International Journal of Soil and Crop Sciences
Abbreviated Key Title: Int. J. Soil and Crop Sci.
ISSN-2360-7963 (Print) & Open Access
v11: (7): Pp: 5-14

Volume-11 | Issue-7| July-August, 20223 | *Full length Research Paper*

The pesticidal activity of Spider plant, Cleome gynandra L, plant tissue on soil borne pathogens

By

Farai Shelton Chihobvu[a,b], Elizabeth Ngadze[b], Stanford Mabasa[b] and Maxwell Handiseni*[c, d]

[a]Horticulture Research Institute, P. O. Box 810 Marondera, Zimbabwe,
[b]Department of Crop Science, University of Zimbabwe, P.O. Box MP. 167 Mount Pleasant, Harare, Zimbabwe
*[c]Faculty of Natural Resources Management and Agriculture, Midlands State University, P. Bag 9055, Senga, Gweru, Zimbabwe
*[d]Department of Plant Pathology and Microbiology, Texas A & M University, College Station, TX 77840, USA
Corresponding author: maxhandiseni@gmail.com

***Corresponding author: Handiseni et al.,** **Accepted:** 4/8/.2023 | **Published:** x//8/2023 |

Abstract: Commercial management of soilborne pathogens is often reliant on use of synthetic chemicals. These chemicals are expensive and not easy to use for most resource poor farmers. Biofumigation represents a potential alternative because of its less heath impacts, less cost and its less harm to the environment. This work was initially done in the laboratory and then continued in the greenhouse tunnel and field. The study aimed to determine the efficacy, produced by Cleome gynandra species, on soil borne fungal pathogens. In the laboratory, green C. gynandra was effective against purple C. gynandra. In the greenhouse seedlings inoculated with Rhizoctonia solani and Pythium ultimum were effectively controlled by purple C. gynandra and it was comparable to the control treatments. Statistical differences gave major evidence of disease suppression in plots amended with purple C. gynandra species in yields of both tomato and sweet pepper grown in the field. The results also show that the two C. gynandra specie types had potential as control for soilborne pathogens. The results are discussed in detail in this paper.

Keywords: biofumigation, glucosinolates, isothiocyanates, Cleome gynandra, pathogens

Quick Response Code

Journal Name

https://www.springjournals.net/ijscs

Citation: Handiseni et alThe pesticidal activity of Spider plant, Cleome gynandra L, plant tissue on soil borne pathogens. Int. J. Soil and Crop Sci. 11(7) Pp 5-14, 2023

Published By IJARR

1.0 INTRODUCTION

Soil borne pathogens are the major constraint in establishing sustainable pest management systems (Prasad et al., 2015). The continuous use of synthetic chemicals may lead to the development of resistant soil pest populations, environmental contaminations and health problems. Smallholder farmers lack and cannot afford most of the soil fumigation options available to conventional growers (Vaughn et al., 2005). Alternative control measures, such as bio fumigation, has to be used in an integrated approach as it is an environmentally friendly option in the fight against soil-borne pathogens (Angus et al., 1994; Edwards and Ploeg, 2014; Fan et al., 2008). Bio fumigation is a novel pest management technique attaining great relevance for producers and consumers (Lord et al., 2011).

The use of plant tissue bio fumigants in the soil has been shown to significantly reduce a wide range of pathogens (Alves et al., 2014; Karavina and Mandumbu, 2012a; Watts et al., 2014). Methyl ITC suppress growth and germination of many pathogen species (Brown and Morra, 1995). Contrary to the problems associated with the use synthetic chemicals, plant products are environmentally friendly, renewable, easily accessible, largely non-phytotoxic, systematic ephemeral, thus readily biodegradable, relatively cost effective and hence constitute a suitable plant protecting agents in the strategy of disease management (Fahey et al., 2001; Hanschen et al., 2015; Matthiessen and Shackleton, 2005; Ngadze, 2014). However, there is need for an in-depth study on the use of biofumigation to suppress soil-borne pathogens (*Pythium, Phytophthora* and *Rhizoctonia* species) that infect plants. The objective of the study was to determine the efficacy of *C. gynandra* green manuring on suppression of soilborne pathogens (*Pythium, Phytophthora* and *Rhizoctonia* species).

