat, since it directly relates to the presence of paraffin in the vicinity of the seed during the crucial phase of germination initiation (Kelly et al. 1992).

The seed water content of the crop seeds was measured using a SINAR Grain Pro 6310 moisture analyzer at the Agricol Seed Company in Brackenfell. One hundred grams of seeds were placed in the container of the analyzer, which displays the percentage water content in the seed. This ensured that the seed water content was less than 20% mass weight, the value under which results would be less erroneous (Sørensen et al. 1996). Seeds were washed with distilled water to remove any chemical product or dust, which would negatively influence the results. Ten grams of seed from the analyzed seeds, were poured into each of twenty 100 ml flasks containing 50 ml of distilled water/paraffin mixtures with concentration percentages of 0, 25, 50, 75 and 100% as described above. The immiscibility of paraffin-water (USEPA 2011) was overcome by continuous shaking of the paraffin-water solution 80 times per minute. After 30 minutes of imbibition the flasks were emptied and immersed seeds were dried with water absorbent paper and then placed in a 100 ml graduated cylinder containing 50 ml of distilled water. After twenty hours the seeds were removed from the water. The water in the cylinder, which contained some electrolytes leaked from the seed, was then shaken to homogenize the solution and the EC was determined by means of an EC meter (Metrohm 644).

3.2.3 Water/paraffin uptake by seeds

The experiment was conducted under room temperature conditions of $18\text{-}22^{0}$ C. The aim was to determine how much water, and how much paraffin, had been absorbed by the different crops seeds during 30 minutes of imbibition in different paraffin concentrations. One hundred millilitres of distilled water/paraffin mixture with concentrations of 0, 25, 50, 75 and 100%

(as described above) were measured into a 200 ml graduated cylinder. The mixture was left to settle and the relative amounts of distilled water and paraffin in the mixtures were confirmed by taking readings in the graduated cylinder. The mixtures were poured into 350 ml Erlen Meyer flasks that was rinsed with distilled water and then mounted in the shaker. To this mixture 10 g of seeds of each of the crop species tested were added separately.

The shaker was activated to facilitate the emulsion of the water and paraffin for a period of 30 min at a frequency of 80 cycles per minute. After 30 minutes the seeds were carefully removed by using a tweezer and shaken to leave as much of the mixture behind as possible. The mixture was then thoroughly drained into the graduated cylinder, left to set for ten minutes and the readings of the amounts of paraffin and water in the mixture were taken again. The amounts of water and paraffin absorbed by the seeds were then calculated by comparison with the original readings before the immersion of the seeds.

3.2.4 Crude fat content of the seeds

Samples of the seeds were analysed at the Department of Animal Sciences of the Stellenbosch University for crude fat content. The samples were milled with a Knifetec™ 1095 Sample Mill and thereafter analysed for crude fat percentage by making use of the Ether extraction method with Petroleum ether (Soxhlet method) (Robinson et al. 2008).

3.3 Treatment and experimental design

3.3.1 Germination test

The experiment was a factorial experiment laid out as a fully randomised design whereby a petri dish was considered as an experimental unit. Three factors: Time of immersion (TOI) (having four levels: 1, 5 10 and 30 min), Paraffin concentrations (PC) (having five levels: 0, 25, 50, 75 and 100 %) and Crop species (CS) (canola, groundnut, common bean, maize, soya bean, sunflower and wheat) were used. Each experimental unit was replicated four times. All the treatment combinations were completely randomized in the incubator. The analysis of variance used Statistica software version 2011 (Statsoft 2011) with a confidence level of 95% ($p < 0.05$). Where the results showed significant interaction or differences within main effects, the means were separated using Post-Hoc Fisher's LSD test at $p = 0.05$.

3.3.2 Electrical conductivity and paraffin-water uptake

For both experiments (EC and paraffin-water uptake), a two-way factorial experiment laid out as a fully randomized design with factors Paraffin concentrations (PC) (with five levels:

0, 25, 50, 75 and 100 %) and Crop species (canola, groundnut, common bean, maize, soya bean, sunflower and wheat) was applied. Every treatment combination was replicated four times and the analysis of variance used Statistica software version 2011 (Statsoft 2011) with a confidence level of 95% ($p < 0.05$). Where the results showed significant interaction or differences within main effects, the means were separated using Post-Hoc Fisher's LSD test at $p = 0.05$.

3.4 Results and discussion

3.4.1 Germination

3.4.1.1 Germination percentage

There was a difference in the germination percentage between different types of seed at different times used for different paraffin concentrations. This was revealed in a significant ($p < 0.05$) three-way interaction between PC, TOI and CS (Appendix 1 Table 1). For all the TOI treatments, the common bean, wheat and maize showed a significant drop in germination percentage from 0% to 25% paraffin concentration (Figure 3.1). This was caused by the direct action of paraffin on the seed in particular on the seed coat which is the part of the seed in direct contact with paraffin (Agarry et al. 2010).

Soya bean and canola were affected least by paraffin at the different concentrations. The lowest mean germination percentage (11.25 %) was recorded at five minutes of imbibition with 75% paraffin concentration on common beans. Paraffin treatment had a beneficial effect on sunflower germination because all the mean values for different paraffin concentrations (even at 100%) were higher than the control values. Paraffin in this case reacted as germination activator or seed priming chemical.
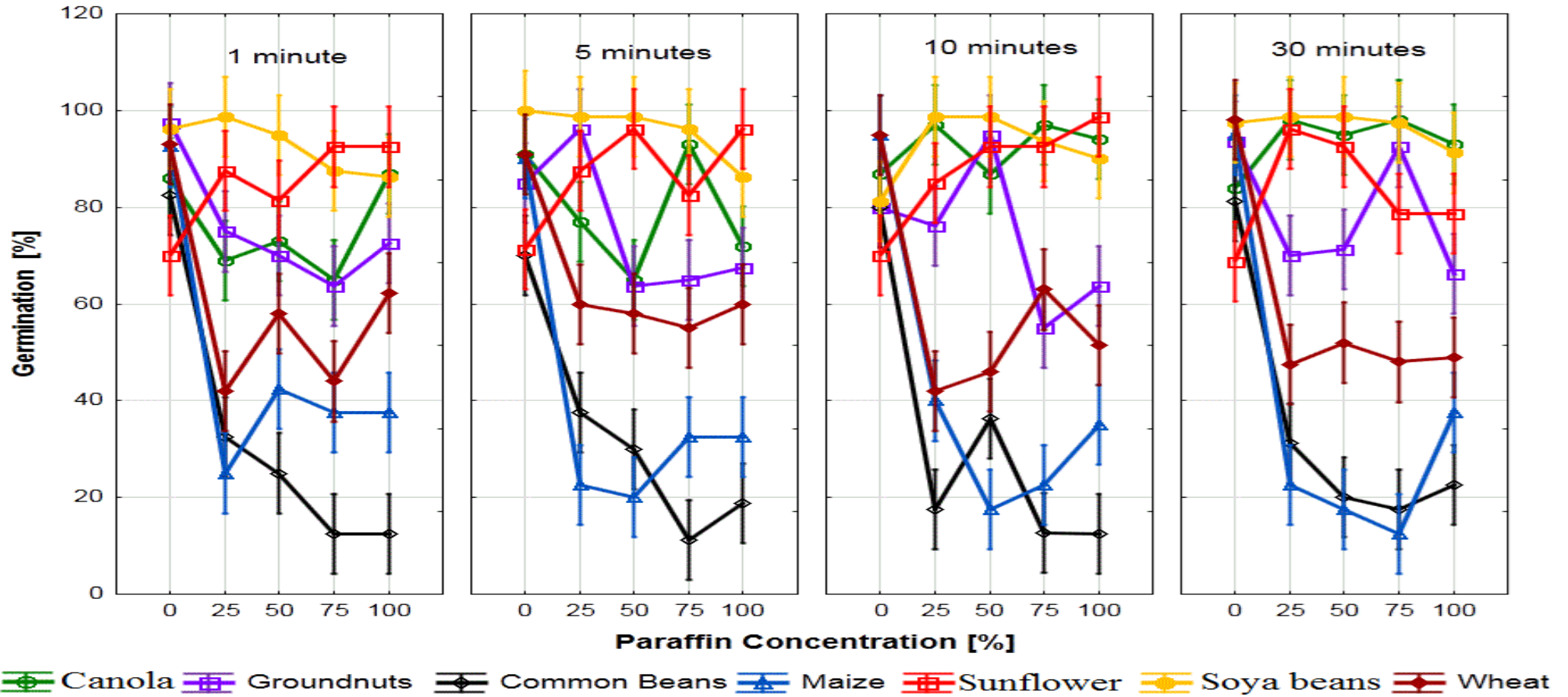
Figure 3.1: Germination percentages of different crop species after seed treatment with different paraffin concentrations at different times of immersion. The vertical bars represent the standard error of the mean.

Although TOI as a factor cannot be considered separately because of the significant interactions with the other factors, Fig 3.1 does indicate that time of immersion did not have such a big effect on germination (also indicated by the non-significant effect of TOI in Appendix 1 Table 1). It appears as if the crop species least influenced by the paraffin treatments were those with a relatively high crude fat content (Table 3.1). Apart from groundnut that was moderately affected (about 20-30% reduction in germination from the control treatment) by the paraffin treatments, the other three least affected species (canola, soya bean and sunflower) had higher (>10%) crude fat content. The three species with <10% crude fat content were moderately (wheat: > than 50% reduction) to severely (maize and common bean: > than 60% reduction) influenced by paraffin treatments. A possible reason why groundnut was more affected could be due to the very thin papery seed coat which provided very little protection of the embryo from the paraffin mixtures. Furthermore the embryo is prominent and not protected by cotyledons as in the case for soya bean seeds (Sharonova and Breus 2012).

Table 3.1: Percentage moisture and crude fat content of different crop seeds

Seed type	Beans	Maize	Wheat	Soya beans	Groundnuts	Sunflower	Canola
Moisture content in % (28*)	12.9	9.8	13.3	5.8	10.5	8.0	6.8
Seed crude fat content in %	0.85	3.51	1.70	14.00	44.13	34.38	39.09

3.4.1.2 Rate of Germination

There was no three way interaction ($p < 0.05$) between TOI, PC and CS that influenced the rate of germination. There was no any two way interaction between factors but both CS and PC significantly ($p < 0.05$) affected the rate of germination (Appendix 1 Table 2). The germination rate is influenced by CS where there is an increasing trend from groundnut (27.12%), beans (43.36%), maize (43.96%), wheat (50.68%), soya bean (69.59%) and sunflower (70.52%) to canola (85.31%) (Figure 3.2). The homogeneity grouping by the use of Fisher's LSD test showed that beans and maize did not differ statistically from each other as did sunflower and soya bean. The groundnut takes long to germinate while canola responds quickly.
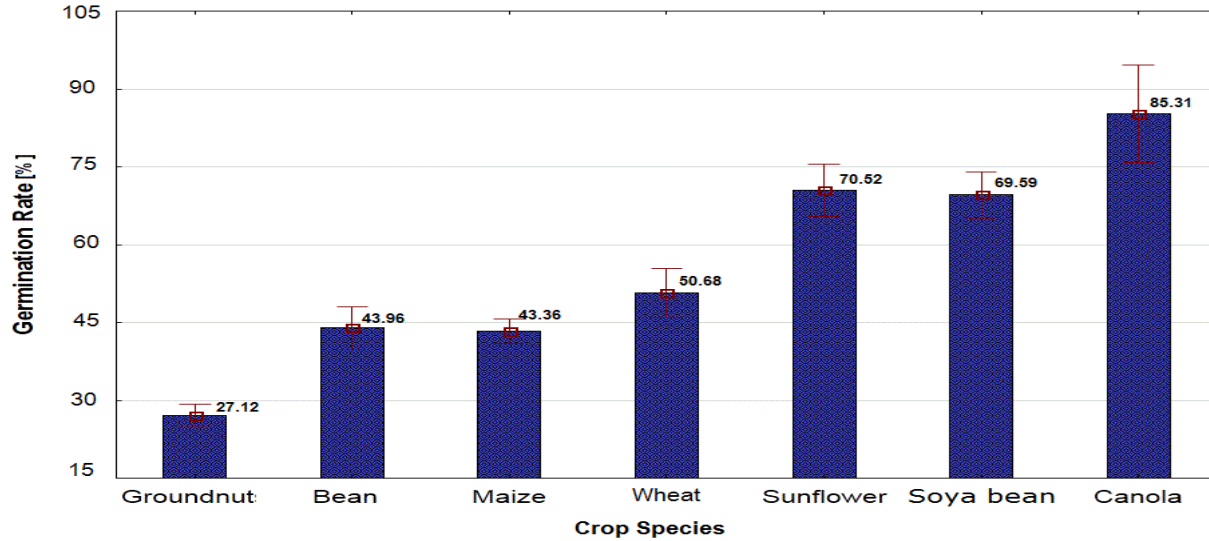
Figure 3.2: The influence of crop species on germination rate of different seeds after being treated with different paraffin concentrations. The vertical bars represent the SE of the man.

There was a significant regular drop in germination rate from the control (0% PC) (which has the highest germination rate) to 100 % PC (Figure 3.3). Post-hoc comparison tests with the Fischer's LSD test showed that there were statistically significant decreases from 0% to 25% PC, then from 25% to 50% PC, but from 50% to 100% PC the rate of germination decreased, but not statistically significant. At 0% and 25% of PC, the germination rates did not differ significantly (Figure 3.3). Control demonstrated the highest germination rate, meaning that paraffin affects negatively the germination rate of the selected crop seeds in general. A high concentration of paraffin decreases the germination rate of all seeds (Appendix 1 Table 2). For agricultural purposes, it would be profitable to select 25% PC as it would require minimum quantity of paraffin and has the highest germination rate amongst all PC still significantly lower than control.
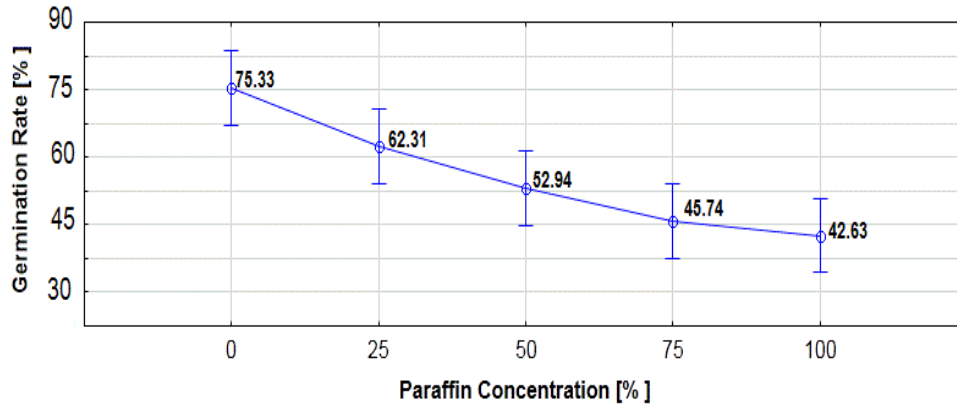
Figure 3.3: Effect of Paraffin Concentration used as seed pre-treatment as a main effect on mean germination rate. The vertical bars represent the standard error of the mean.

