University of Pretoria etd - Van der Laan M 2006

CHAPTER IV – PERSISTENCE OF PARTHENIN IN SOIL

4.1 Introduction

Parthenin has been identified as one of the major allelochemicals in *Parthenium hysterophorus* and the phytotoxicity of this compound has been investigated on a variety of test species (Datta & Saxena, 2001; Batish *et al*., 2002b; Belz *et al*., 2006). Although parthenin has been found in all parthenium plant parts it occurs most abundantly in trichomes on the surfaces of the leaves (Rodriguez *et al*., 1975, Kanchan, 1975; Reinhardt *et al*., 2004). Reinhardt *et al*. (2004) observed a parthenin concentration of 24.3 mg g^{-1} in the capitate-sessile trichomes (virtually 100% of trichome contents) occurring on leaves. Individual trichome parthenin content was measured at 0.3 µg. When plant residues decompose they can release secondary metabolites that are phytotoxic on other plant species (An *et al*., 2002). In CHAPTER III it was observed that at senescence, parthenium plants grown under controlled conditions have total parthenin content in leaves of 267.1 mg $plant^{-1}$, with smaller amounts from the achenes and other plant parts potentially adding to this volume. It was concluded that a parthenin amount of more than 267 mg would therefore potentially be available for release into the environment by a single plant in a growing season.

Although there is an abundance of literature on allelopathy, few reports have addressed the fate of allelochemicals in the soil environment (Cheng, 1992). Thompson (1985) emphasized the importance of understanding the effects of soil and microbial flora on allelochemical activity in the natural environment. In turn it can be expected that secondary compounds released from plants will also influence microbial ecology, as well as resource competition, nutrient dynamics, mycorrhizae and abiotic factors (Wardle *et al*., 1998). Once a chemical enters the soil a number of interacting processes may take place, some of which may transform or degrade the allelochemical. These are influenced by the nature of the compound, organisms present, soil properties (mineral and organic matter contents, particle size distribution, pH, ion exchange characteristics, oxidation state) and environmental factors (Cheng, 1992). These abiotic and biotic soil factors can influence and limit the quality and

University of Pretoria etd - Van der Laan M 2006

quantity of alleochemical required to cause plant injury (Inderjit, 2001). Therefore, the accumulation of chemicals at phytotoxic levels and their fate and persistence in soil are important determining factors in plant interactions (Inderjit, 2001).

The main objective of this study was to investigate the persistence of parthenin in soil.

4.2 Preliminary experiments

4.2.1 Preliminary experiment 1: Extraction of parthenin from compost soil

4.2.1.1 Materials and methods

Biologically active soil [hereafter referred to as compost soil (CS)] was obtained from the University of Hohenheim store. The equivalent of 50 g of dry soil was added to glass jars. Deionized water together with parthenin dissolved in acetone was added to each soil sample to achieve a parthenin concentration of 10 µg g^{-1} (10 µl acetone g^{-1}) in the soil and a water-holding capacity (WHC) of 40%. The soil was then stirred thoroughly with a spatula to ensure an even distribution of parthenin within the soil. Loose-fitting glass lids which allowed air circulation were placed on each jar and jars were kept at 20°C in darkness. Sampling was done after one hour incubation time to determine the recovery rate, and thereafter daily for one week. Samples were frozen at -20ºC until extraction and analysis. One soil sample was sterilized by autoclaving at 120ºC for two hours and then air-dried, treated with parthenin and sampled after 14 days.

Extraction technique

Deionized water was added to the soil to obtain a final volume of 15 ml water in the sample. Acetone was pre-warmed to 40ºC and 85 ml was then added to each sample after which the samples were subjected to four minutes of ultra sound followed by 30 minutes of shaking extraction on a mechanical shaker at 200 rpm. A 30 minute sedimentation period was allowed following shaking. The supernatant of each sample was filtered over two spoons of both Na_2SO_4 and quartz sand into Erlenmeyer flasks. A 50 ml aliquot was then removed and added to a separating funnel, followed by the

University of Pretoria etd - Van der Laan M 2006

addition of 50 ml H_20 and a small amount of NaCl. A liquid-liquid extraction was conducted with 50 ml TBME using a two minute shaking period. The TBME phase was filtered over Na_2SO_4/quartz sand into a round-bottomed flask and a second liquid-liquid extraction was repeated in the same way as described above. Supernatants were pooled and concentrated in a rotary vacuum evaporator followed by vacuum centrifugation at 40ºC until a volume of less than 250 µl was obtained. Acetonitrile was added to take the total volume up to 500 µl and samples were subsequently centrifuged at 28 000 rpm for 20 minutes at 4ºC. Finally, samples were transferred to glass vials and subjected to HPLC analysis.

Quantification of parthenin

HPLC analysis for the determination of the parthenin concentration was done using the method described in CHAPTER III (see 3.2.2.2 - 3.2.2.4).