2.0 MATERIALS AND METHODS

2.1 Study site

The research was carried out at Horticulture Research Centre ($18^0$11'S and $31^0$28'E), near Marondera, which is in agro-ecological region IIa and is at an altitude of 1630 m above sea level. Horticulture Research Centre has an average day-length of 13.2 hours in summer and 11.1 hours in winter (Mhazo, 2011; Vincent and Thomas, 1962). The mean annual rainfall is approximately 873 mm, but this is subject to wide fluctuations and in the last 30 years has varied between 430 mm and 1320 mm. Mean minimum temperature ranges from 19.5 ^{0}C (July) to 24.6 ^{0}C (January). Hot summer is between September and December with October being the hottest month of the year with maximum temperatures above 30 ^{0}C. Slightly more than two thirds of the total rainfall normally falls during the months of December, January and February (Mhazo, 2011).

2.2 Pathogen greenhouse experiment

A tomato variety, Rodade, and a sweet pepper variety, Califonia Wonder, were used in this study. Seeds were primed before sowing by placing them on a wet filter paper 12 hours prior to planting. *Pythium ultimum* and *Rhizoctonia solani* AG1-1A strain virulent to both tomato and sweet pepper were provided by the Kutsaga Research Station, Plant Pathology Clinic, in Harare. The strains were cultured on a semi-selective medium at 25^{o}C for 5 days. Crushed pine bark media collected from Horticulture Research Center, was pasteurized at 121^{o}C for 24 hours prior to use in the experiment.

A Randomised Complete Block Design was used and the treatments were replicated 3 times. The *C. gynandra* plants grown as described previously were harvested at flowering stage and ground using a blender. Pasteurized pine bark media was weighed and ground *C.gynanadra* tissue was applied at a rate of 0 %, 5 %, 10 % and 15 % (w/v) as per treatment and dispensed into plastic pots. The seedlings were then planted therafter. A no amendment treatment in pathogen inoculated media and a no amendment treatment in non-inoculated soil were used as controls. Two chemical controls Ortivar® and Uniform were also used at rates of 30 ml per 3 liters of water for Ortivar®; and 60 ml and 80 ml per 3 liters water for the chemical Uniform (not yet registered in Zimbabwe). These were applied just after planting and watering the plants.

Sterile plastic pots (10 cm in diameter by 25 cm tall) were filled with pasteurized media (1kg). Ten discs of *P. ultimum* and/or *R. solani* agar plugs (5 mm diameter) were added to 1kg of soil. The pathogen discs were placed in soil at a depth of 10 cm below the surface. Controls were made up of soil without *C. gynandra* plant tissue amendment. In addition, due to known phytotoxicity of glucosinolate containing tissue which may be mistaken for disease effect, *C. gynandra* tissue amendment treatments with no pathogen inoculum were included to differentiate between the growth-limiting effect arising from the pathogen and that from potential phytotoxicity of the tissue amendment. The inoculated soil was incubated for 7 to 10 days to increase the pathogen population to 10^6 spores per plastic pot before the C. gynandra plant tissue amendment and chemical treatment

2.3 Field trial

Field studies were conducted at Horticulture Research Centre in fields inoculated with *Rhizoctonia solani* and *Pythium ultimum*. The pathogen species were inoculated on sterilized wheat seed with its husks. Wheat seed, 20 kgs, was inoculated with *Rhizoctonia solani* and another 20 kgs of wheat seed was inoculated with *Pythium ultimum* for ease of spread and distribution in the field. The wheat seeds were soacked with water (quarter

volume) before the sterilization process. The seeds were left covered for 24 hours after autoclaving. The same process was repeated after 24 hours and the seeds were allowed to cool down. The final autoclave made sure that the wheat seed was moist with no residual water so as to avoid rotting. The growing pathogens on PDA were cut into small pieces to increase the surface area and were mixed with the sterilized wheat seed. The mixture was then covered to avoid contamination. The mixture was opened and mixed again after five days to redistribute the growing pathogens. One Petri dish of PDA with growing pathogens was used to inoculate a kilogran of wheat seed. The pathogen inoculum was spread in the field at five weeks after planting when the *C. gynandra* plants were at full flowering stage.

A split plot design was used, with biofumigant crop treatments: glucosinolate containing green-stem and purple-stem *Cleome gynandra,* a non glucosinolate containing kale crop, and zero biofumigant (control) as the main plots and the *Rhizoctonia solani* and *Pythium ultimum* pathogens as subplots. Main plots were 4.5 m by 1.8 m and each cover crop treatment replicated four times. Within each plot, crop species' locations were completely randomly assigned. The *C. gynandra* plants and kale crops were grown were grown from seedlings grown for six weeks in the nursery. The plants were drenched a day after planting after the first irrigation with a chemical Actara for the control of cut worms, aphids and leaf minors. The plants were grown with a basal dressing of 10 grams per plant of Compound C fertilizer and a top dressing was done using 10 grams of Ammonium nitrate, split applied at 3 and 5 weeks after planting.