3.4.2 Electrical conductivity

The results showed a significant two-way interaction between PC and CS (Appendix 1 Table 3). The EC of soya bean and canola decreased with the increase of PC as did groundnut, but to a lesser degree (Figure 3.4).
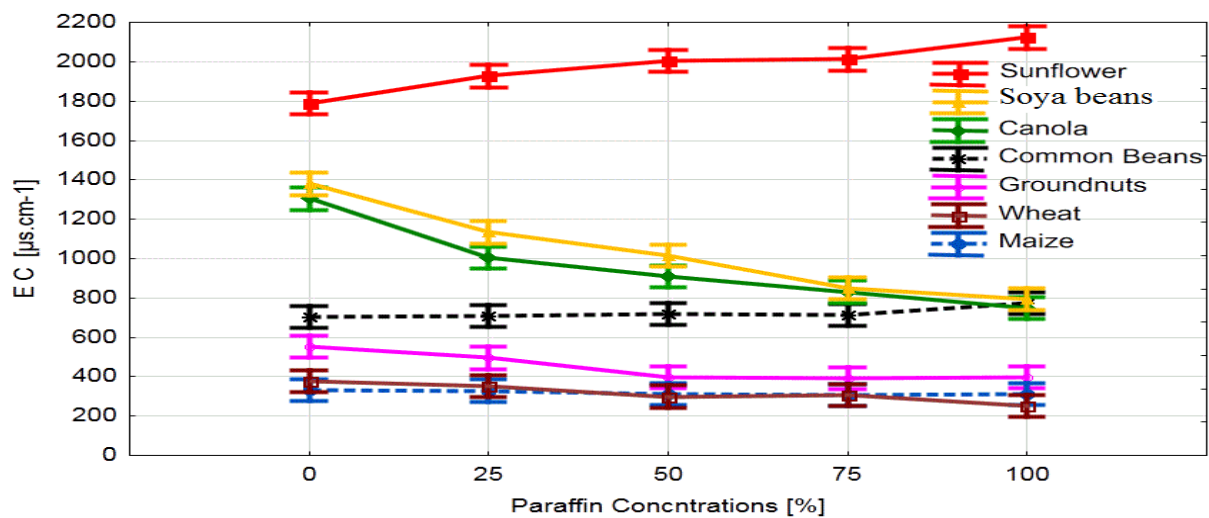
Figure 3.4: EC readings of different crop species after being treated with different paraffin concentrations. The vertical bars represent the standard error of the mean.

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Sunflower had a three to four times higher EC than the lowest amongst the group. Wheat and maize had the lowest EC mean values and were not influenced by the paraffin treatments. There is no clear trend in terms of the effect of paraffin treatment on EC values of the seed and it appears as if paraffin treatment does not really affect the leakage of electrolytes from the seeds.

Electrical conductivity being one of the indexes used to indicate damage, aging of the seed or malfunction of the seed coat; it is not clear how seeds with lower EC such as maize, wheat and common beans had such poor germination percentage after treatment with paraffin (Figure 3.1). Tajbakhsh (2000) indicated that under optimal conditions, the EC of wheat with 99% germination success was 412.7 μS cm^{-1} g^{-1} while naturally dormant aged wheat seed recorded 552.5 μS cm^{-1} g^{-1}. This experiment showed a value of 382.42 μS cm^{-1} g^{-1} for the wheat indicating seeds in good condition. Therefore, seed of particular species have different ranges of EC wherein they are expected to be viable and in good condition. It is therefore erroneous to compare two different types of seed with similar EC values as the electrolytes leaked are not the same and the leak level varies from type to type (Sørensen 1995).

In the conventional process, EC data are recorded after about 16 to 24 hours of imbibition of seeds which originally had less than 20% moisture content (Sørensen 1995). For this experiment, a twenty hours period was selected. The moisture contents measured on the sample seed are shown in Table 3.1. However, the reason of using EC method in this experiment was to investigate whether leakage of solutes at different paraffin concentrations had any correlation to the germination of the seeds treated in a similar way. There is no clear trend visible and it therefore does not look as if the paraffin has a significant negative effect on the membranes of the seed. The poor germination of some of the crop species must therefore be due to another unknown effect of the paraffin.

3.4.3 Imbibition of paraffin and water by seeds

The analysis of variance (p = 0.05) of the mean values of water or paraffin uptake during thirty minutes of imbibitions showed there is significant interaction between PC and CS in terms of water as well as paraffin uptake (Figure 3.5, Appendix 1 Table 4 and 5).
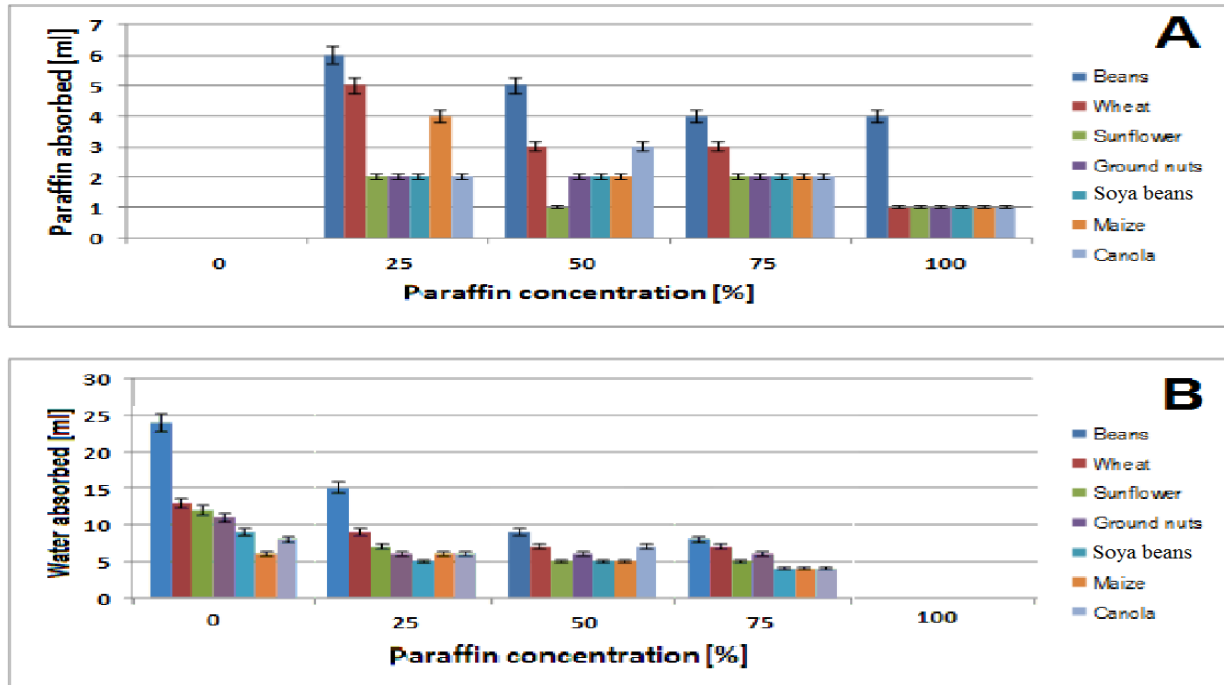
Figure 3.5: Quantity of paraffin (A) or water (B) absorbed by different crop seeds (10 grams each) while immersed in different paraffin concentrations for thirty minutes.

Generally the amount of paraffin absorbed decreased with increasing paraffin concentration (Figure 3.5 A). At 100% paraffin concentration only about 2 ml of paraffin was absorbed by all seeds except for common beans, that absorbed 4 ml. This could be because the common bean seed was much bigger than the rest of the crop species but in all cases only 10 g of seed were aded to the mixture. It is also possible that the seeds may have absorbed zero pure paraffin and that the about 2 ml could have been lost either when the seeds were removed from the mixture and dried on the absorbent paper or by evaporation. At the 25 to 75% paraffin concentrations, it appears as if most of the crop species absorbed about 3 ml of paraffin except for common beans and wheat where more paraffin appeared to be absorbed by the seed at lower concentrations.

Water uptake levels in pure 100% water were significantly higher than in the 25 to 75% water concentrations which in most cases did not differ from each other (Figure 3.5 B). One notable exception was common bean, that showed a constant decrease in amount of water imbibed with lower water concentrations. The fact that less water was imbibed at lower water concentrations (higher paraffin concentrations) may be an indication that paraffin acts as a priming agent and therefore in the case of some seeds the paraffin treatments actually improved germination

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percentage compared to the control of pure distilled water. There was no relationship between water content in the seed and the water uptake for the thirty minutes of immersion. This is proven on beans and wheat which had the highest percentage of water content and were expected to demonstrate less osmotic forces compared to other seeds. However, they are the biggest water absorbers of the species tested. The analysis of other factors such as seed coat, its thickness, its chemical nature and its impermeability (Hilhorst and Toorop 1997, Asli and Houshmandfar 2011) could help to explain differential water and paraffin uptake from mixtures.

3.5 Conclusions

In this study, it was found that the germination of some seeds such as maize and common beans was severely influenced by exposure to paraffin while others such as wheat and groundnuts were moderately influenced, while on others, such as canola and soya bean the influence was almost negligible. On sunflower, exposure to paraffin improved germination of the seeds. The exposure to paraffin had very little effect on electrolyte leakage of the seeds and it does therefore not appear as if the paraffin negatively influenced the cell membranes of the seeds. More work is to be carried out to determine what mechanism is responsible for the negative influence of paraffin on the germination of seeds.

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Chapter 4

Effects of paraffin on seedling establishment and vigour of different crops grown in a glasshouse

Abstract

Establishing seedlings are vulnerable to predation. It is claimed that paraffin repels phytophages when applied to seeds before planting. In this study the effect of paraffin seed treatment on canola (*Brassica napus* L.), common beans (*Phaseolus vulgaris* L.), ground nuts (*Arachis hypogea* L.), maize (*Zea mays* L.), soya beans (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L.) was investigated. Seeds were subjected to a 7x5x4 completely randomised factorial design with factors crop species (CS) (see above), paraffin concentrations (PC) (0, 25, 50, 75 and 100% of commercial paraffin diluted with distilled water) and time of immersion (TOI) (1, 5, 10, and 30 minutes). Treatments were repeated four times. After immersion seeds were dried and 10 seeds were sown into 8 cm x 8 cm plastic pots filled with coarse sand and seedling establishment (emergence) was monitored daily. The final percentage of establishment was calculated after emergence stopped. Seedlings were thinned to retain only the seedling judged strongest per pot which was harvested seven weeks after planting to determine plant height, root length and dry mass. Generally, establishment (emergence) of maize, common beans, wheat and groundnuts were severely reduced by paraffin treatment but growth of the established seedlings was less influenced by the paraffin treatments. CS and PC showed a significant two way interaction on the dry mass at the thinning stage (three weeks) and after seven weeks of growth. Groundnuts showed a distinctive low establishment percentage (under 40%) compared to other crop species having establishment percentages over 80%. The comparison of stem length between different types of crop species showed that there was a two-way interaction between CS and TOI and differences within PC as main factor. Paraffin treatment influenced wheat severely for all times of imbibition. The tolerance indices had three way CS x PC x TOI interaction with good high indices at one, five and 30 minutes on sunflower, at one and five minutes on canola and soya beans and at 10 minutes for groundnuts.

4.1 Introduction

Many authors consider germination as starting with the increase of metabolic activities in the seed and ending with the appearance of the radicle (Sarkar et al. 2009). The growth stages, which follow germination, are characterized by simultaneous extension of the radicle and rapid appearance and growth of the plumule (Mei and Song 2008). These two new organs continue their development marked by remarkable differentiations whereby the radicle develops into a root system and the plumule into a stem. The response of the new plant to gravity causes the roots to penetrate deep into the soil while the plumule grows in the opposite direction towards the soil surface (Roberts 1986).

It is generally agreed that germination success is indicated by the appearance (emergence) of the plumule from the soil. However, the number of plants that emerged from the soil indicates the establishment percentage of the crop. This is not necessarily equal to germination because seedlings can die off under the soil surface before emerging. These stages of the plant's life are the most vulnerable as the least injury, disease or water stress may lead to death or open the way to opportunistic parasites (Kulik and Yaklick 1991). Thus the establishment and the vigour of the seedling are evidence that the seed survived destructive factors in the soil.

In Central Africa paraffin is used by smallholder farmers to repel predators from emerging crop species (personal observation). However paraffin has been shown to have negative impacts on germination and growth of plants when spills occur in nature. The residue of paraffin stuck on the seed surface during seed imbibition is quantitatively insignificant to impact continuously on growth. Radicles and roots are the most affected parts of the plant as they are in permanent contact with the contaminant (Baud-Grasset et al. 1993). Obviously the above ground plant parts which are dependent on the root system suffers consequences such as chlorophyll degradation (Malallah et al. 1998) and disturbance of stomatal mechanism, leading to reduction of photosynthesis in its different stages and respiration (Baker 1970). Species resist pollutants differently but generally the increase of petroleum derivatives, paraffin in this case, specifically in the vicinity of the germinating seed or the seedling has negative impacts on the entire development of new plants (Luhach and Chaudhry 2012).

Depending on how seeds and seedlings can resist contaminants in the growth medium, a scale can be established. That scale could help to identify species, varieties or cultivars which could be used in bioremediation. Sharonova and Breus (2012) rated plant species to be more tolerant

when more than 70% of seeds germinated in a polluted soil, less tolerant when 30-70% germinated and intolerant when less than 30% germinated.

Because it is important to know how paraffin affects seedling establishment and seedling vigour before it is considered as animal deterrent in crop production (Aveling et al. 2012), a study was conducted to determine the effect of paraffin seed treatment on the establishment and growth of different crop species during initial vegetative growth stages.

4.2 Material and methods

4.2.1 Treatment and experimental design

Seeds of seven crop species: canola (*Brassica napus* L.), common beans (*Phaseolus vulgaris* L.), ground nuts (*Arachis hypogea* L.), maize (*Zea mays* L.), soya beans (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L) were treated by immersing them in one of five paraffin concentrations (0, 25, 50, 75 and 100 % of commercial paraffin diluted with distilled water) for one of four time periods (1, 5, 10 and 30 minutes). The paraffin/water mixtures had to be agitated continuously by means of a shaker with minimum frequency of 80 shakes per minute to form an emulsion into which the seeds were immersed. Every treatment was repeated four times.