4.2.1.2 Results and discussion

Parthenin was extractable from the soil, and the concentration of parthenin in the samples could be detected without any interference from other compounds in the soil. The CS soil was therefore judged suitable for use in further degradation experiments. However, a recovery rate of 70% was decided to be inadequate to allow for an accurate study of parthenin at very low concentrations and, therefore, the extraction technique needed to be improved.

From this preliminary experiment it could be determined that parthenin degraded relatively quickly in the soil, with a half-life (DT_{50}) value of less than three days when applied at a concentration of 10 µg g^{-1} (Figure 4.1). By day 14 the parthenin concentration in the soil was measured at 0.14 µg g^{-1}. After 14 days the sample which had been initially sterilized had a considerably higher parthenin concentration than the non-sterilized sample, and it was decided to include a sterilized treatment in the main degradation experiment.

University of Pretoria etd - Van der Laan M 2006

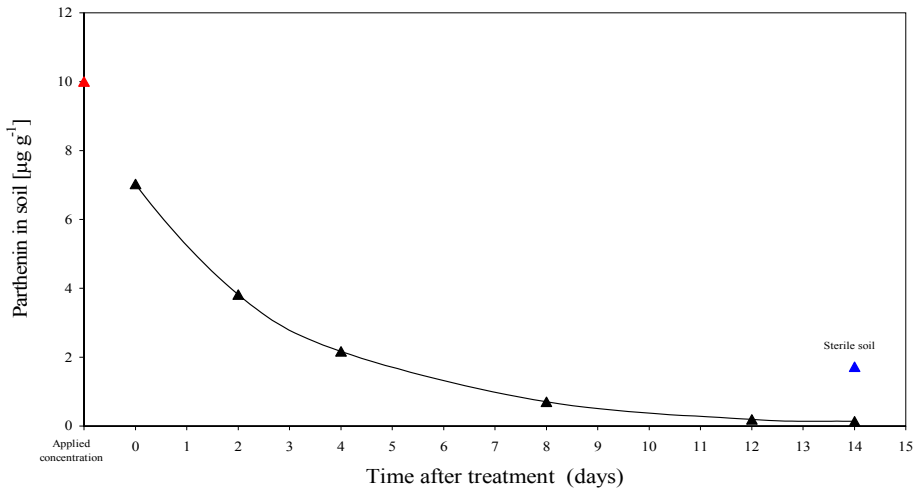


Figure 4.1 Disappearance of parthenin at 20°C in darkness over a period of 14 days added at an original concentration of 10 µg g-1 in sterilized (▲) and non-sterilized (▲) soil

4.2.2 Preliminary experiment 2: Extraction of parthenin from three different soil types

4.2.2.1 Materials and methods

Three different standard soils types, labelled 2.1, 3A and 5M were obtained from the 'Landwirtschaftliche Untersuchungs- und Forschungsanstalt – Speyer' (LUFA – Germany). Properties for the soils are presented in Table 4.1.

Table 4.1 Properties for the different soil types provided by LUFA and the compost soil (CS) provided by the University of Hohenheim

Soil	Org C in %	pH value (0.01 M $CaCl_2$)	CEC (mval 100 g^{-1})	Soil type (USDA)	Water-holding capacity (g 100 g^{-1})
2.1	1.21±0.27	6.1 ± 1.0	7 ± 1	Sand	34.7 ± 5.0
5M	1.56 ± 0.3	7.1 ± 0.3	13 ± 2	Sandy loam	42.1 ± 1.8
3A	2.2 ± 0.1	7.1 ± 0.1	19 ± 5	Loam	49.4 ± 5.5
CS	5.12	6.9	23.2	Very loamy sand	54.7

University of Pretoria etd - Van der Laan M 2006

The experiment was conducted as described in 4.2.1.1. Two replicates for each soil were used and the recovery rate of parthenin was calculated after one hour incubation time using the same technique as described in 4.2.1.1. Untreated soils were subjected to the same analysis in order to determine whether any other compounds present in the soil would interfere with parthenin detection by HPLC.

4.2.2.2 Results and discussion

Parthenin was successfully extracted and detected in all three of the soils. Recovery rates for the three soils are presented in Table 4.2. It was therefore decided that all three soils, in addition to the CS soil could be used for the main degradation experiment. Recovery rates varied between the soils and were less than desired (64.6±3.6%) which necessitated an improvement in extraction technique.

Table 4.2 Recovery rates of parthenin from three different soil types

Soil type	Recovery Rate [%]
2.1	59.2
5M	69.9
3A	64.7

4.2.3 Preliminary experiment 3: Evaluation of different extraction techniques for obtaining the highest recovery rate

4.2.3.1 Introduction

After previous experiments yielded less than desirable parthenin recovery rates it was decided to conduct and compare five different extraction techniques to maximize the recovery rate of parthenin from soil.