The biofumigant crop treatments were grown up to flowering stage, mowed and incorporated by use of a tractor drawn disc harrow to a depth of 15 cm. Before mowing and incorporation, biofumigant crops were sampled, freeze dried, ground into powder using a grinding mill and the glucosinolate content was determined. Above ground biomass of the biofumigants was assessed in the field by collecting biomass from two 0.5 m x 0.5 m quadrats per plot. Samples were dried for 7 days at 65°C and weighed. Two weeks after biofumigant incoporation, four lines with a spacing of 0.9 m by 0.3 m were made in each main plot and a six week old tomato and pepper seedlings were transplanted. Control plots which had no biofumigants incoporated were treated with the chemical Ortiva as the positive control. The tomato and sweet pepper plants were grown with a basal dressing of 20 grams per plant of Compound C fertilizer and a top dressing was done using 10 grams of Ammonium nitrate, split applied when the fruits were marble size. Insecticide treatments were done across all plots using Actara (Thiamethoxam), Dynamec (Abamectin), Ampligo (Lambda-cyhalothrin), Dimethoate (Dimethoate) and Proclaim (Emametin benzoate) chemicals. Fungicide treatments were done as at when necessary after scouting. Scouting for forlia diseases was done weekly.

2.5 Greenhouse data collection

Seedling establiment was determined from seven days after planting both for tomato and pepper. Root systems from surviving eight seedlings per treatment were measured. A ruler was used to measure the total root length, a set of vernier calipers was used to determine the root diameters and a count of number of root tips was done manually. *Pythium ultimum* and *Rhizoctonia solani* AG1-1A in the soil and plant roots from each treatment were quantified by scoring the severity using a scale of 1 to 9, the score 1 being a healthy root system and score 9 being a dead plant.

2.6 Field trial data collection

At 16 weeks after planting the end of the trial, four soil cores were sampled randomly from each plot to make a composite sample per treatment. Pathogens were isolated from soils in amended and non-amended plots and a comparison of pathogen populations was done for both environments in order to etablish the effects of the various plants on pathogen dynamics. Serial dilutions were used to assess the severity of the pathogen in the soil. The *Rhizoctonia* and *Pythium* were distributed evenly on a surface of Potato Dextrose Agar media, a small drop of the suspension of the pathogens, made from one gram of soil, were spread on the media using a sterile bend glass rod. The pathogens were then quantified basing on the counts of the colonies that emerged in the petri dishes. In the first 6 weeks disease scouting for *R. solani* and *P.ultimum* was also done, any plants which showed symptoms consistent with *Rhizoctonia* or *Pythium* damage were collected and cultured on *Rhizoctonia* and *Pythium* selective media and also on standard water and Potato Dextrose Agar. At maturity the crops were harvested, counted, graded and weighed.

2.9 Data analysis

Data was analyzed using the Genstat 17th edition (VSN International, 2015). Significant differences between treatments means were examined using Fishers protected LSD multiple range test(VSN International, 2015).

3.0 RESULTS

3.1 Results greenhouse pathogen experiment

The green, and purple spider plant *(C. gynandra)* at rates 0, 5, 10, 15 % w/v, and the chemical controls Ortivar® and Uniform, a non- chemical and no pathogen control and a non -chemical inoculated with *Rhizoctonia solani* control had significantly different effects on the root

diameter of tomato ($p < 0.05$) and sweet pepper ($p < 0.001$) seedlings (table 4.1). There were significant effects noted on tomato ($p < 0.01$) and on sweet pepper seedlings ($p < 0.001$) inoculated with *Rhizoctonia solani* on disease damage (table 4.1).

Table **4.1**. Analysis of variance (*P* value) for the effects of two pathogenic species (*Pythium ultimum* and *Rhizoctonia solani* AG1-1A) on tomato and sweet pepper seedling growth on root length, number of root tips per plant, root diameter and disease damage at 28 days after planting.