After immersion seeds were dried with water absorbent paper and immediately planted into plastic pots of 8 cm x 8 cm that were filled with coarse sand and put in a temperature controlled glasshouse. Ten seeds per pot of each crop species investigated were sown at a depth of 1 cm and watered daily with tap water until establishment of seedlings ceased. The irrigation was done manually with a watering can. The glasshouse temperature was controlled at a continuous day/night temperature of approximately 20^0 C and positions of pots were randomly changed five times during the experimental period. The numbers of emerged seedlings were counted daily and the percentage establishment was calculated. The formula for calculating establishment percentage was as follows (Sousa et al. 2012):

$$\text{Estabishment} = \frac{\text{Number of seedlings emerged from the soil}}{\text{Number of seeds planted in the soil}} \times 100$$

Seeds were considered established when the plumule or coleoptile emerged from the soil wherein seeds were planted. After establishment, plants were daily watered with a standard nutrient solution until harvested (Table 4.1).

Table 4.1: Composition of nutrient solution (meq.L^{-1}) used to water plants in the glasshouse

Na$^+$	NH$_4^+$	K$^+$	Ca^{2+}	Mg^{2+}	NO$_3^-$	H$_2$PO$_4^-$	SO$_4^{2-}$	Cl$^-$	HCO$_3^-$
0.109	0.046	5.805	7.570	3.329	9.907	0.804	5.839	0.35	0.054

Three weeks after planting, the plants were thinned, leaving only the tallest and most vigorous growing plant per pot to continue growing. Relevant information such as the lengths of the root and the stem from three random uprooted plants from each pot were recorded at this stage. Thinned plants were dried at 50^0 C for 48 hours to be weighed and the mean dry mass (total of dry mass of thinned plants per pot divided by the number of those plants thinned) per plant was calculated. The remaining plants were harvested four weeks after thinning and the stem length and length of the longest root were recorded before the plants were dried at 50^0 C for 48 hours to be weighed to determine dry mass. In order to compare data of different crops scale indices of dry mass, stem length and root length were calculated. Calculations consisted of expressing measurements of treated seedlings as a percentage of the untreated control treatments. The calculations were done as shown by the formula below:

$$\text{Index Value (\%)} = \frac{\text{Mean value of treatment for particular CS}}{\text{Mean value of Control for particular CS}} \times 100$$

The data of the root length were used to calculate the tolerance indices (TI) using the following formula (Asli and Houshmandfar 2011).

$$\text{TI at defined conditions (D)} = \left(\frac{\text{Length of the root at certain conditions D}}{\text{Root length at the control}}\right) \times 100$$

The percentage oil content of seeds from all crops before treatment was determined at the Department of Animal Sciences of Stellenbosch University and the moisture was calculated using a SINAR Grain Pro 6310 analyzer.

Table 4.2: The percentage moisture and oil content of different crop species

Seed type	Common Beans	Wheat	Maize	Soya beans	Sunflower	Canola	Groundnuts
Moisture content in %	12.9	13.3	10.5	9.8	8.0	6.8	5.8
Seed crude fat content in %	0.85	1.70	3.51	14.00	34.38	39.09	44.13

4.2.2 Statistical analysis

The experimental layout was a completely randomized factorial design (Clewer and Scarisbrick 2001) whereby a pot was considered an experimental unit. Three factors; Time of immersion (TOI) (1, 5, 10 and 30 minutes), Paraffin concentration (PC): (0, 25, 50, 75 and 100 % of commercial paraffin) and Crop Specie (CS) (Seven crop species described above) were used. The experimental data was subjected to analysis of variance (ANOVA) using STATISTICA, software version 2011 programme (Statsoft 2011). Where the experiment showed significant interaction, the means were separated using Post-hoc Fisher's LSD test at $p = 0.05$. Because of the large number of missing plots (no plants established), data on plant components (root lengths, stems lengths and dry mass) of crop species with establishment percentages of < 20 % were not statistically analysed and discussed.

4.3. Results and discussion

4.3.1 Establishment

No significant (p > 0.05) three-way interaction occurred between CS, PC and TOI (Appendix 2 Table 1). The only significant two-way interaction was between CS and PC and there were significant differences within TOI as main factor. The establishment of seedlings of the seven crop species shows distinctive differences in response to increasing paraffin concentrations (Figure 4.1).
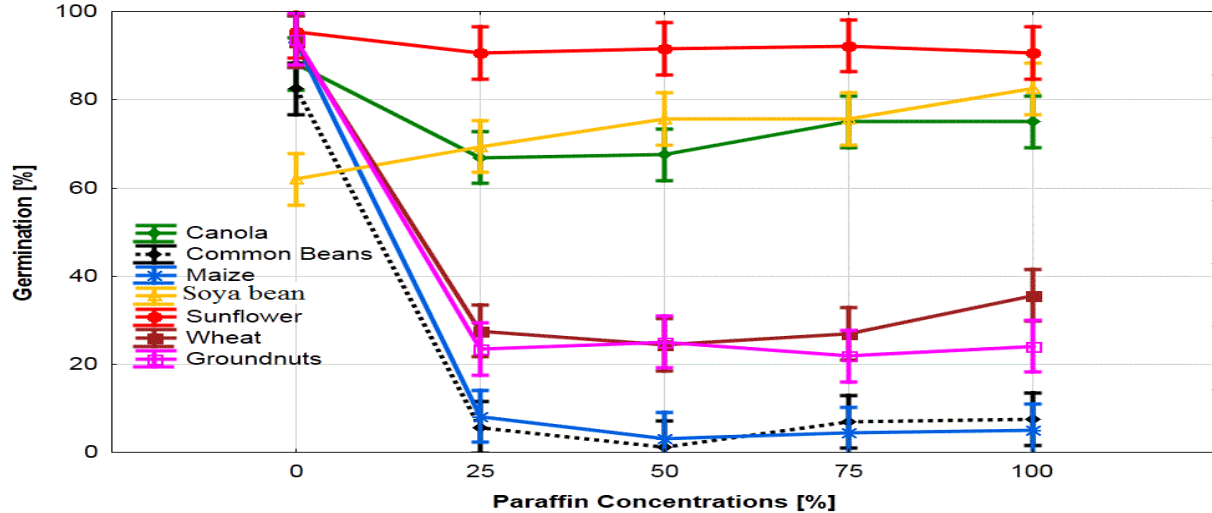
Figure 4.1: Establishment percentage of different crop species as influenced by different paraffin concentrations (0, 25, 50, 75 and 100%) in a glasshouse at 20^0 C. The vertical bars represent the standard error of the mean of each treatment.

Common beans and maize show only satisfactory establishment (>80%) in the control treatments (Figure 4.1) and establishment were reduced to a very low percentage with the rest of the PC (<20%). Figure 4.1 shows moderate percentage establishment of wheat (31.50 %) and groundnut (27.63 %). Presumably, the original water content in the seed is important because it is by its increase (water uptake) that the germination activities are initiated. On the other hand, the percentage of oil in seed would susceptibly influence paraffin absorption by the seed. Results shown in Table 4.2 showed that groundnuts seed oil content (44.13%) is 52 times higher than that of common beans (0.85%) while common beans (12.9%) had twice the moisture content of groundnuts (5.8%). The mean percentage of germination of different crop seeds indicated variable tolerance to paraffin toxicity in the seven crop species.

According to the classification provided by Sharonova and Breus (2012) soya bean, canola (at 75 and 100% PC) and sunflower are more tolerant (>70% germination), wheat at 100 % PC is less tolerant (30-70% germination), groundnuts, wheat (25, 50 and 75 % PC), maize and common beans are intolerant (<30% germination) to paraffin. The use of paraffin as repellent in the intolerant crops will therefore not be economically feasible because very high seeding rates will be needed due to poor establishment (Figure 4.1).

Sunflower showed no influence at all when treated with different paraffin concentrations while soya bean establishment was significantly improved by paraffin treatment. Compared to the control, canola establishment was significantly reduced by paraffin treatments but the establishment percentage was still at about 75% with all paraffin concentrations of 25 to 100% (Figure 4.2). Common bean establishment was inhibited by all paraffin concentration.
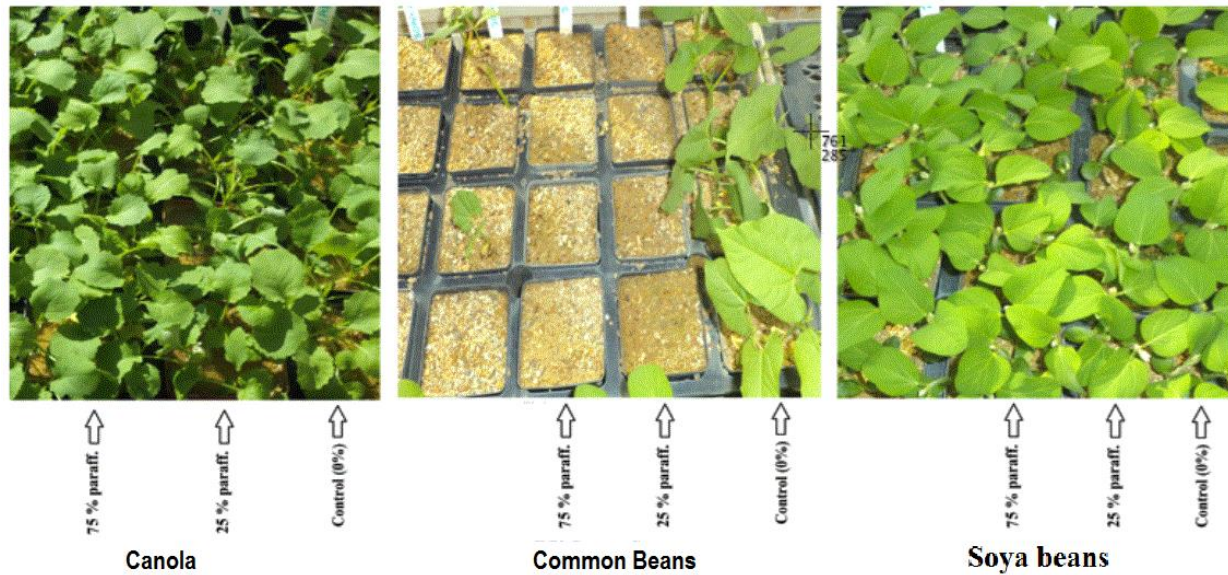

Figure 4.2: Seedling establishments for different crop species at 25 days after planting in response to seed treatment with different paraffin concentrations.

Variable responses of different crop species to paraffin could be attributed to the nature of the seed coat (Sharonova and Breus 2012), the position of the embryo within the seed and the seed oil content (La Scala et al. 1999, Dübbern de Souza and Marcos-Filho 2001). Some seed coats are thick and hard such as the case of sunflower while some, such as wheat, present thin layered bran which is vulnerable to external factors. Other features may be specific to certain species causing the seed to be either resistant or vulnerable; for example the hilum on common bean seed is considered to be the most permeable port of entry or leak for the seed (Roberts 1986).

From Table 4.2 it is clear that soya beans, sunflower, canola and groundnuts have high oil contents compared to beans, wheat and maize. Nevertheless, if the seed oil content was the only defining factor by which the increase of seed resistance towards paraffin would proportionally depend on, then groundnuts would have the highest establishment percentage. The high

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sensitivity of groundnuts to paraffin treatments may be due to the protrusion of the embryo which allows it to be easily accessed by paraffin (Figure 4.3) combined with its thin tegument. During the germination and establishment processes, paraffin causes the groundnuts seedling to develop slowly as illustrated in Figure 4.3.

Figure 4.3: Growth comparison between groundnuts washed with control (0% paraffin for 10 minutes) and 25% paraffin 10 minutes, dried and planted in a uniformly watered soil.

Because of this retarded development some embryos were eventually killed and decomposed in the soil. The decomposing fungi and bacteria that multiply exponentially around the seed are facilitated by the biochemical state of the seed. It is that state of the seed which conditions the seed-soil exchange of elements (Matthews and Powell 2006). The leaked nutrients from the seed feed the decomposing bacteria thus increasing the potential attack of seeds that are slow in establishing.

The TOI as a factor significantly ($p < 0.05$) influenced the establishment percentage of different crop species (Figure 4.4). A significant reduction in percentage establishment was recorded when TOI increased from one minute (53.79%) to five minutes (52.13%), and another significant drop from ten minutes (51.78%) to 30 minutes (49.26%).
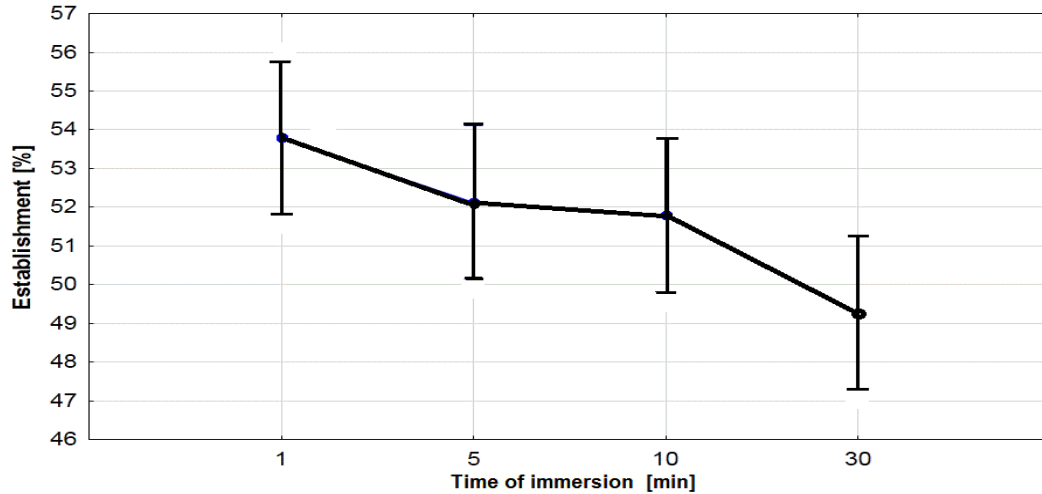
Figure 4.4: Establishment percentages of different crop species as influenced by Time of immersion (TOI) (1, 5, 10 and 30 minutes) in the glasshouse at 20^0 C. The vertical bars represent the standard error of the mean of each treatment.

4.3.2 Stem length

4.3.2.1 Stem lengths at the first harvest

There was no significant three-way interaction ($p < 0.05$) between PC, CS and the TOI shown in terms of stem length at the thinning time (3 weeks after planting) (Appendix 2 Table 2.). However, a significant two-way interaction between PC and CS was observed (Figure 4.5).

Figure 4.5: The effect of different paraffin concentrations on stem length (indicated as percentage of the control) of different crop species. The vertical bars represent the standard error.