University of Pretoria etd - Van der Laan M 2006

4.2.3.2 Materials and methods

For each sample, 50 g of dry compost soil was added to glass jars. A volume of 15 ml H_20 containing 500 µl parthenin in acetone was then added to the soil and mixed thoroughly with a spatula to attain homogenization. Three replicates were measured for each of the five methods. An incubation period of one hour was allowed before different extraction methods as described below were utilized.

Method 1 and 2: Acetone was warmed to 40ºC and 85 ml was added to each soil sample. Samples were then shaken for 30 minutes on a mechanical shaker at 200 rpm and allowed to sediment for a further 30 minutes. The supernatant from each sample was filtered over Na_2SO_4 and quartz sand. The aliquot was transferred to a separating funnel and 50 ml H_20, a small amount of NaCl, and 50 ml TBME added and a liquid-liquid extraction with a two minute shaking period conducted. The TBME phase was then transferred to a round-bottom flask while another 50 ml of TBME was added to the water phase and a second liquid-liquid extraction conducted. The TBME phases were pooled and then concentrated in a rotary vacuum evaporator and transferred to calibrated test tubes and vacuum centrifuged until a final volume of less then 250 µl was obtained.

Method 1: acetonitrile (ACN) added to attain a final volume of 500 µl.
Method 2: ACN:H_2O added to attain a final volume of 2000 µl.

In both methods samples were centrifuged for 20 minutes at 28 000 rpm before transferring 500 µl to glass vials for HPLC analysis.

***Method 3 and 4*:** 85 ml of extraction solvent [Method 3: acetone; Method 4: acetone:TBME 1:1 (v/v)] was added to each soil sample and samples were shaken for 30 minutes on a mechanical shaker at 200 rpm. After shaking, 15 minutes of sedimentation was allowed before the supernatant was filtered over Na_2SO_4/quartz sand. Aliquots of 40 ml were pipetted into round-bottom flasks and the aliquots were concentrated in a rotary vacuum evaporator. Concentrated samples were then transferred to graduated centrifuge tubes. Additional TBME was used to remove any remaining residues of the sample from the walls of the round-bottomed flasks.

University of Pretoria etd - Van der Laan M 2006

Samples were then vacuum-centrifuged at 40ºC to obtain a final volume of less than 600 µl. Deionized water was added to take samples to 600 µl, and 400 µl ACN added to obtain a final volume of 1000 µl. The samples were then centrifuged and 500 µl was transferred to glass vials for HPLC analysis.

Method 5: 85 ml acetone (at 40ºC) was added to the soil, followed by 30 minutes of shaking extraction at 200 rpm and 15 minutes of sedimentation. The supernatant was then filtered over NA_2SO_4/quartz sand. The soil remaining in the glass jar together with any remaining acetone was transferred to a 50 ml test tube and centrifuged at 4000 rpm for 20 minutes. The initial filtrate together with the supernatant from the centrifugation process was then conveyed to the rotary evaporator followed by vacuum centrifugation until <600 µl of solution was left. This was then taken up to 600 µl with deionized water and 400 µl ACN added to obtain a final volume of 1000 µl. The sample was then centrifuged and 500 µl was transferred to HPLC vials for analysis.

4.2.3.3 Results and discussion

Recovery rates (Table 4.3) varied considerably between the different methods tested. Through Methods 3 and 4 the highest recovery rates were achieved, and it was decided to use Method 4 (acetone:TBME as extracting solvent) in the main degradation experiment.

Table 4.3 Mean recovery rates for parthenin from 'CS' soil using different extraction techniques

Method used	Recovery rate [%]
Method 1	51.6
Method 2	80.8
Method 3	106
Method 4	97.9
Method 5	67.2

University of Pretoria etd - Van der Laan M 2006

4.2.4 Preliminary experiment 4: Determination of the consistency of recovery rates

4.2.4.1 Introduction

In order to obtain useful and consistent data the reliability of the extraction technique and consistency of recovery rates were investigated.

4.2.4.2 Materials and methods

For each of the four soil types given in Table 4.1, the equivalent of 50 g dry soil was added to glass jars and deionized water together with parthenin dissolved in acetone was added to obtain 40% WHC and 10 µg g^{-1} parthenin concentration in the soil. Extraction Method 4 (see 4.2.3.2) was used to extract parthenin from the soil and to assess consistency and reliability of the recovery rates.

4.2.4.3 Results and discussion

Mean recovery rate and standard deviation across the four replicates for the four soils is presented in Table 4.4. Recovery rates were judged to be sufficiently consistent and reliable.