		Root length		Number of root tips per plant		Root diameter		Disease damage	
Source of variation	Df	Tomato	Sweet pepper	Tomato	Sweet pepper	Tomato	Sweet pepper	Tomato	Sweet pepper
Rhizoctonia solani	10	0.167	0.185	0.107	0.184	0.01	<0.001	0.024	0.001
Pythium ultimum	10	0.026†	0.146	0.002	0.224	<0.001	0.045	<0.001	0.049

Table 4.2**.** The effect of *Rhizoctonia solani* rate of amendment on root length, number of root tips, root diameter and disease damage at 28 days after planting tomato and sweet pepper seedlings.

	Rhizoctonia solani					
	Root length		Number of root tips per plant		Root diameter	
Treatment	Tomato	Sweet pepper	Tomato	Sweet pepper	Tomato	Sweet pepper
No chemical + Pathogen	6.80	3.75	2.375	1.812	0.32^{ace}	0.1738^{bceg}
10% Green *C. gynandra*	5.50	2.97	1.875	2.188	0.26^{ceg}	0.165^{cegik}
10% Purple *C. gynandra*	7.00	4.16	2.75	2.062	0.407^{a}	0.1663^{cegi}
15% Green *C. gynandra*	5.00	3.41	1.5	1.562	0.21^{ceg}	0.1288^{cegikm}
15% Purple *C. gynandra*	5.00	2.44	1.813	1.562	0.277^{ceg}	0.1155^{degikm}
5% Green *C. gynandra*	4.20	3.56	1.625	1.938	0.191^{dfg}	0.1619^{cegik}
5% Purple *C. gynandra*	5.40	3.12	2.313	1.688	0.309^{aceg}	0.2025^{ac}
No chemical + No Pathogen	5.60	3.16	2.313	2.125	0.327^{ac}	0.2631^{a}
Ortivar 30ml/3L	6.70	3.56	2.00	1.688	0.282^{bceg}	0.1944^{ace}
Uniform 60ml/3L	5.40	3.66	1.75	1.125	0.174^{h}	0.03^{m}
Uniform 80ml/3L	5.20	3.06	1.75	1.438	0.221^{ceg}	0.0575^{fhjlm}
LSD	2.00	1.094	0.8415	0.759	0.1235	0.0849
CV%	6.30	2.30	12.50	6.10	12.00	8.50

†The means followed by the same letter in a column are not significantly different from each other according to the LSD value. NS: Not significant

The effect of rate of amendment of green spider plant and purple spider plant (*C. gynandra)*, at rates 0, 5, 10, 15 % w/v, together with the chemical Ortivar®, Uniform, and no chemical treatment with no pathogen control and a no chemical treatment inoculated with *Pythium ultimum* control had significantly different effects noted on seedling early growth. The root length of tomato seedlings was significantly ($p < 0.01$) affected as shown above (table 4.1). The number of root tips per plant were significantly influenced ($p < 0.01$) by the treatments (biofumigants and the chemical controls) on the tomato seedlings. The root diameter and disease damaged seedlings were also significantly affected at $p < 0.001$ and $p < 0.05$ for tomato and sweet pepper seedlings respectively.

The effect of rate of amendment on *Rhizoctonia*

solani significantly ($p < 0.05$) affected root diameter of both tomato and sweet pepper seedlings. On root diameter of tomato seedlings, the chemical Uniform at rate 60 ml/3L had the least mean diameter on the crop (0.174 cm) and the bio fumigant with 10% Purple *C. gynandra* had the biggest root diameter (0.407 cm) which was statistically similar to 5% purple *C. gynandra* (0.309 cm), and the controls with pathogens amended and no chemical treatment and also a control with no pathogen amended and non biofumigant/ chemical treatment (0.32 cm and 0.327 cm respectively). The treatment effects were all similar except for tomato for rate 5 % green *C. gynandra* and Uniform 60ml/3L which decreased root diameter.

On sweet pepper seedlings the treatment effects were similar in on root diameter, root length and disease damage. The root diameters of plants treated with no chemical/ bio fumigant and no pathogen had the largest diameter (0.2631 cm) which was statistically the same with a bio fumigant, purple *C. gynandra* 5% w/v, and the chemical control of Ortivar® at rate 30ml/3L water. The chemical control, Ortivar® (30 ml/3L), performed statically the same with the biofumigant of both green and purple *C. gynandra* at rates 5, 10 and 15 % w/v (table 4.2). The chemical control, Uniform at rate 60ml/3L, had the least mean diameter (0.03 cm) on sweet pepper seedlings and this was statistically similar to the treatment 15% green and purple *C. gynandra.*