Regardless of different paraffin concentrations and times of immersion, groundnuts showed the shortest stems lengths at 3 weeks after planting. Canola stem length was not affected by the PC in contrast to soya beans where the increase in PC caused a constant decrease in stem length. In sunflower, paraffin treatment appeared to stimulate the growt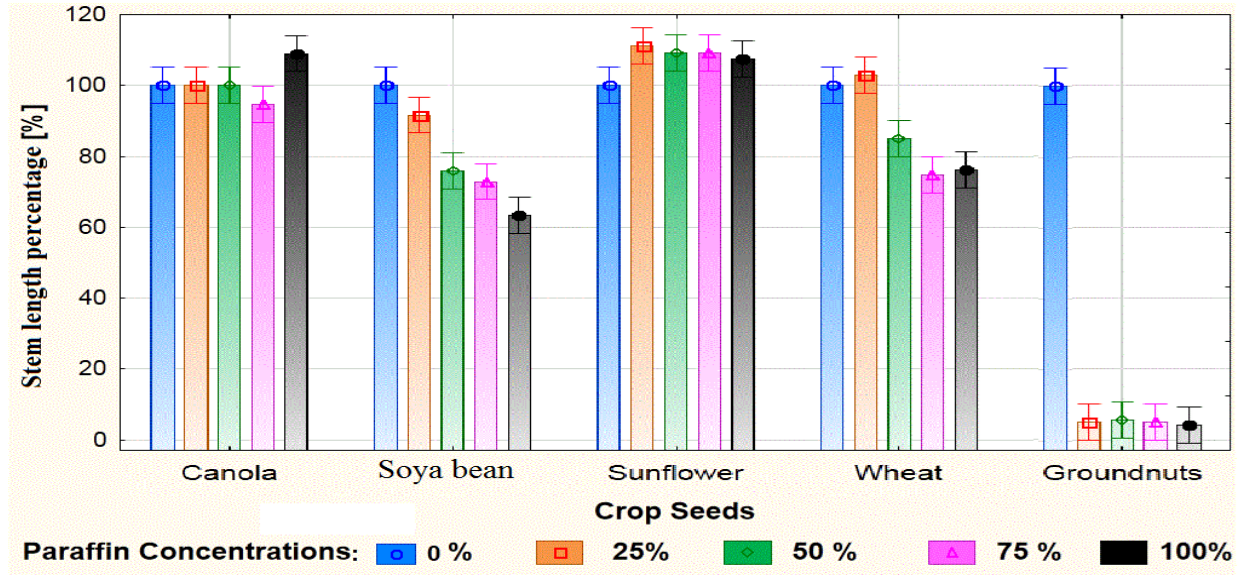h of the stem because the stems of seedlings developing out of paraffin immersed seeds grew longer than the control (Edwards 2011). This is an unusual observation showing positive action of paraffin on seedling growth. This is contrary to results obtain by bioremediation researchers (Adekunle and Adebambo 2007, Njoku et al 2009, Saadoun and Al-Ghazawi 2010, Sharonova and Breus 2012) who are interested in the quantity limit of hydrocarbons above which the seedlings may not survive. Paraffin treatment manifested a negative effect on the stem length of wheat from 50% PC and higher.

There is also a two way interaction ($p < 0.05$) between TOI and CS where the groundnut marks the lowest index of 4.9 % of stem length percentage. There is no significant difference with groundnuts stem length percentages. This clustering of data is also observed on soya beans between 77 % and 83 %. It showed that there is no significant difference in stem length percentage on different indicated times on groundnuts and on soya beans. Again, the graph at 30 minutes showed that the stem length percentages on wheat, canola and soya beans are nor significantly different. The stem length percentages of sunflower and canola at one minute, five

minutes and ten minutes are not significantly different. (Figure 4.6; Appendix 2 Table 2.). The Fischer's LSD test showed that values of stem length percentage of groundnuts are significantly different between different CS and the values of wheat at 10 minutes are different from the rest of other values on wheat within CS.
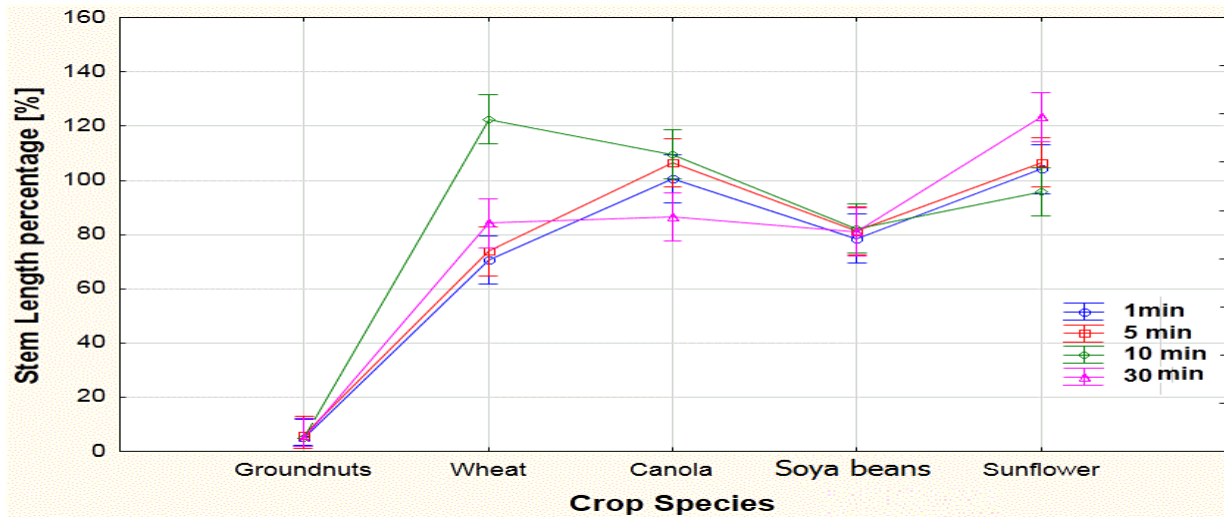
Figure 4.6: The effect of different timings of imbibition of commercial paraffin on stem length (indicated as percentage of the control) of different crop species. The vertical bars represent the standard error.

4.3.2.2 Stem lengths at the second harvest

There was no significant ($p < 0.05$) three-way interaction between CS, PC and TOI with regard to stem lengths at seven weeks after planting (Appendix 2 Table 3.) but a significant two way interaction between TOI and CS was observed. At seven weeks after planting, paraffin concentrations which mainly affected the seedlings at the early stage did not significantly affect the stem growth of the crops. Stem growth was rather affected by the TOI and CS. Soya bean growth was inversely proportional to the TOI where the growth index was 90 % at 30 minutes, while it was 110% at one minute (Figure 4.7). Mean values of wheat at one minute showed the lowest growth at seven weeks, with the percentage under 50 %. Overall, the best growth index was observed at five minutes of TOI for all CS except for the wheat. Canola seedlings at 30 minutes TOI did suffer the influence of paraffin and developed slowly compared to shorter periods of imbibition.
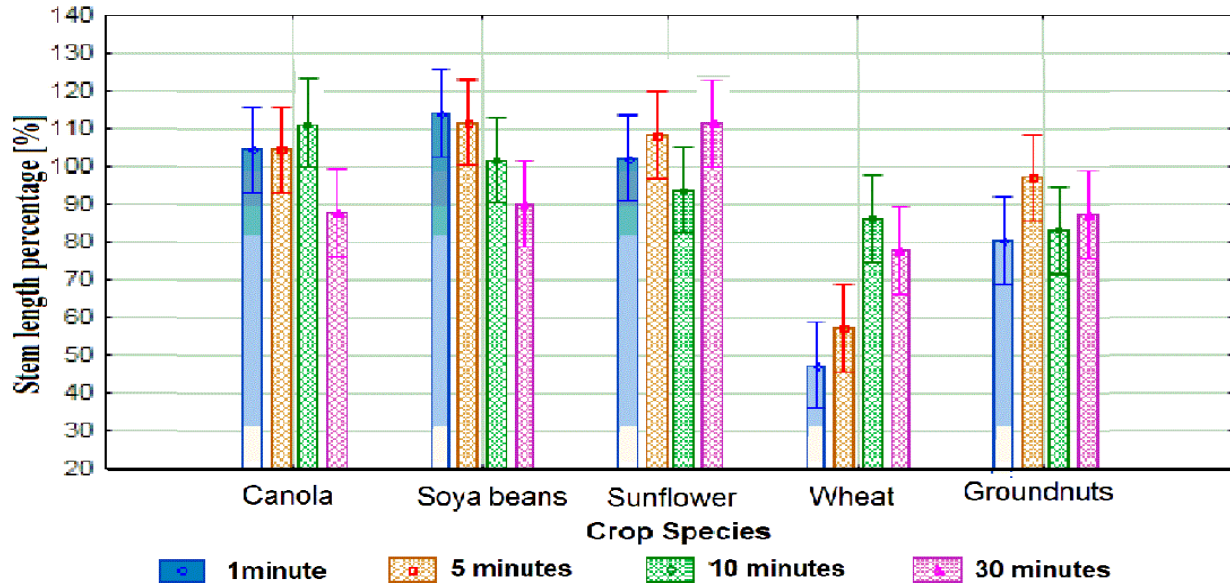
Figure 4.7: The effect of different period of paraffin imbibition on stem growth (presented as percentage of the control) of different seed species seven weeks after planting. The vertical bars represent the standard error.

4.3.3 Tolerance index

A significant ($p < 0.05$) three-way interaction between CS, PC and TOI occurred in terms of tolerance index of the roots. The analysis of the effect of treatments on the root system which is considered to be the port of entry for the nutrients in the plants showed that the soya bean roots are negatively affected by the increase of the period of immersion for all paraffin concentrations (Figure 4.8). Nevertheless, a higher tolerance index was shown at one minute of immersion when compared to the control. This is a supportive argument on possible seed priming by paraffin at one minute of immersion (Wahid et al. 2008). Tolerance index values of wheat and groundnuts indicated that root systems of the control are well developed in length in comparison with other PC apart from 10 % PC on groundnuts where the length generally exceeds the control.

Figure 4.8: Tolerance indices comparing lengths of the roots of canola, soya beans, sunflower, wheat and groundnuts seven weeks after planting. Error bars displayed are 5% value.

On canola there is no distinctive pattern which would help to draw a distinctive conclusion but the index values oscillate around the control, indicating that paraffin concentrations do not exert a significant impact o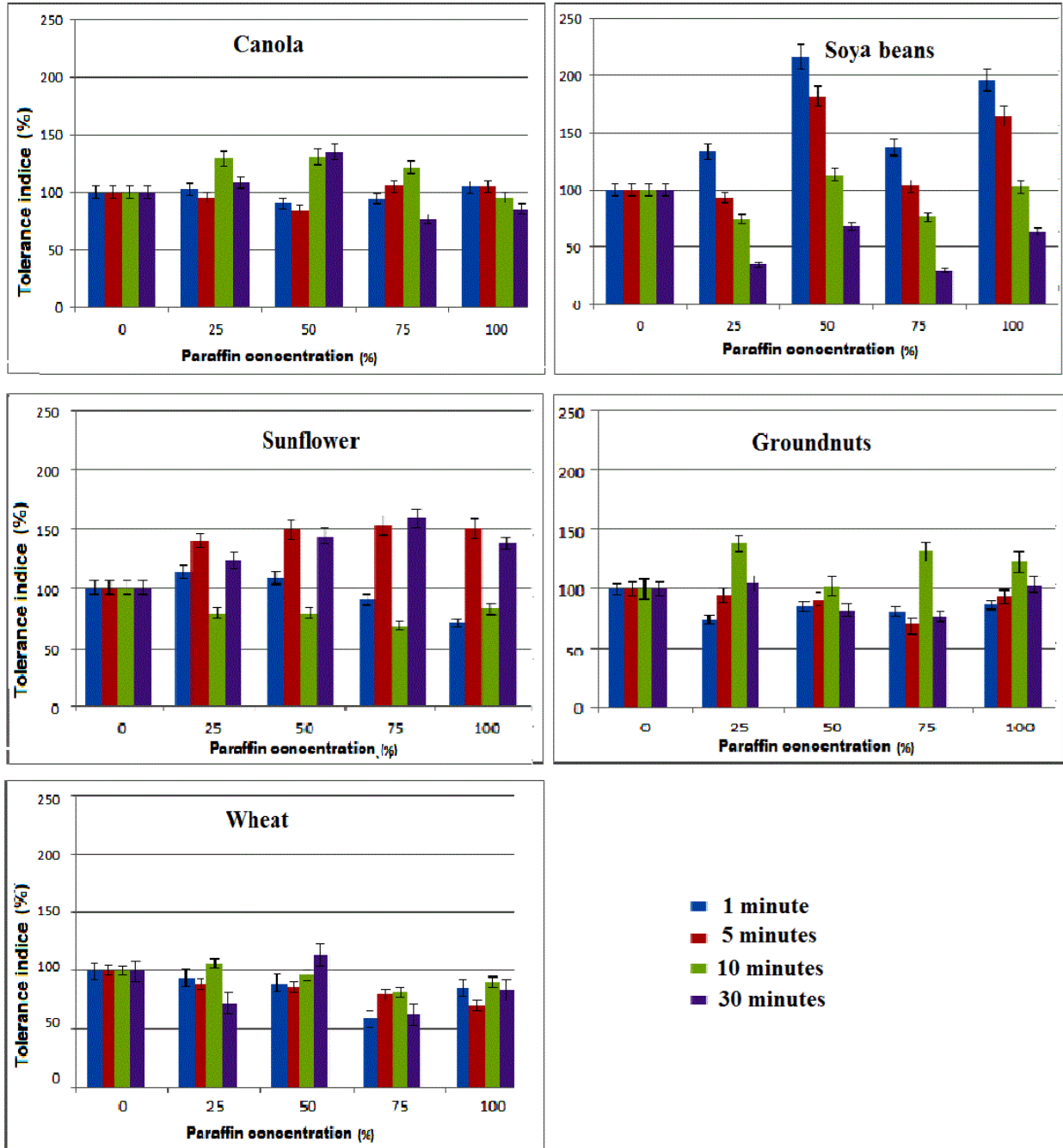n the length of canola root systems. In sunflower roots, the five and 30 minute values are higher than the control but indices of 10 minutes are low. This phenomenon is inexplicable and can probably be ascribed to experimental error. These variations do not proportionally influence the plant canopies because eventually short roots may be compensated by effective branching systems which may create a dense root system. The small pots used in these experiments did not facilitate free root growth and these values, although informative should be considered with care taking into account the effect of pot bound root systems.