Table 4.4 Mean parthenin recovery rates with standard deviations for the four soil types

Soil type	Mean recovery rate [%]
2.1	95.8 ± 2.8
5M	105.2 ± 6.1
3A	96.6 ± 4.5
CS	109.2 ± 2.9

University of Pretoria etd - Van der Laan M 2006

4.2.5 Preliminary experiment 5: Persistence of pure parthenin at different concentrations in soil

4.2.5.1 Introduction

The phytotoxicity of herbicides in the soil is correlated with the concentration of the herbicide in the soil water but not with amount of herbicide per entire soil mass (Kobayashi *et al*., 1994; Kobayashi *et al*., 1996). Ito *et al*. (1998) observed that the amount of dehydromatricaria ester (DME) adsorbed to the soil solids depended on the concentrations applied. The objective of this experiment was to determine the persistence of parthenin applied at three different concentrations (in magnitudes of ten) to study the effect of concentration and to determine at which concentration the main degradation experiment should be conducted.

4.2.5.2 Materials and methods

Fifty grams of soil was placed into each glass jar and deionized water was added to achieve a 40% WHC. Aliquots of a stock solution of parthenin in acetone (10 mg ml^{-1}) were added to the soil to obtain parthenin concentrations of 100, 10 and 1 µg g^{-1} respectively. An additional treatment was prepared at the 100 µg g^{-1} concentration, using soil that had been sterilized by autoclaving for two hours at 120ºC and then left to air-dry. Samples were kept in the dark at a constant temperature of 20ºC. Sampling occurred after one hour incubation and then regularly over a one week period.

4.2.5.3 Results and discussion

Parthenin proved to degrade slower when applied at 100 µg g^{-1} than at 10 and 1 µg g^{-1} (Figure 4.2). Chemicals have often been observed to degrade slower in soil when present at higher concentrations, as has also been noted for allelochemicals by Fomsgaard *et al*. (2004) and Weidenhamer & Romeo (2004). Ito *et al*. (1998) observed that the higher the DME concentration in the soil, the longer the DME concentration was maintained in the soil water. Parthenin applied at 1 and 10 µg g^{-1} degraded at a similar rate initially, having a similar DT_{50} value, but after four days degradation rate in soils to which 1 µg g^{-1} parthenin was much faster than at 10 µg g^{-1}

University of Pretoria etd - Van der Laan M 2006

(Figure 4.2). Parthenin at 100 µg g^{-1} also began degrading rapidly after four days, prior to which, very little degradation had taken place. In the initially sterilized soil to which parthenin had been added at a concentration of 100 µg g^{-1} no degradation was evident within the seven day period examined.

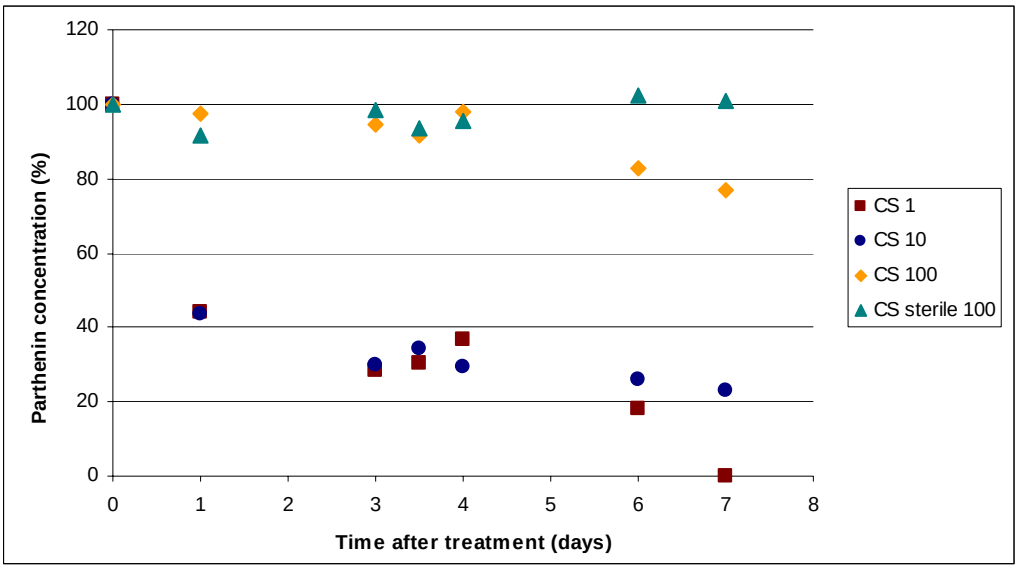


Figure 4.2 Disappearance of parthenin at 20°C in darkness over a period of seven days added at an original concentration of 1, 10 and 100 µg g^{-1} to non-sterilized soil and in sterilized soil at 100 µg g^{-1}

4.3 Main experiment

4.3.1 Introduction

Based on results of the preliminary experiments described above it was decided to use a parthenin concentration of 10 µg g^{-1} in the soil for the main experiment and a sampling period of 22 days.