The effect of rate of amendment with green and purple *C. gynandra* species (at rates 0, 5, 10 and 15 % w/v) and the chemical control Ortivar®, Uniform, a non-chemical and no pathogen control and a non-chemical inoculated with pathogen control for *Rhizoctonia solani* significantly ($p < 0.05$) damaged the roots of both tomato and sweet pepper seedlings. The chemical control, Uniform at rate 60 ml/3L, had the worst disease damage score (7.19) which was similar statistically with other treatments (Uniform at rate 80 ml/3L water and the two bio fumigants, green and purple *C. gynandra* at rate 5% w/v) for tomato. The chemical Ortivar® (30 ml/3L) had an excellent control on disease damage which was also similar to the green and purple *C. gynandra* biofumigants at different rate of amendment (5, 10, 15 % w/v) on sweet pepper seedlings (table 4.2).

The effect of rate of amendment with green and purple *C. gynandra* species (at rates 0, 5, 10 and 15 % weight to volume (w/v)) and the chemical control Ortivar®, Uniform, a non- chemical and no pathogen control and a non -chemical inoculated with pathogen control on *Pythium ultimum* had significant differences ($p < 0.05$) noted for root length, number of root tips, root diameter and disease damage score at 28 days after planting (table 4.3).

The chemical Uniform at rate 80ml/3L had the longest root length mean (5.50 cm) which was similar to the biofumigants, purple *C. gynandra* at rate 10% w/v (4.06 cm), green and purple *C. gynandra* at rate 5% w/v (4.34 cm and 5.03 cm respectively) and the controls with no chemical amendments plus pathogens (5.31 cm) and no chemical/ bio-fumigant without pathogens (5.28 cm). There were no significant differences ($p < 0.05$) noted on root length for the sweet pepper seedlings (table 4.3).

The effect of rate of amendment with green and purple *C. gynandra* species (at rates 0, 5, 10 and 15 % weight to volume (w/v)) and the chemical control Ortivar®, Uniform, a non- chemical and no pathogen control and a non -chemical inoculated with pathogen control on *Pythium ultimum* had significant differences ($p < 0.05$) root length number of root tips at 28 days after planting (table 4.3) for the tomato crop seedlings. The biggest mean number of root tips was observed on the control with no chemical amendment and no pathogens inoculated (2.875 cm). The least mean number of root tips per plant was observed on the biofumigant green *C. gynandra* at rate10% wt/v (1.375). There were no significant differences ($p < 0.05$) noted on the number of root tips for sweet pepper seedlings

.

Table 4.3. The effect of rate of amendment for root length number of root tips, root diameter and disease damage at 28 days after planting tomato and sweet pepper seedlings.

	Pythium ultimum							
	Root length		Number of root tips per plant		Root diameter		Disease damage	
Treatment	Tomato	Sweet pepper	Tomato	Sweet pepper	Tomato	Sweet pepper	Tomato	Sweet pepper
No chemical + Pathogen	5.31^{ac}	3.44	2.25^{bc}	2.50	0.31^{ac}	0.19^{a}	4.62^{fhilm}	4.87^{g}
10% Green *C. gynandra*	3.38^{i}	2.78	1.38^{f}	1.63	0.15^{h}	0.12^{bc}	7.62^{ac}	6.31^{aceg}
10% Purple *C. gynandra*	4.06^{acegi}	3.19	1.63^{ce}	2.06	0.19^{fh}	0.09^{c}	6.62^{acegi}	5.94^{aceg}
15% Green *C. gynandra*	3.44^{hi}	3.03	1.75^{ce}	2.19	0.22^{bcfh}	0.07^{c}	6.38^{acegik}	6.62^{aceg}
15% Purple *C. gynandra*	3.81^{bdfgi}	2.25	1.38^{f}	1.94	0.18^{fh}	0.09^{c}	7.12^{aceg}	5.06^{fg}
5% Green *C. gynandra*	4.34^{acegi}	2.59	1.69^{ce}	1.69	0.21^{efh}	0.12^{c}	$6.19^{acegikm}$	5.62^{bdeg}
5% Purple *C. gynandra*	5.03^{aceg}	2.38	2.13^{ce}	1.81	0.25^{ach}	0.05^{c}	$5.75^{bdegikm}$	7.00^{ace}
No chemical + No Pathogen	5.28^{ace}	2.97	2.88^{a}	2.13	0.34^{a}	0.11^{c}	4.5^{n}	6.37^{aceg}
Ortivar 30ml/3L	4.75^{acegi}	3.66	1.81^{ce}	2.13	0.15^{gh}	0.08^{c}	6.38^{acegik}	6.44^{aceg}
Uniform 60ml/3L	4.16^{acegi}	3.09	1.44^{de}	1.31	0.13^{i}	0.07^{c}	7.81^{a}	7.62^{a}
Uniform 80ml/3L	5.50^{a}	2.31	1.56^{ce}	1.31	0.17^{fh}	0.08^{c}	7.25^{ace}	7.50^{ac}
LSD	1.46	NS	0.73	0.91	0.10	0.08	1.65	1.81
CV%	10.40	7.90	13.6	6.60	5.40	14.00	10.40	6.20