4.3.4 Dry mass (per plant)

4.3.4.1 Dry mass (per plant) at the first harvest

A significant ($p > 0.05$) three-way interaction occurred between CS, PC and TOI with regard to dry mass index at three weeks after planting (Table 4.10, Appendix 2 Table 5.). This means that all factors (CS, PC and TOI) play influential roles in determining the mass at three weeks of plantation. At one minute of imbibition, canola showed the highest dry mass value in the control treatment with the smallest value displayed by sunflower. The dry mass of canola decreased with the increase in paraffin concentrations. Sunflower demonstrated a general drop of the dry mass index from a value of 100 % at control to 60 % and around that percentage at 25 % PC. Soya bean and wheat appears to be the species with a stable dry mass index between 80 % and 120%. Groundnuts produced the lowest dry mass mean values for all TOI from 25% to 100%.
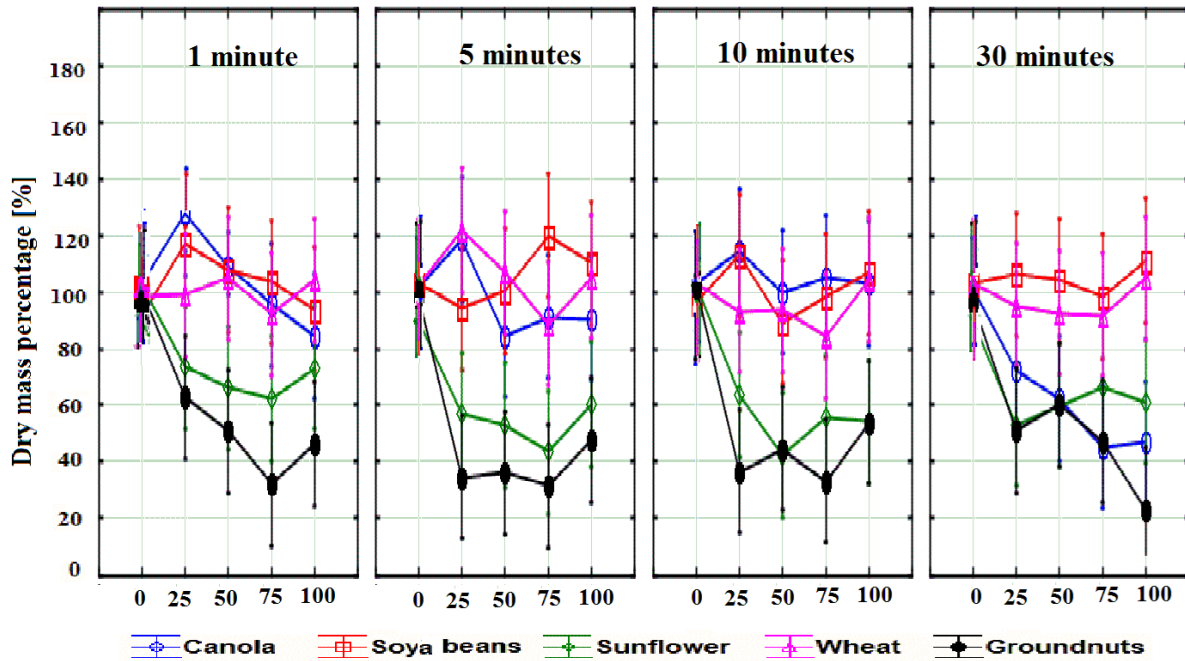
Figure 4.9: Effect of paraffin concentration and time of immersion on dry mass of different crop seedlings at 3 weeks after planting. The vertical bars represent the standard error.

Dry mass of wheat also showed no significant response to paraffin concentration, which may be ascribed to the early tillering of the stem that causes the dry mass to increase (Assuero and Tognetti 2010). This means that paraffin inhibits the establishment but does not negatively influence the autotrophic plant's growth. Dry mass of groundnuts treated with paraffin was significantly reduced when compared to the control treatment (Figure 4.9). Paraffin treatment of groundnut seeds therefore has a severe effect on growth of the established seedlings.

4.3.4.2 Dry mass at the second harvest

There was no significant ($p > 0.05$) three-way interaction between CS, PC and TOI with regard to dry mass per plant at 7 weeks after planting (Appendix 2 Table 6.). However there was a two-way interaction between CS and PC. Groundnuts at 25, 50, 75 and 100 % PC were significantly more influenced by paraffin treatments than the other crop species. Apart from groundnuts, seeds immersed in 25 % PC generally produced seedlings with a high dry mass index. Within species, canola mean values decrease with the increase of PC and it is the same with Soya beans and wheat. Sunflower did not have any particular pattern. Studies done by Asli and Houshmandfar (2011) on safflower showed that the damage by diesel (close to paraffin) increased simultaneously with the diesel concentration. Luhach and Chaudhry

(2012), concluded that the germination stage is the most negatively affected of during the whole development of maize, vigna, pennisetum and sorghum.
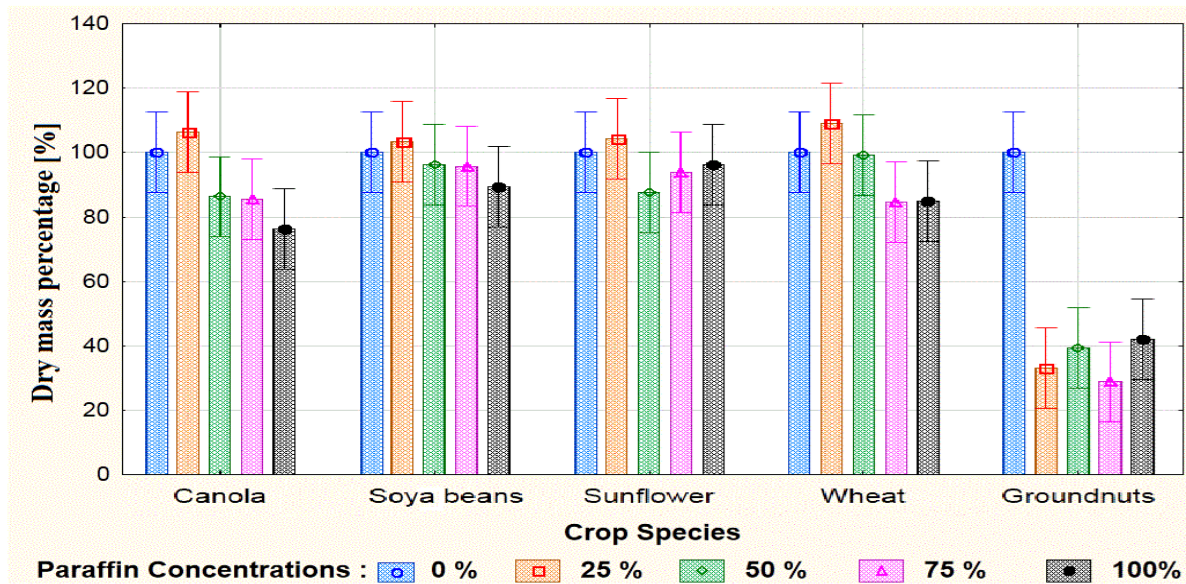
Figure 4.10: Effect of different paraffin concentrations and different crop species on the dry mass index of the seedlings. The vertical bars represent the standard error.

4.4 Conclusions

In order to be capable to germinate, the seed survives many damaging factors. Some side effects of pesticides used on the farm may influence the germination and the growth of the seedlings to large extent. This was the case with paraffin which is suspected to have pesticidal properties on seeds and plants. The results in this chapter has shown a strong negative effect on germination and/or establishment of common beans and maize, moderate negative influence on wheat and groundnuts but proved to be benign to canola, soya beans and sunflower with possible positive influence on germination and establishment of the seedlings. According to the Sharonova and Breuss (2012) scale, maize and beans are intolerant (under 30% germination/establishment), groundnuts and wheat are less tolerant (30-70%) and soya beans, sunflower and canola are classified amongst tolerant plants. Paraffin acted as germination activator on soya beans, sunflower and canola but mostly where the paraffin treated seeds germinated much better than the control (Arif et al. 2010).

In this study, paraffin influenced germination and establishment of the seedlings and the early stage of plant autotrophy. At a certain stage, the plant grows and develops without any consequence of having been treated with paraffin. If it is to be used as a pest repellent, it

would be required to increase the sowing density of the crop to a certain extent, depending on the susceptibility of the specific crop to paraffin treatment. Factors that need to be further investigated are the interaction of paraffin with the seed coat constitution, the oil seed content and the protrusion of the embryo. Such research could determine the most influential amongst those factors and could explain why groundnut seeds do not show a higher percentage of germination since they are bigger in size with the highest oil content compared to seed from the species investigated in this chapter. Further seed testing could confirm the relationship between seed oil content and its resistance to PAH (polycyclic aromatic hydrocarbon) in general and paraffin in particular. This could contribute to select suitable annual seeds potentially usable in bioremediation (Sharonova and Breus 2012), but could also give an indication of which crop species could be treated with paraffin as a pest repellent should it prove to be successful.

4.5 References

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Chapter 5

Detection of paraffin residues within plants growing from paraffin-treated seeds

Abstract

Paraffin is used in some cultures to treat crop seeds before sowing to repel birds and insects from attacking the germinating seeds and young seedlings. The question arises whether paraffin is taken up by the seed and if it remains in the tissues of the ensuing seedling. This study attempted to locate traces of paraffin within tissues of seeds and seedlings of selected crop species. This was done by fluorescence and confocal laser scanning microscopy. Seeds of soya beans were imbibed in commercial paraffin for 30 minutes, dried with a drying paper and germinated in pots filled with sand. In order to have a clear idea of how paraffin residue persists within the growing plant, transversal specimens were collected respectively after two, seven, 14, 28 and 56 days of planting. At two days samples were collected from the emerging plumule and the cotyledons while specimens were from the stem of the growing seedling at the later dates. Specimens were mounted on slides and stained with a solution of 100 µg.ml^{-1} of Nile Red for fifteen minutes. The excess of Nile Red was flushed with distilled water. The preparation was covered and the cover was fixed at the corners by soft glue. The slides were then observed in confocal and fluorescence microscopes which showed the presence of paraffin stained in golden yellow. The observed images were captured with the software ZEN 2011. The results showed droplets of paraffin in the seed coat of the soya bean seed germinating in the petri dish and residues in the endoderm of the soya bean stem.

5.1 Introduction

Seed in a paraffin polluted medium may germinate (Agarry et al. 2010), may die (Saadoun and Al-Ghazawi 2010) or may have low percentage germination (Luhach and Chaudhry 2012) depending on the type of seed or the concentration of the pollutant in the soil. There are different reactions from seed imbibed with paraffin, then planted in paraffin free soil and seed planted in soil polluted by paraffin (Sharonova and Breus 2012). In the second case, paraffin constantly disturbs the growth. The seed coat is permeable, capable of absorbing substances from the solution surrounding it or leaking electrolytes into the environment (Roberts 1986). The absorbed substances are capable of being metabolized by the plant or in

some cases can remain recalcitrant in the plant system, preferably translocated into specific tissues.

Paraffin, as derivative of petroleum, consisting solely of a mixture of hydrocarbon chemicals, does not have affinity to water (Njoku et al. 2009). Therefore it is not easy for paraffin to penetrate the seed in high concentrations as water remains the main medium to transport it through. Nevertheless minimal quantities of paraffin or its components are capable of forcing entry and cross the seed coat which has normally developed a type of selective permeability (Beeckman et al. 2000). The aim of this study was to determine if paraffin is taken up by seed when imbibed in paraffin and if it persists in the seedling.

5.2 Materials and methods

Soya bean was the only crop seed chosen to be used in this experiment as it contains a thin seed coat which would facilitate paraffin absorption into the seed. Fifty soya bean seeds in good condition were immersed in commercial paraffin for 30 minutes. The seeds were than taken from the paraffin and dried by on absorbent paper tissue. Dried seeds were planted in five plastic pots filled with sand where every pot contained ten seeds and the pots were watered with normal tap water till establishment. After two days the first germinating seed specimens to be studied under the microscope were collected from the soil and the plumule cotyledons examined for traces of paraffin. The remaining seedlings were then watered with a standard nutrient solution and the seedling specimens were collected 7, 14, 28 and 56 days after planting. At every collection, cross sections were cut with a sharp blade from the stem of the seedlings and four specimens were analyzed on the day of collection but the clearest one was retained as a figure in the text.

Microscopic experiments were carried out under facilitation of the Central Analytical Facilities (CAF) of the University of Stellenbosch. With a sharp razor blade, a thin cross section from the stem of each of the soya bean seedlings (apart from the first specimen which was obtained from the cotyledon of the germinating soya bean) was removed and mounted in the center of a glass slide by means of a needle. A solution of 10 µl Nile Red were diluted in 500 µl of phosphate –buffered saline (PBS) in a room with dim light at 22 ^{0}C. A droplet of the solution was added to every specimen and fixation took place for fifteen minutes (Kathryn and Oparka 1996, Tan et al. 2005). Nile red (9-diethylamino-5H-benzo[α] phenoxazine-5-on) is a hydrophobic probe having properties to depict the passage of hydrophobic chemicals such as paraffin (Figure 5.1).
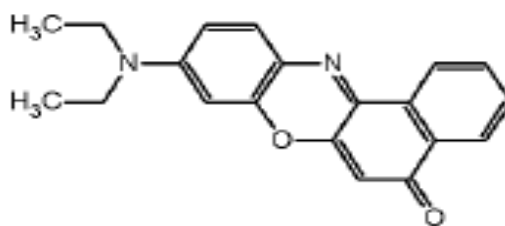
Figure 5.1: The structure of a Nile red molecule (Barasubramaniyam 2012).

It is nearly insoluble with water or hydrophilic chemicals (only soluble with organic solvents) and strongly fluorescent in the hydrophobic solvent. It does not stain fluorescence to lipids in the plants as it dissolves in lipids which diminish its fluorescence. Depending on the level of hydrophobicity of the stainable chemical, the observation in the microscope emits a red to yellow fluorescence (Greenspan et al. 1985, Barasubramaniyam 2012).

The excess of Nile red solution was removed by flushing three times with distilled water and dried with an absorbent paper tissue. A slide cover was fixed on the slide with soft glue (necessary because the slide is mounted upside down on the microscope to avoid glass refraction). The slide was mounted in the microscope, type LD Plan-Neofluar 60X/0.6 at the arbitrary fluorescence excitation intensity of 150 nm and the photos were captured with ZEN 2011 Software.

5.3 Results and discussion

At two days after planting of the soya bean, the seed had already germinated and the radicle showed some three millimeters outside the surface of the cotyledons. At that stage, biochemical activity has to convert the reserves in the seed into nutrients for the new seedling to expand at its full potential. Figure 5.2 shows some trail of yellowish color spread on the surface of the seed coat of the specimen without any defined pattern. This showed that during the imbibition period, the seed absorbed some paraffin which is retained mainly in the seed coat. Different densities on this figure express a differential in paraffin quantity distribution within the parts of the seed coat. In some areas the dots are deep and dense in yellow color (as it is shown with red letter **a** in the figure), but in some others they are very shallow (letter c), irregular and small (letter b).

Figure 5.2: Microscopic observation of the cross section of the seed coat of soya bean seed immersed in paraffin for 30 minutes and submitted to a germination process for two days. The yellow stains show residues of paraffin disseminated within the seed coat of the germinating seed. The letter A in the figure showed the outer part and B showed the inner part of the seed coat.