4.3.2 Materials and methods

The WHC of the four soil types classified as: sand (labelled 2.1), sandy loam (5M), loam (3A) and compost soil (CS), was determined. For each of the soils, the equivalent of 50 g of dry soil was placed into glass jars and the correct volume of deionized water together with parthenin dissolved in acetone was added to achieve a

University of Pretoria etd - Van der Laan M 2006

WHC of 40% and a parthenin concentration of 10 µg g^{-1}. The soil was then thoroughly mixed with a spatula to achieve homogenization. Jars were closed with loose fitting glass lids which allowed for free air movement. Samples were placed in permanent darkness at a constant temperature of 20ºC. In addition to the above treatments, both sterilized and non-sterilized CS soils were incubated at 20, 25 and 30ºC in order to determine parthenin degradation in initially sterilized and non-sterilized soils at different temperatures. Sterilization was achieved through autoclaving the soil at 120ºC for two hours and then allowing the soils to air-dry. For each treatment a total of 15 samples were taken over 22 days with sampling frequency decreasing over time. Water was replenished every 3-4 days to maintain the soil moisture at 40% WHC. Any seedlings that germinated in the soil were immediately removed. Sampling was done by replacing the glass lid with a tight fitting plastic lid and freezing the sample at -20ºC until analyzed.

Parthenin extraction

Samples were removed from refrigeration and defrosted in a heat bath at 30ºC. All samples were at 40% WHC and an additional volume of deionized H_2O, depending on the soil type, was added to attain a final volume of 15 ml H_2O in the soil. A volume of 85 ml of 1:1 acetone:TMBE was added to each sample. Plastic lids lined with parafilm were placed over the jars and samples were shaken for 30 minutes on a mechanical shaker at 150 rpm. After shaking and 30 minutes of sedimentation the supernatant was filtered over Na_2SO_4/quartz sand. A 40 ml aliquot was then transferred to flat-bottomed flasks and the sample was concentrated in a rotary vacuum evaporator. The concentrated sample was transferred to graduated centrifuge test tubes. A small amount of TBME was used to rinse the flat-bottom flasks to ensure transferral of the entire sample to the centrifuge tubes. Samples were then vacuum-centrifuged at 30ºC for 20 minutes, and then at 45ºC with the cooling unit switched on until a volume of less than 600 µl was obtained. Deionized H_2O was added to obtain 600 µl, and then 400 µl ACN. Samples were centrifuged at 28 000 rpm for 20 minutes before transferral to glass vials for HPLC analysis.

Parthenin quantification

Parthenin concentration in the samples was determined using the method described in Chapter III (see 3.2.2.2 – 3.2.2.4). Nonlinear regression analysis was done using

University of Pretoria etd - Van der Laan M 2006

SPSS® regression models and degradation curves were compared using *F* test for lack-of-fit based on analysis of variances (P≤0.05).

4.3.3 Results and discussion

4.3.3.1 Parthenin degradation in different soil types

Parthenin was quickly degraded in all four soils tested under the particular experimental conditions used. The degradation curves for the soils tested were parallel indicating a similar degradation mechanism in all soils (Figure 4.3).

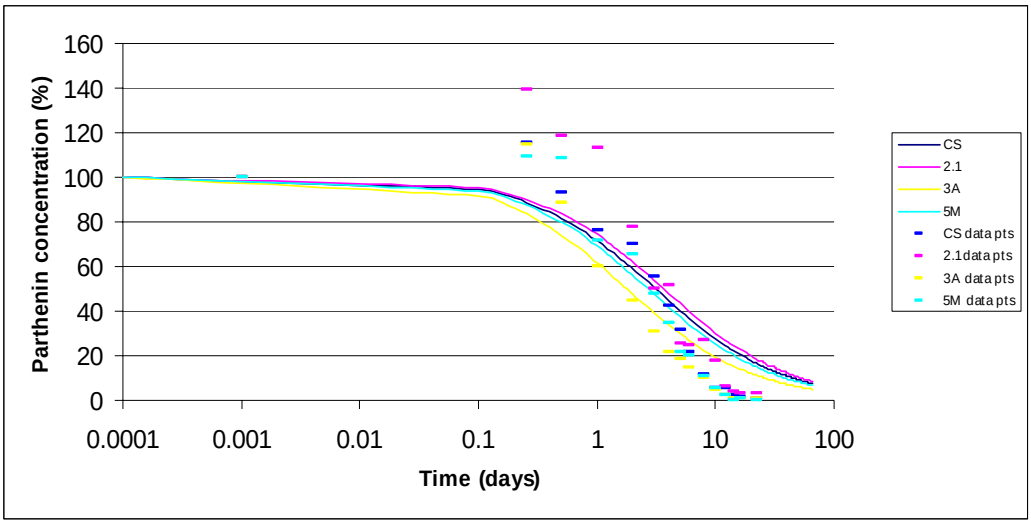


Figure 4.3 Disappearance of parthenin at 20°C in darkness added at an original concentration of 10 µg g^{-1} to four different soil types

DT_{50} values for the soils ranged from 1.78 to 3.64 days and differed significantly (Table 4.5). DT_{10} and DT_{90} values (also presented in Table 4.5) are representative of the time of degradation onset and the end of the degradation process, respectively.