However, paraffin components gradually moved to the interior of the seed (Figure 5.3) within the cotyledon tissue or in the embryo. During the mixing paraffin-seed process, the short chain molecule components, under 10 atoms of carbon (Tidd 2011), evaporate. The long chain molecules persist and penetrate through the seed coat in a small quantity as observed in chapter 3 where 10 grams of soya beans absorbed only 1 ml of pure paraffin.
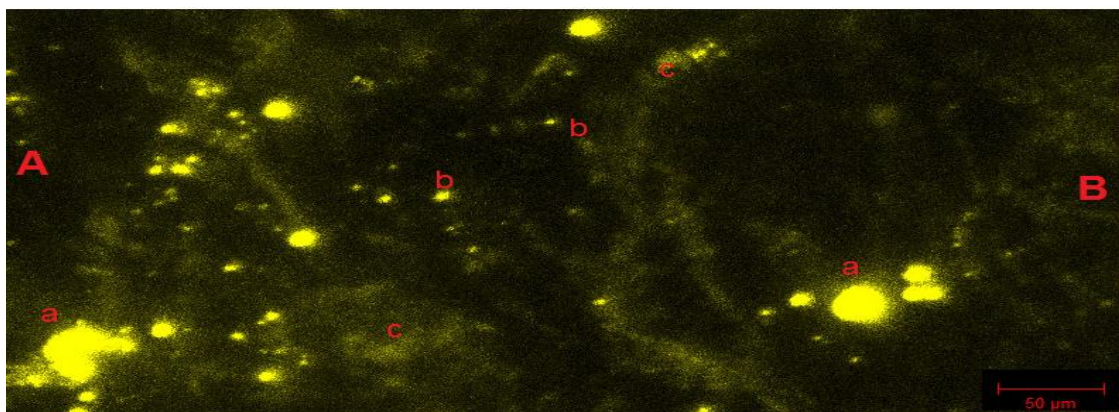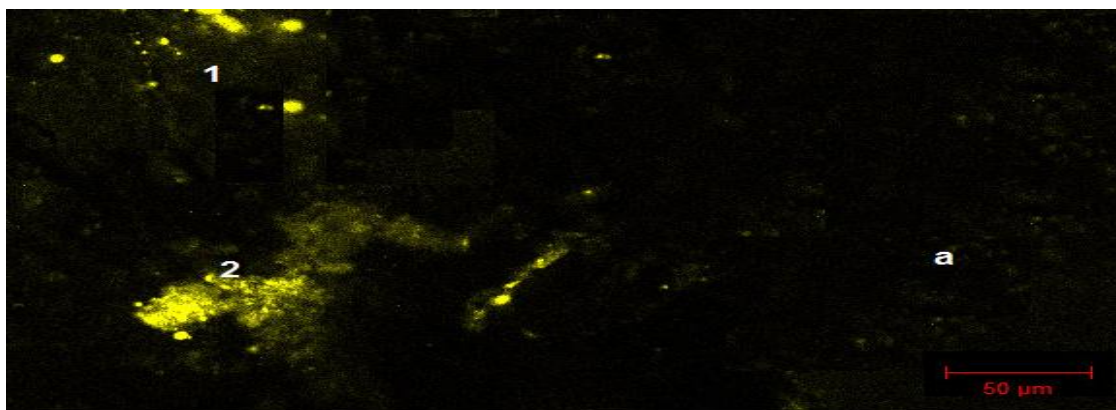
Figure 5.3: Microscopic observation of the cross section of the cotyledon of soya bean seed immersed in paraffin for 30 minutes after germination for two days. The yellow densely stained area (left side of the photograph marked by numbers 1and 2) is the outer part of the seed and the interior is marked by a poor paraffin residue presence (with letter a in the figure).

The paraffin uptake (Baek et al. 2004) makes it available within the tissues of the plant as they develop. As the seedling developed, the paraffin in the seed might have continued to move slowly within the seed as some residues were also absorbed even within the plumule of the seedling (Figure 5.4). Obviously the more the paraffin gets distributed within different parts of the germinating seedling system, the quantity of paraffin indicated by the deepness and density of yellow stains in the figures was reduced. There was no distinctive localization of wide stains or small residues of paraffin within the regions of the cross sections of the plumule.
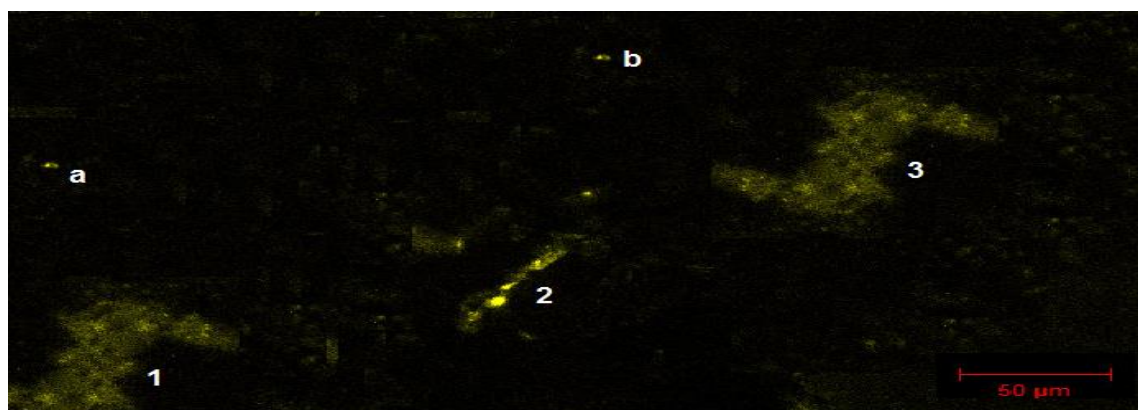
Figure 5.4: Microscopic observation of the cross section from the plumule of germinating soya bean after the seed was immersed in paraffin for 30 minutes. Letters showed small quantities of paraffin residues dispersed within the plumule while the numbers showed wide stains where paraffin residues are concentrated.

After one week of growth, the paraffin quantity had dropped substantially as shown by the small and dispersed yellow dyed residues (Figure 5.5). The center of the herbaceous stem appears to be free of paraffin droplets. Only ten small dots of yellow stains were observed in Figure 5.5. The ideal amount of paraffin acting as a pesticide would have been indicated by the presence of a quantity that would have spread through the whole specimen coloring all the cells in yellow.

Figure 5.5: Microscopic observation of the cross section of the stem of seven day old soya bean seedlings grown from seed immersed in paraffin for 30 minutes. Yellow stains in the black background show droplets of paraffin. The specimen was collected from half way up the stem.

However, as repelling chemical, the low presence would suffice as its mode of action is to act on the nervous system of a predator as a warning method. That warning system conditions a response of avoiding such food which would create discomfort (Avery et al. 2001, Werner and Clark 2003).

However, the concern is what happens to paraffin in the system and what are the physiological consequences, Paraffin migrates within the seedlings accelerated by the metabolic activities in the plant (Rice and Rohrbaugh 1953). Paraffin residues were found within the stems of soya beans as shown in Figure 5.5. The droplets of paraffin staining observed in the plant systems were transported throughout the intercellular space of the phloem parenchyma (Rice and Rohrbaugh 1953) and not in the xylem vessels. In her research on fescue (*Festuca arundinacea*), Barasubramaniyam (2012) confirmed the findings of Rice and Rohrbaugh (1953). She planted *Festuca* on two different media, one contaminated with hydrocarbon and the control (hydrocarbon free). At a certain stage of growth (136 days from planting), the watering system was interrupted and the two groups of plants started to wilt in different ways. Control plants were quick to wilt and dry while the contaminated plants survived much longer. Microscopic observations showed that the cell walls of the endodermis (part of phloem) were thickened by the presence of the hydrocarbon (a sign of adaptation to the presence of a xenobiotic element) slowing down the wilting capacity (Barasubramaniyam 2012). The hydrocarbon was easily detectable because *Festuca* had absorbed enough of it as it was planted in the soil contaminated by long chained hydrocarbons.

Two weeks after planting, soya bean stems had reached a length of 15 cm with six leaves. Figure 5.6 highlights the gradual exhaustion of the paraffin in the system. The contact of paraffin with the plant leads to its absorption by the plant and spread in the plant system via the epidermis. Eventually the excessive dosage of paraffin in the vicinity of the root leads to its adaptation or at the extreme level toxicity and death of the plant. The adaptation to the stress caused by the hydrocarbon may manifest in a three-way reaction: lignification of the cell walls, shortening of the root system and increase of root diameter (Bouma et al. 2000). The components of paraffin in the plant system experience three destinies: translocation activated and maintained by transpiration (Barasubramaniyam 2012), accumulation within the endodermis or/and catabolism which breaks n-Alkanes (C_{10} - C_{22}) into non-toxic products like CO_2 and H_2O (Fismes et al.2002, Robson 2003).
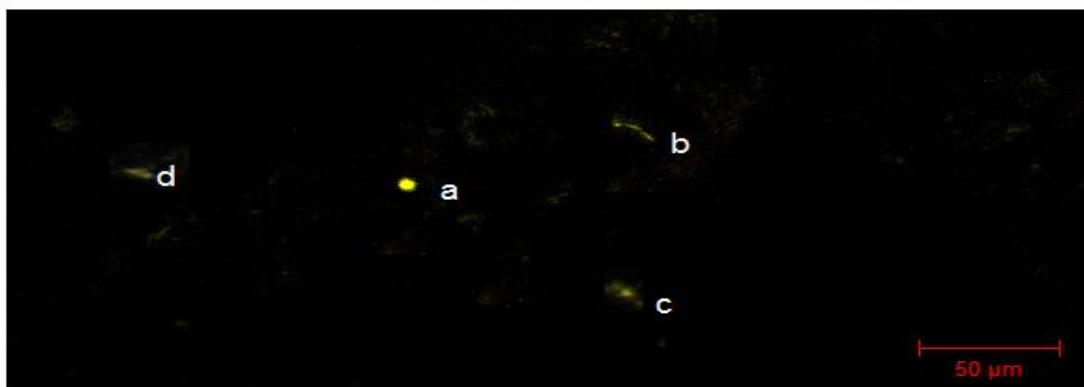
Figure 5.6: Microscopic observation of the cross section from the stem of 14 days old soya bean seedling grown from seed immersed in paraffin for 30 minutes. Yellow stains in the black background show droplets of paraffin. The specimen was collected from half way up the stem.

Aromatic hydrocarbons do not metabolize easily (Phillips et al. 2006). There are few angiosperms that have the ability to break down hydrocarbon molecules into simpler and less toxic molecular compound reusable by the plant. Edwards et al (1982) mentioned soya beans and bush beans while Durmishidze (1977) highlighted walnuts, quince and grapes. They proposed that the metabolic model of degradation of n-alkanes where complex enzymatic oxidation, reduction and hydrolysis reactions take place to reduce toxic plant substances into normal compounds (Walton et al 1994). Eweis et al (1998) described the pathway in a growing plant as follows: N-alkane ---> primary alcohols ----> acetyl-CoA ---> various compounds. The biodegradability of paraffin in any soil at a certain dosage is a good indication of its possible use in the farm without any fear of pollution.

The microscopic observations of specimens collected at 28th and 56th days after planting did not show any relevant sign or traces of paraffin in the plant system. The samples were similar to the specimen collected from the control treatment control which showed just a dark color without any trace of fluorescent product. Lots of unanswered questions about the toxicity of hydrocarbons, particularly paraffin, on plants or plant systems and the plant defensive reactions are still speculative (Bona et al 2011). However, there is one certainty: continuous exposure of the plant root system to hydrocarbons damages the plant health and decreases its sustenance growth with a general decrease of biomass (Baek et al. 2004, Asli and Houshmandfar 2011, Ehiagbonare et al. 2011, Luhach and Chaudhry 2012).

Paraffin absorbed by the plant during imbibition should not be a threat to human health (OJEU 2009) because it gets reduced by plant metabolism as the plant grows. Results in Chapter 4 show that imbibed paraffin ceases to affect vegetative growth of the plant significantly a few weeks after germination.

5.4 Conclusions

This study showed that a very small amount of paraffin is taken up into the seed endosperm and is present in very small amounts in the stem of two weeks old seedlings. These amounts are probably not enough to harm the growth of the plant or to act as pesticide in the plant but may be enough to act as repellent to predators of the seed and young seedlings. However a thorough study to determine the threshold values of different crop plants to paraffin exposure is needed before paraffin can be considered as a pest repellant.

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Chapter 6

Influence of paraffin on early canola growth and its possible pest repellency in the field

Abstract

The period from seed germination to establishment of the seedlings is a critical period for crop plants in particular, due to mortalities caused by phytophage pests. Canola (*Brassica napus* L.) is no exception and serious stand losses can occur during this phase.

Paraffin has been described as a pest repellent of crops during the establishment and early growth stages of crop plants in rural areas in Africa and is used intensively in Canada to stop different species of aphids that are vectors of potatoes and soya beans viruses. In this study, seeds of canola were treated with paraffin just before planting for protection against insects, slugs and snails. Canola seeds were immersed in two liquids (water and paraffin) for different periods of time (0, 1, 5, 10 and 30 minutes). The treatments were repeated four times. The seeds were planted in the field approximately four hours later. Stand density was assessed at 12 weeks after planting and leaf area and dry mass were determined. After 21 weeks the second harvest to determine dry mass was carried out. At the 27th weeks the plots were harvested to determine yield at maturity. Paraffin treatments significantly improved stand density and plant size after 12 and 21 weeks respectively. At 27 weeks the differences disappeared. No significant differences with regard to final yield were observed. Paraffin appears to hold promise as a pest repellent in canola in areas where severe seedling predation occurs.

6.1 Introduction

Canola (*Brassica* napus L.), a plant in the Brassicaceae family is a winter crop (Chastain n.d.) frequently attacked by pests including larva or adult insects, snails, slugs, flea beetles, grasshoppers, cutworms, aphids, sugar beetroot fly and maggots (Pekrun et al. 1998). Some of those pests survive more than one season on weeds or other crops in a rotational system. In South Africa canola is susceptible to red-legged earth mite, blue oat mite and cabbage aphid and lucerne flea in the early growth stages and diamond-back moth and cotton bollworm in the later growth stages. Snails and isopods are particularly troublesome predators that attack the crop in the germination and establishment phase (Anonymous 2013).

The fragility of the crop seed and seedling (Bybordi and Tabatabaei 2009) to chemicals makes the application of any chemical applied on it risky as it may impact on the yield (Mauromicale and Licandro 2002). Only chemicals which cause minor damage to the crop and increase yield are acceptable as pesticides (Rojas et al. 1999). However, environmentalists and nature conservation specialists advocate the use of pest repellents because they are more environmentally friendly (Whitford et al. 2003).

In repellency, touch and chemical senses; olfactory, gustatory and chemesthetic systems (irritation and/or pain) are the key paired points of parasites to be stimulated particularly in systemic repellency where the active ingredient of the repellent is mixed with the material to be protected (Najar-Rodriguez et al. 2008). Parasites have developed specialized neurons called nociceptors capable of providing translated information about noxious chemicals (Werner and Clark 2003). Nociceptors provide consequential information of potential tissue damage etc. as precursors of adaptive function within the parasite physiology (Clark 1998). Some researchers have even started to investigate the efficacy of the long chained hydrocarbon chemical found in paraffin oils, such as nC$_{24}$ as pest repellents (Najar-Rodriguez et al. 2007, Kreiss and Cullen 2008, Najar-Rodriguez et al. 2008).

In an attempt to deter crop predators, small scale farmers in Central Africa have utilized commercially obtainable paraffin as a pest repellent to protect soya bean (*Glycine max* L. Merr) seeds and seedlings throughout the vegetative stage of the crops (Personal observation). The seeds are washed with paraffin and left to dry in the sun before planting. The possibility therefore exists that crops that are not sensitive to paraffin seed treatment may be treated with paraffin to repel predators in the early phases after planting. The aim of this experiment was to investigate the potential of paraffin as a pest repellent on canola grown in a field in the South Western Cape region of South Africa.

6.2 Materials and methods

6.2.1 Experimental procedure

Seeds of canola (44C11) were immersed in either commercial paraffin or distilled water for zero, one, five, ten or thirty minutes. The treatments were repeated four times. After immersion seeds were dried with water absorbent paper. Approximately four hours after the treatments were applied in the laboratory the seeds were planted on the Department of Agriculture: West Cape experimental farm of Langgewens located at 33^0 26' S, 17^0 70' E. Seeds were planted in 40 plots of 1.36 m X 5 m each grouped in two blocks separated by a

1.5 meter path to avoid possible influence of paraffin on water treated plots. In each block (water treated and paraffin treated) the time treatments were randomly applied. In every plot 4 g of canola seeds were planted on May 8th 2013. Plots were treated only with herbicides and no pesticides were applied. Fertilizers (limestone ammonium nitrate and potassium nitrate) were applied according to prescribed fertilization regimes.

Stand density was recorded 12 weeks after sowing by randomly placing a 50 cm X 100 cm rectangle in each plot and counting all the plants in the frame. At the same time the leaf area and dry weight of plants was determined. By harvest, 10 randomly selected plants from each plot. The leaf areas of the plants were determined by means of a Li-Cor Model 3100 area meter and the mean leaf area per plant for each treatment was calculated. Plants were dried in a drying oven at 80º for 48 hours to determine dry mass and the mean dry mass per plant for each treatment was calculated.

The second destructive harvest after 21 weeks focused on comparison of the dry mass of ten plants randomly harvested from every plot. Leaf area was not measured because most of the leaves had dropped by that stage with green pods in the maturing phase. The final data, which was recorded, was the yield by harvesting each plot with a plot combine harvester 27 weeks after planting. The data was calculated as gram canola seed produced per m^2.

6.2.2 Statistical analysis

A randomized complete block experimental design was used, with treatment laid in a split plot. Forty plots were spatially grouped into two separate blocks. Twenty plots received the five paraffin treatments replicated randomly four times and the other twenty plots received the five water treatments also replicated randomly four times. The treatment liquids were distilled water and paraffin and Time of immersion (0, 1, 5, 10 and 30 minutes) replicated four times. The collected data were analyzed using STATISTICA, software version 11. Wherever main effects or interaction effects were found to be significant, examination of the post-hoc tests was done to determine between which levels of each factor the differences lie, after adjusting for multiple comparisons at $p = 0.05$ (Clewer and Scarisbrick 2001, Rossiter 2006).

6.3 Results and discussion

6.3.1 First harvest

6.3.1.1 Stand density

The ANOVA table (Appendix 3, Table 1) showed that there was a significant interaction between Time of immersion and Treatment liquid. This means that both treatment liquid and time of immersion have a combined effect on the stand density of canola. The post-hoc analysis using Fisher's LSD test showed that the control (0 min TOI) in the paraffin treatment was significantly ($p < 0.05$) different from 5 min TOI and 30 min TOI. Immersion in paraffin appeared to stimulate establishment of canola while immersion in water appeared to inhibit establishment of canola. Canola establishment after immersion in water however, was not statistically ($p > 0.05$) different from establishment percentages in the control treatment.

The paraffin treated seeds resulted in a mean stand density (38 plants m^{-2}) almost double that of the water treated seeds (22 plants m^{-2}) (Figure 6.1). This results in a cumulative difference of 26.6% which is high enough to indicate that paraffin appears to have a positive effect on the establishment of canola or else it has a protective effect on the seed and seedlings that could result in a higher stand density (Micic 2005). Canola seed therefore probably have similar properties than seeds that are used in phytoremediation of paraffin contaminated soil (Robson 2003).

Figure 6.1: The effect of time of pre-treatment of canola seed with water and paraffin on the stand density per m^2 of canola plants 12 weeks after planting on Langgewens Experimental Farm. Whiskers represent the standard error of the means.

6.3.1.2 Leaf area

No two way interaction was recorded between the Time of immersion and Treatment liquid but treatment liquid had a significant ($p < 0.05$) effect on the mean leaf area of the plants. (Appendix 3, Table 2). The water treated seeds resulted in a mean leaf area (2133.86 cm^2) that was significantly less than the leaf area from the paraffin treated seeds (2807.71 cm^2) (Figure 6.2). It would be expected that the lower plant density in the water treatment would result in compensatory growth and the leaf area would not be smaller than the paraffin treatment but this was not the case. It therefore appears as if the paraffin in some way or other could have stimulated growth of the seedlings too. The small amount of damage from predation in some of the water treatment plots (Figure 6.4) could probably not have caused such a big difference in leaf area between the two treatments.
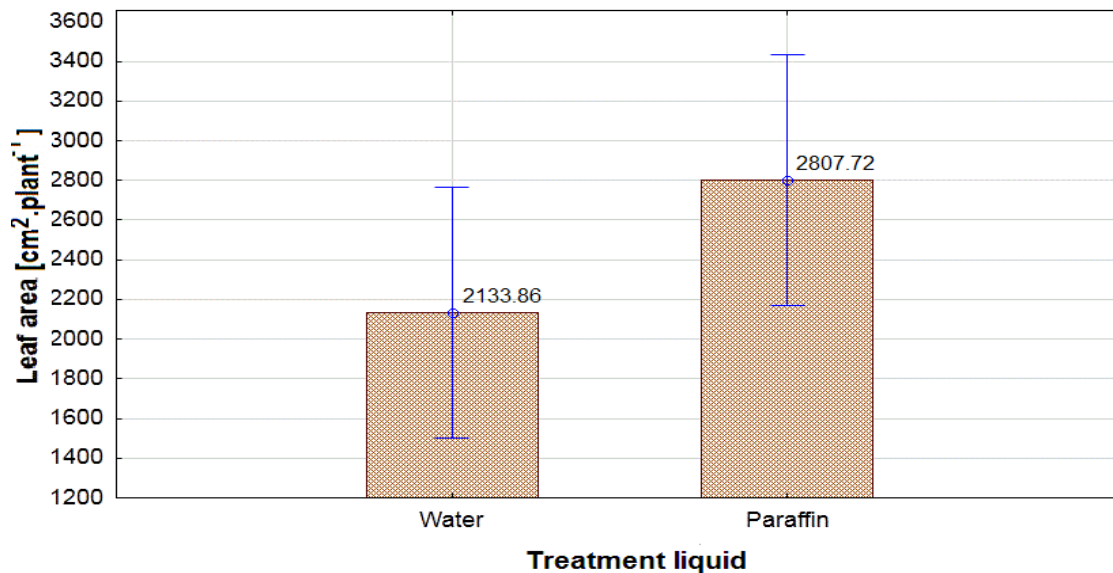
Figure 6.2: The effect of pre-treatment of canola seed with water and paraffin on the mean leaf area plant^{-2} of canola plants 12 weeks after planting on Langgewens Experimental Farm. Whiskers represent the standard error of the means.

6.3.1.3 Dry mass at first harvest

No two way interaction was recorded between the Time of immersion and Treatment liquid, but treatment liquid had a significant ($p < 0.05$) effect on the mean dry mass of the plants. (Appendix 3, Table 3). The paraffin treated seeds resulted in plants with 45.7% more dry

mass than the water treated plants (Figure 6.3). This supports the trend that was observed in stand density and leaf area.
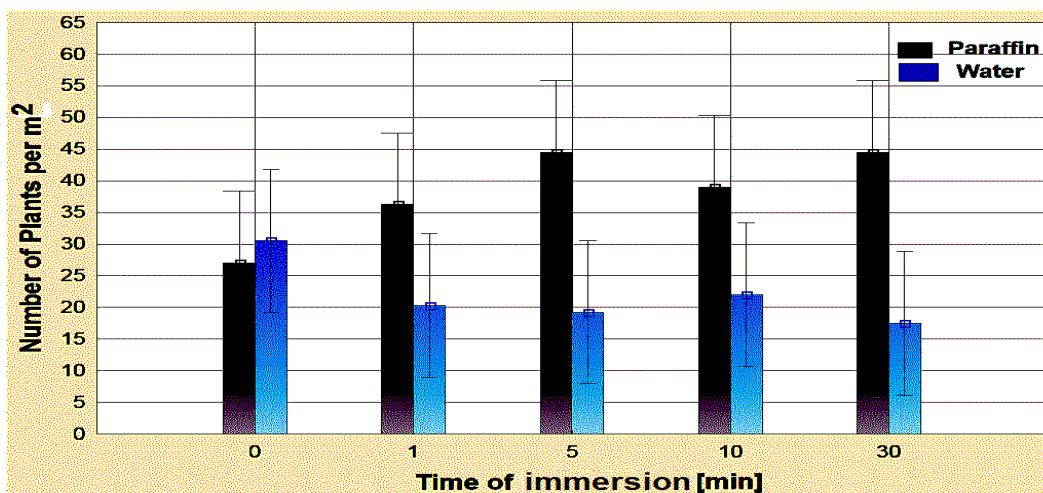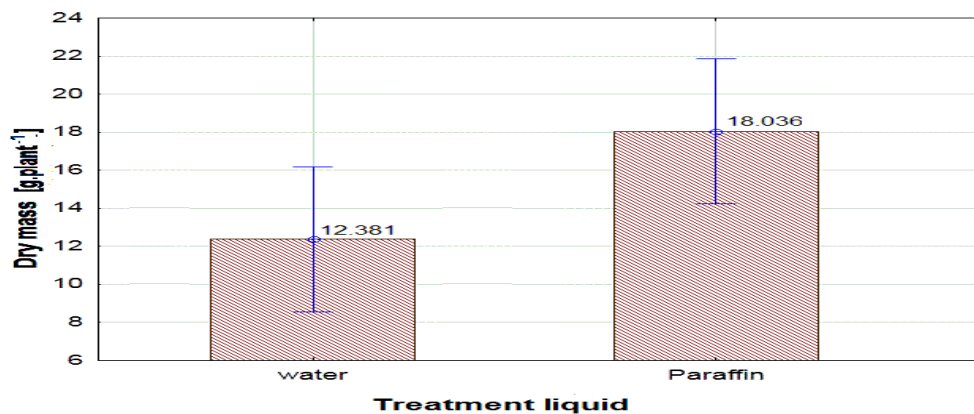

Figure 6.3: The effect of pre-treatment of canola seed with water and paraffin on the mean dry mass plant^{-2} of canola plants 12 weeks after planting on Langgewens Experimental Farm. Whiskers represent the standard error of the means.

6.3.1.4 Visual appearance

There were not any malformation or abnormality of leaves, stem or roots visible 12 weeks after planting in the paraffin treated plants. In three of the water treated plots signs of insect damage on the leaves were observed (Figure 6.4) with visible holes in the middle and on the edges of the leaves.

No insect damage was observed in the paraffin treated plots 12 weeks after planting. Very little pest infestation of canola was observed (even in the control plots) during the 2013 season on Langgewens Experimental Farm. It is therefore assumed that the positive influence of the paraffin treatment on canola establishment and early growth are more a result of increased vigour after paraffin treatment than protection against pests but this would need to be confirmed in follow-up studies.
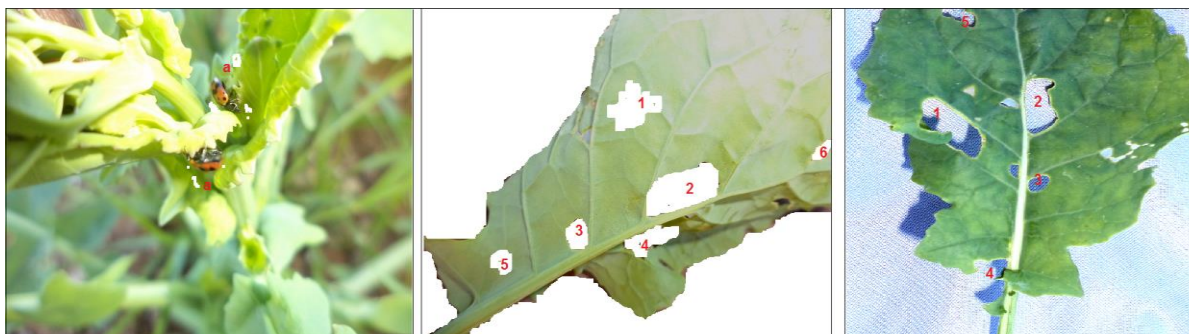
Figure 6.4: Insect damage on leaves of canola in the plots where seeds were treated with water. The red letters on the figure show insects which were found feeding on the leaves of canola and the numbers show holes within the leaves made by parasites.

6.3.2 Second harvest

In terms of total dry mass plant^{-1} there were no significant interactions between Time of immersion and Treatment liquid as well as no significant differences within factors (Appendix 3, Table 4). Mean dry mass values per plant were 186.04 g in the water treatment and 205.89 g in the paraffin treatment with the highest mean dry mass value of 231.50g on plants from seeds that received ten minutes of paraffin imbibition and the lowest on paraffin control with 169.46g (Results not shown). It appears as if the beneficial effect of paraffin treatment of the seeds observed at 12 weeks after planting disappeared at by 21 weeks after planting. The plants in the paraffin treatment were still bigger than those in the water treatment but the difference was not significant and the 45.7% increase in dry mass observed after 12 weeks had dwindled to 10.7% after 21 weeks. This could be a result of the compensatory growth by the canola plants where plants in a less dense stand branched more and became bigger and thus compensated for the lower stand density at establishment (Koenig et al. 2011).