University of Pretoria etd - Van der Laan M 2006

Table 4.5 Disappearance-time (DT) for 10, 50 and 90% degradation for the four different soils used in the experiment

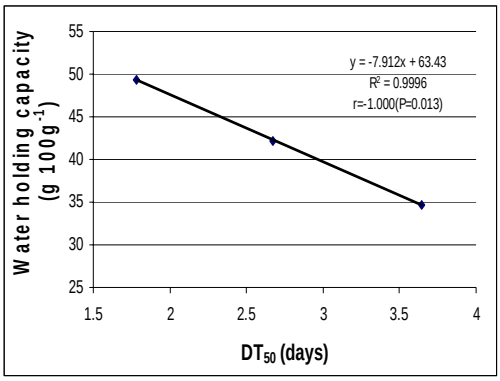
Soil		DT_{10}	DT_{50} (Days)	DT_{90}
3A	(loam)	0.34	1.78a	27.24
5M	(sandy loam)	0.50	2.67ab	40.81
CS	(very loamy sand)	0.58	3.10b	47.32
2.1	(sand)	0.69	3.64b	55.58

Means followed by different letters differ significantly (F-test, P=0.05)

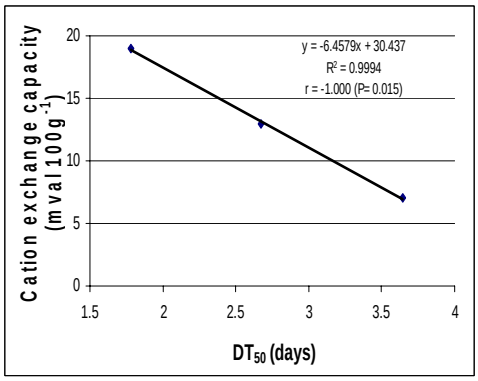
Correlation between soil characteristics and DT_{50} values were found to be negative and significant for WHC and soil cation exchange capacity, but not significant for pH and organic carbon content (Figure 4.4). PH values for the different soils were relatively close together which may be the reason for the non-significant correlation. Calvet *et al*. (1980) pointed out that for non-ionic herbicides, correlation between degradation and soil organic matter is not always very good across the range of 0 to 4% organic matter. This range includes most temperate arable soils and it is likely that the soils used in this study contained too little organic carbon for a significant correlation between DT_{50} values and organic carbon percentage. Soils with higher clay and organic matter contents generally have greater adsorptive power.

Although analyses were performed for the CS soil, it was not included in the correlation analysis due to the unnatural constitution of this “soil”.

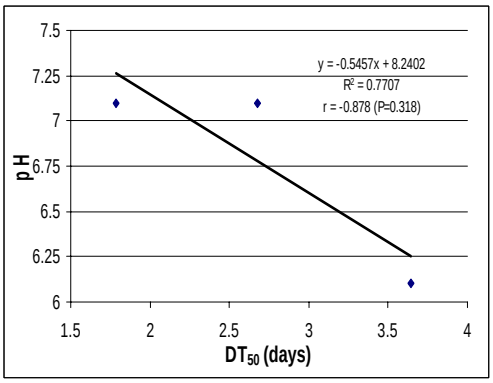
University of Pretoria etd - Van der Laan M 2006



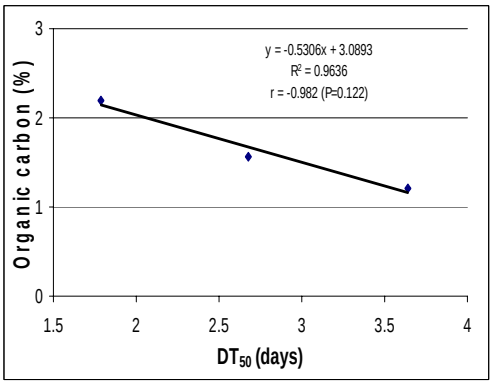
(a)



(b)



(c)



(d)

Figure 4.4 Correlation between DT_{50} value and (a) water holding capacity, (b) cation exchange capacity, (c) pH, and (d) organic carbon percentage for the degradation of parthenin in the 3A, 5M and 2.1 soils

4.3.3.2 Parthenin degradation in sterilized and non-sterilized compost soil at three different temperature regimes

Similar to parthenin degradation in different soil types, the sterilized and non-sterilized compost soil placed at different temperatures all had parallel curves but different DT_{50} values (Figure 4.5).