6.3.3 Final harvest

There was no two-way interaction between Time of immersion and Treatment liquid and no differences within the main effects on the yield of canola (Appendix 3, Table 5). This indicates that the yield was not affected statistically by the treatment factors. Figure 6.5 shows that the yield in the paraffin treatment is only about 4.20 % higher than the yield in the water treatment.
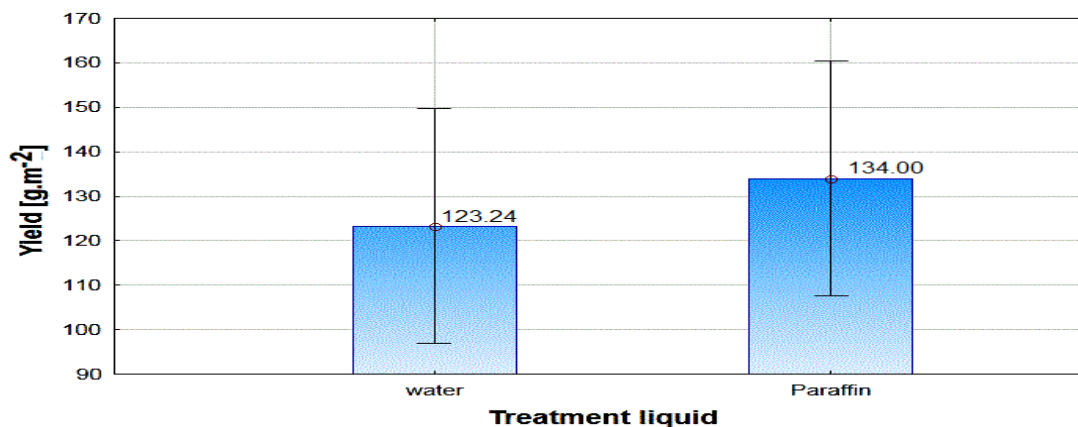
Figure 6.5: The effect of the pre-treatment of canola seed with water and paraffin on the mean seed yield at final harvest on Langgewens Experimental Farm. Whiskers represent the standard error of the means.

6.4 Conclusions

The paraffin applied to canola seeds did not cause any damage, but rather increased the seedling density on the farm where lots of factors can cooperate to disturb seed germination and seedling development. Paraffin treatments stimulated the establishment of canola plants and increased the leaf area and the dry mass production of canola in the early growth stages. However, the paraffin treatments did not affect subsequent vegetative growth or seed yield of the plants. Paraffin did not negatively impact in any way the growth and seed production of the plant. Since insect damage was minimal on this particular farm in this particular season the repellent effect of paraffin could not be clearly established. Similar experiments should be carried out in various localities and seasons to test the repellent action of paraffin and also to confirm that the paraffin does not have any negative impact, but indeed positive impacts on establishment and early growth of canola under various climatic and soil conditions.

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Chapter 7

General conclusions

Since its discovery paraffin has been a commodity with a wide range of uses, from domestic (medicine, household cleaning, lighting and cooking) to industrial (source of energy, organic solvent in industries as well as in agriculture). These multiple uses are due to its properties which are linked to its nature as mixture of hydrocarbons: cyclic and aliphatic chains between 10 and 16 carbons (Leifer 2006, Tidd 2011). Industrial development resulted in research providing substitutes for paraffin that are generally more efficient with fewer side effects. This did not deter some people from African countries (Osekre and Opoku-Agyeman 2005, Wale 2003) and some horticulturists to continue to use paraffin as pesticide or pest repellent, particularly against insects. Seeds are washed with commercial paraffin before they are planted. However, doubts remain whether paraffin is not detrimental to the germination and growth of seed and seedlings and its efficiency as pest repellent.

This study was initiated to try to provide clarity on the effect of paraffin on germination and growth of seven crop species and also to test the repellency value of paraffin on a canola crop. A series of experiments were conducted on crop seeds viz. canola (*Brassica napus* L.), common beans (*Phaseolus vulgaris* L.), ground nuts (*Arachis hypogea* L.), maize (*Zea mays* L.), soya beans (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and wheat (*Triticum aestivum* L.). Investigations focused on the effects of pre-treatment in different paraffin concentrations for different periods of time on germination percentage and vigour of the resulting seedlings. Canola was selected as a crop produced in the Western Cape to test the pest repellency potential of paraffin.

Canola, soya beans and sunflower germinated and established very well (over 70% germination), with a severe sensitivity for wheat and groundnuts (around 40% germination) and a severe drop in germination for maize and beans (under 30%). According to Sharonova and Breus (2013) these three groupings of crops could be classified as more tolerant, less tolerant and intolerant of paraffin respectively. It was also found that as the seedling grows, some residues of paraffin are detected within the endodermis of the growing plant and this confirmed findings of Robson (2003) and Barasubramaniyam (2012). Repellency was tested in a field experiment and although the crop was pest-free to a large extent, some observations on the farm showed that paraffin probably repelled pests on canola.

It was concluded that paraffin would not be successful as a pesticide but rather as pest repellent as the quantity retained by the seed coat and endosperm during imbibition is too small to kill parasites. However, as it gets transferred within the plant system, its repellency potential could be explained, as parasites might feed on the plant and develop malaise. The malaise would create a learning action in the nervous system which helps the pest to avoid feeding on the plant. It is strongly believed that this type of action causes paraffin to act as a repellent.

However, the mode of action of paraffin as repellent and its spectrum of action in general should be investigated further. Though repellents are normally environmentally friendly, many farmers do not trust them because in their opinion, not killing the parasite could end up creating a resistance mechanism within the parasite (Isman 2006). They therefore simply turn to the available pesticides that would give quick and efficient control of the parasites.

This study simply introduced the subject; there is a need to extend the investigation to a wide range of seed species even cultivars within the species. The response of more crop seeds to paraffin in terms of germination, vigour and establishment could help to explain the various responses observed in this study. Such research should also investigate the mechanism which for instance, causes canola to resist paraffin treatment but which causes beans and maize germination to stop almost completely.

As paraffin taken up by the seed from the imbibition process get translocated and distributed within the plant system (Kathryn and Oparka 1996, Barasubramaniyam 2012), detailed quantifying analyses should investigate its presence in the grain harvested. There is a need to investigate the pest repellency of paraffin on crop species and different varieties to observe the possible internal variations within the same species. Experiments extended to different crops in different seasons on a farm heavily infested with pests would bring more clarity about the pest repellent actions of paraffin. It is known that the lipophilic sites on the hydrocarbon nC_{24} have pesticidal properties (Najar-Rodriguez et al. 2007, Najar-Rodriguez et al. 2008). It remains questionable whether some close molecules such as nC_{20} would not have similar properties since the active principal is simply based on lipophilic properties. Analytical investigations would also help to determine which of the components of paraffin mixture remain efficient as repellent in the plant.

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Appendices

Appendix 1

Table 1: ANOVA for the germination percentage of different crop species after seeds were immersed in different paraffin percentages for different time periods

Effect	Df	SS	MS	F	p
Intercept	1	2598288	2598288	36957.80	0.000000
Time of immersion (TOI)	3	292	97	1.39	0.246719
Paraffin concentration (PC)	4	48349	12087	171.93	0.000000
Crop species (CS)	6	268009	44668	635.36	0.000000
TOI*PC	12	2030	169	2.41	0.005068
TOI *CS	18	6878	382	5.43	0.000000
PC *CS	24	85051	3544	50.41	0.000000
TOI*PC*CS	72	20493	285	4.05	0.000000
Error	420	29528	70		

Table 2: ANOVA for the germination rate of different crop species after the seeds were immersed in different paraffin percentages for different time periods

Effect	SS	Df	MS	F	p
Intercept	3098834	1	3098834	870.5030	0.000000
Time of immersion (TOI)	25367	3	8456	2.3753	0.069559
Paraffin concentration (PC)	140816	4	35204	9.8893	0.000000
Crop species (CS)	344235	6	57373	16.1167	0.000000
TOI*PC	22689	12	1891	0.5311	0.894550
TOI *CS	67049	18	3725	1.0464	0.405925
PC *CS	104257	24	4344	1.2203	0.218452
TOI*PC*CS	173562	72	2411	0.6772	0.978194
Error	1495125	420	3560		

Table 3: ANOVA for EC of seeds of different crop types treated with different paraffin percentages for thirty minutes

Effect	SS	Df	MS	F	p
Intercept	47595912	1	47595912	31131.98	0.000000
Paraffin concentration (PC)	216869	4	54217	35.46	0.000000
Crop species (CS)	20420283	6	3403381	2226.12	0.000000
PC *CS	788277	24	32845	21.48	0.000000
Error	53509	35	1529		

Table 4: ANOVA for the quantity of paraffin absorbed by seed of different crop species during the 30 minutes of immersion in different paraffin concentrations

Effect	SS	Df	MS	F	p
Intercept	343.8095	1	343.8095	2123.529	0.000000
Paraffin concentration (PC)	115.0476	4	28.7619	177.647	0.000000
Crop species (CS)	30.8571	6	5.1429	31.765	0.000000
PC *CS	34.9524	24	1.4563	8.995	0.000000
Error	11.3333	70	0.1619		

Table 5: ANOVA for the quantity of water absorbed by seed of different crop species during the 30 minutes of immersion in different paraffin concentrations

Effect	SS	Df	MS	F	p
Intercept	2419.200	1	2419.200	527.0041	0.000000
Water Concentration (WC)	872.610	4	218.152	47.5228	0.000000
Crop species (CS)	168.533	6	28.089	6.1189	0.000034
WC*CS	272.324	24	11.347	2.4718	0.001791
Error	321.333	70	4.590		

Appendix 2

Table 1: ANOVA of the effect of the pre-treatment of different seeds of crop species with different paraffin concentration percentages at different times of immersion on establishment percentages in the glasshouse

Effect	Df	SS	MS	F	P
Intercept	1	1499405	1499405	10484.34	0.000000
Crop species (CS)	6	376045	62674	438.24	0.000000
Paraffin concentration (PC)	4	173853	43463	303.91	0.000000
Time of immersion (TOI)	3	1470	490	3.43	0.017220
CS *PC	24	128324	5347	37.39	0.000000
CS *TOI	18	2715	151	1.05	0.396819
PC *TOI	12	2947	246	1.72	0.060609
CS*PC *TOI	72	11912	165	1.16	0.194024
Error	420	60066	143		

Table 2: ANOVA of the effect of the pre-treatment of different seeds of crop species with different paraffin concentration percentages at different times of immersion on the stem length three weeks after plantating

Effect	SS	Df	MS	F	p
Intercept	2332447	1	2332447	5597.479	0.000000
TOI	6777	3	2259	5.421	0.001209
CS	542632	4	135658	325.556	0.000000
PC	8209	4	2052	4.925	0.000737
TOI*CS	41865	12	3489	8.372	0.000000
TOI*PC	2738	12	228	0.548	0.882399
CS*PC	19957	16	1247	2.993	0.000118
TOI*CS*PC	19505	48	406	0.975	0.524186
Error	125009	300	417		

Table 3: ANOVA of the effect of the pre-treatment of different seeds of crop species with different paraffin concentration percentages at different times of immersion on the stem length seven weeks after plantating

Effect	SS	Df	MS	F	p
Intercept	4026449	1	4026449	5610.882	0.000000
Time of immersion (TOI)	203	3	68	0.094	0.963069
Crop species (CS)	27798	4	6949	9.684	0.000000
Paraffin concentration (PC)	2880	4	720	1.003	0.405985
TOI*PC	4992	12	4160	5.797	0.375984
TOI*CS	5703	12	475	0.662	0.787172
PC*CS	32079	16	2005	2.794	0.000318
TOI*PC*CS	44430	48	926	1.290	0.106706
Error	215284	300	718		

Table 4: ANOVA of the effect of the pre-treatment of different seeds of crop species with different paraffin concentration percentages at different times of immersion on the tolerance index of the roots three weeks after plantating

Effect	SS	Df	MS	F	p
Intercept	4156912	1	4156912	1.844565	0.00562
Time of immersion (TOI)	10172	3	3391	1.504615	0.00436
Paraffin concentration (PC)	16990	4	4247	1.884744	0.00000
Crop species (CS)	31620	4	7905	3.507702	0.00190
TOI*PC	7473	12	623	2.763476	0.00238
TOI*CS	162195	12	13516	5.997641	0.00253
PC*CS	48743	16	3046	1.351823	0.00000
TOI*PC*CS	71105	48	1481	6.573268	0.00124
Error	476925	300	24376		

Table 5: ANOVA of the effect of the pre-treatment of different seeds of crop species with different paraffin concentration percentages at different times of immersion on the dry mass three weeks after plantating

Effect	SS	Df	MS	F	p
Intercept	3877243	1	3877243	5764.072	0.000000
TOI	11599	3	3866	5.748	0.000779
SC	43094	4	10774	16.016	0.000000
PC	2975	4	744	1.106	0.353978
TOI*SC	29681	12	2473	3.677	0.000035
TOI*PC	4112	12	343	0.509	0.908201
SC*PC	32182	16	2011	2.990	0.000120
TOI*SC*PC	45386	48	946	1.406	0.048172
Error	201797	300	673		

Table 6: ANOVA of the effect of the pre-treatment of different seeds of crop species with different paraffin concentration percentages at different times of immersion on dry mass seven weeks after plantating

Effect	SS	Df	MS	F	p
Intercept	2892117	1	2892117	4488.959	0.000000
TOI	3913	3	1304	2.024	0.110520
CS	135108	4	33777	52.427	0.000000
PC	23612	4	5903	9.162	0.000001
TOI*CS	12630	12	1052	1.634	0.081482
TOI*PC	5612	12	468	0.726	0.725892
CS*PC	49557	16	3097	4.807	0.000000
TOI*CS*PC	27448	48	572	0.888	0.684552
Error	193282	300	644		

Appendix 3
Table 1: ANOVA of the effect of the pre-treatment of canola seed with water and paraffin on the stand density per m^2 of canola plants 12 weeks after planting on Langgewens Experimental Farm

Effect	Num. Df	Den. Df	F	p
Time	4	24	0.19794	0.937007
Treatment	1	3	28.25847	0.013002
Time*Treatment liquid	4	24	3.10362	0.034202

Table 2: ANOVA of the effect of the pre-treatment of canola seed with water and paraffin on the mean leaf area of canola plants 12 weeks after planting on Langgewens Experimental Farm

Effect	Num. Df	Den. Df	F	p
Time	4	24	1.07828	0.389195
Treatment	1	3	10.40785	0.048358
Time*Treatment	4	24	1.08518	0.386019

Table 3: ANOVA of the effect of the pre-treatment of canola seed with water and paraffin on the mean dry mass of canola plants 12 weeks after planting on Langgewens Experimental Farm

Effect	Num. Df	Den. Df	F	p
Time	4	24	0.27265	0.892690
Treatment	1	3	17.45545	0.024977
Time*Treatment	4	24	0.64054	0.638744

Table 4: ANOVA of the effect of the pre-treatment of canola seed with water and paraffin on the mean dry mass of canola plants 21 weeks after planting on Langgewens Experimental Farm

Effect	Num. Df	Den. Df	F	p
Time	4	24	1.363123	0.276143
Treatment	1	3	2.460008	0.214783
Time*Treatment	4	24	0.414180	0.796688

Table 5: ANOVA of the effect of the pre-treatment of canola seed with water and paraffin on the mean yield of canola at final harvest after 27 weeks of planting on Langgewens Experimental Farm

Effect	Num. Df	Den. Df	F	p
Time	4	24	1.363123	0.276143
Treatment	1	3	2.460008	0.214783
Time*Treatment	4	24	0.414180	0.796688