University of Pretoria etd - Van der Laan M 2006

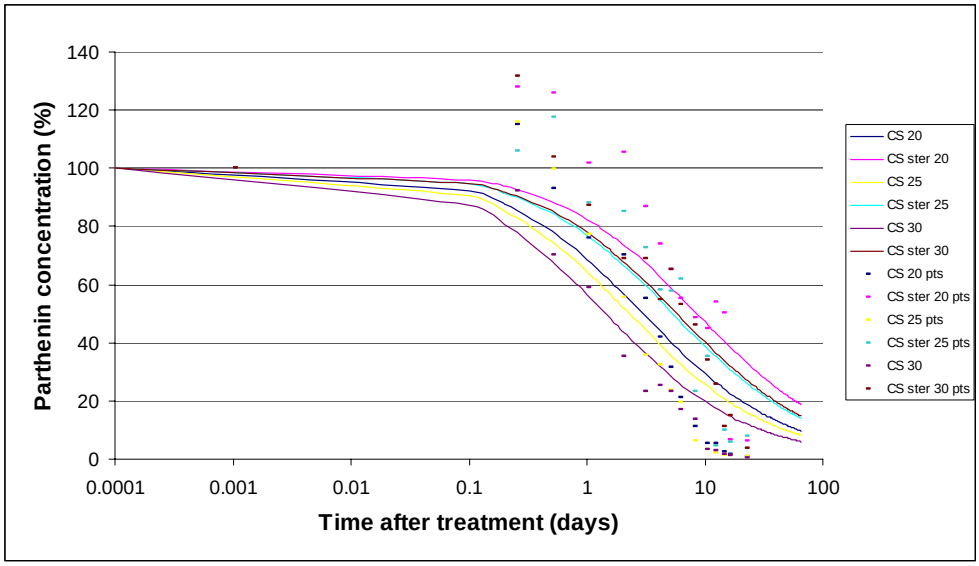


Figure 4.5 Disappearance of parthenin added at an original concentration of 10 µg g^{-1} in sterilized and non-sterilized compost soil (CS) incubated at temperature regimes of 20, 25 and 30ºC in darkness

From Figure 4.5 it is apparent that parthenin degraded faster in soils which were not sterilized than in soils which were autoclaved. Ito *et al*. (1998) also observed that the degradation of the allelochemical dehydromatricaria ester was slowed by autoclaving the soil. According to Grover (1988), chemicals are absorbed, degraded or leached in the soil. Picman (1987) concluded that when isoalantolactone, a sesquiterpene lactone, was added to soil at a concentration of 100 µg g^{-1}, microbial degradation was most likely responsible for the disappearance of this sesquiterpene from the soil. After 90 days isoalantolactone was not detected in the organic soil used and only traces could be detected in the mineral soil used. Picman (1987) suggested that the initial disappearance of the chemical compound from the soils, especially from the organic soil, was due to the compound forming 'bound residues' with humic material in the soil. Inderjit (2001) pointed out the need to evaluate other soil properties including electrical conductivity, inorganic ions, clay minerals and water content. As leaching and light degradation was not possible under the present experimental conditions, it seems plausible that microbial degradation was the predominant cause of the disappearance of parthenin from the soil. It is not entirely certain that all microbes capable of playing a role in degrading parthenin were neutralized during the autoclaving process. It can, however, be expected that microbe numbers were at least drastically reduced. As the sterilized soil was not kept under completely sterile

University of Pretoria etd - Van der Laan M 2006

conditions following autoclaving, it can be expected that microbial populations in the originally sterilized soils would increase in size and diversity over time.

In non-sterilized soils, parthenin degraded significantly quicker in soil kept at 30ºC compared to soils kept at 20 or 25ºC (Table 4.6). There was a trend for faster degradation in soil incubated at 25ºC than at 20ºC but this was not significant. This finding supports the hypothesis that microbial degradation played an important role in degradation as we would expect faster metabolism at higher temperatures. In sterilized soils, parthenin degraded significantly slower in soils incubated at 20ºC than in soils kept at 25 or 30ºC.

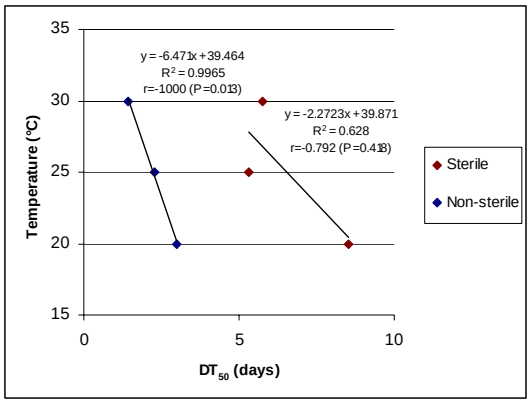
Table 4.6 Parthenin disappearance-time (DT) for 10, 50 and 90 % degradation in sterile and non-sterile compost soil (CS) placed at temperature regimes of 20, 25 and 30ºC

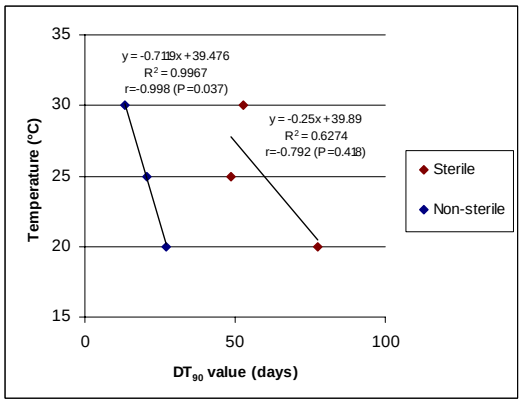
Soil		**DT_{10}**	**DT_{50} (Days)**	**DT_{90}**
CS 20ºC	*Sterile*	0.96	8.54e	77.70
	Non-sterile	0.33	2.98bc	27.10
CS 25ºC	*Sterile*	0.60	5.32cd	48.37
	Non-sterile	0.26	2.29b	20.82
CS 30ºC	*Sterile*	0.65	5.78d	52.56
	Non-sterile	0.16	1.44a	13.09

Means followed by different letters differ significantly (F -test, P=0.05)

Significant correlation was observed between temperature and DT_{50} and DT_{90} values for non-sterilized soils only (Figure 4.6).

University of Pretoria etd - Van der Laan M 2006





(a) **(b)**

Figure 4.6 Correlation between temperature and DT_{50} (a) and DT_{90} (b) values for sterile and non-sterile compost soil placed at 20, 25 and 30ºC

Schmidt & Ley (1999) postulated that allelochemicals may be prevented from building up to phytotoxic levels by microbial activity in natural soils. Limited work has been done on the microbial transformation of parthenin (Bhutani & Thakur, 1991) and further investigation into parthenin transformation and degradation products occurring in the soil will be necessary for increased appreciation of parthenin soil degradation mechanisms. Chemically transformed parthenin products may also display phytotoxic properties. Also, little is known of microbial sensitivity to parthenin and the influences of this on parthenin degradation in the soil. In the CS soil, parthenin DT_{50} was observed to be affected significantly by temperature and under natural conditions we can expect temperature, seasonal temperature fluctuation and amount of precipitation to affect the biochemical degradation of parthenin.

Different soil types also differed significantly with regard to DT_{50} values, reiterating the importance of soil characteristics in allelochemical degradation as has been reported (Dalton *et al*., 1989; Shibuya *et al*., 1994; Takahashi *et al*., 1994; Kobayashi *et al*., 2004). According to An *et al*. (2002), the potential phytotoxicity of plant residues 'is dependent on numerous factors that together govern the rate of residue decomposition, the net rate of active allelochemical production and the subsequent degrees of phytotoxicity'. Although it is difficult to determine parthenin concentrations occurring under natural conditions, it is clear from the DT_{50} values that a continual replenishment of parthenin into the soil will be necessary in order for parthenin to have a phytotoxic effect on other plant species. Little is known about the

University of Pretoria etd - Van der Laan M 2006

necessary parthenin concentrations in the soil required to inhibit plant growth. Investigating the allelochemical dehydromatricaria ester (DME) from *Solidago altissima* L. (Asteraceae), Ito *et al*. (1998) observed that the DME concentration required for 50% growth inhibition was ten or twenty times greater in soil that in agar culture depending on soil type. Herbicide studies have shown that the phytotoxicity of herbicides in soils was highly correlated with soil water concentrations as opposed to amounts per whole soil mass (Kobayashi *et al*., 1994; Kobayashi *et al*., 1996). In studying the disappearance of isoalantolactone, a sesquiterpene lactone occurring mainly in species from the genera *Inula* and *Chrysanthenum*, Picman (1987) concluded that 'sesquiterpene lactones do not accumulate in the soil presumably because they are decomposed'.

4.3.3.3 Conclusions

The disappearance of parthenin from soil can be a result of leaching from the soil, or chemical, biochemical or photochemical transformation. In this experiment, parthenin disappearance due to leaching or photochemical transformation can be ruled out. As parthenin disappeared faster in soils that had not been sterilized than in soils that were autoclaved, it is probable that microbial transformation of parthenin played a role.
Inderjit & Weiner (2001) suggested that in the field, effects of allelochemicals could be due to (i) direct effect of allelochemicals, (ii) effects of degraded or transformed products of the allelochemicals released, (iii) effect of allelochemicals on physical, chemical and biological soil factors, and (iv) chemical induction of release of active chemicals by a third species. Inderjit & Weiner (2001) further proposed 'that the behaviour of vegetation can be better understood in terms of allelochemical interactions with soil ecological processes rather than the classical concept of direct plant-plant allelopathic interference'. Although the phytotoxicity of parthenin on numerous test species has been well demonstrated, less is known about parthenin phytotoxicity in the soil and the effect of parthenin on soil ecology. This requires further research.