†Means followed by the same letter in a column are not significantly different from each other according to the LSD value. NS: Not significant

The mean root diameter on the tomato plants seedlings was also significantly affected ($p < 0.05$) (Table 4.3) at 28 days after planting. The controls (non- chemical with pathogens and non- chemical with no pathogen amendment) had the largest root diameter means (0.314 cm and 0.339 cm respectively) on the tomato seedlings. The two control treatments were statistically similar to a bio fumigant purple *C. gynandra* at rate 5% w/v (0.251 cm). On sweet pepper seedlings root diameter, the largest mean was observed on the control with no chemical plus a pathogen (0.1906 cm) and the least was observed on a bio fumigant green *C. gynandra* at rate 15% w/v.

The effect of rate of amendment with green and purple *C. gynandra* species (at rates 0, 5, 10 and 15 % weight to volume (w/v)) and the chemical control Ortivar®, Uniform, a non- chemical and no pathogen control and a non-chemical inoculated with pathogen control on *Pythium ultimum* had significant differences ($p < 0.05$) on disease damage of tomato and sweet pepper seedlings. The disease damage score varied significantly on both tomato and sweet pepper seedlings, with the worst control observed on the chemical Uniform 60 ml/3L, Uniform 80 ml/3L and Ortivar® 30 ml/3L respectively. The bio fumigants had a good-standard control of the pathogen *Pythium ultimum* on both tomato and sweet pepper seedlings (table 4.3).

4.0 DISCUSSION

The present study demonstrated the functional role of *C. gynandra* as a bio fumigant, and proved that the efficacy of both green and purple stem *C. gynandra* on growth inhibition of *R. solani* AG1-1A, and *P. ultimum* increased with an increase in plant tissue application rate. This study also showed that higher application rate of the chemical Uniform (80 ml/3L) did not offer any benefit to the control of both *R. solani* AG1-1A and *P. ultimum* and that the chemical can also be used successfully at the lower rate (60ml/3L). This is consistent with Handiseni et al. (2011), we observed that the amendment of *C. gynandra* at different levels varies with the species types being used. Some authors have attributed these differences to the type of chemical, cultivation, soil conditions, climate, humidity, photoperiod and several other environmental factors (Fahey et al., 2001).

The lowest level tested (5 % w/v) of green *C. gynandra* did not inhibit the incidence of either of the pathogen species, although root length, number of root tips and root diameter for both tomato and sweet pepper seedling were completely inhibited by both green and purple C. gynandra at 10 and 15 % w/v levels. The differences observed between *C. gynandra* plant tissue types may be partially accounted by the fact that various pathogen strains have different sensitivity to the glucosinolate. This has been confirmed by Handiseni *et al.*, 2013 where different fungal pathogens had varying degrees of sensitivity to allyl glucosinolate.

The amount glucosinolates of the two different *C. gynandra* variants affect *R. solani* AG1-1A, and *P. ultimum* differently. This is in support with the findings by Agostini (2011) who indicated that all glucosinolates were not equal in their effects on weeds. Fan *et al.* (2008) also agrees with the findings in this study were the growth inhibition achieved by *Brassica* species differed with the different fungal species. The explanation mighty be that different species of bio fumigants have different concentrations of the GSLs which also works in a varied fashion (Brown and Morra, 2005; Fan et al., 2008; Sarwar and Kirkegaard, 1998).

5.1 Results field experiment

The pesticidal activity of green and purple *C. gynandra*, rape (*B. napus*) and a non bio fumigant control showed significant differences ($p < 0.05$) on overall yields, number of fruits and number of surviving on sweet pepper (Table 5.1). The effect on the difference of the two pathogens used (Sub-plot) was observed on sweet pepper. There were significant ($p < 0.001$) interactions observed on the effect of the main plot and sub plot treatments on pathogen damage of sweet pepper plants (Table 5.1).

The effect of the different main plot effects showed significant differences on the total number of surviving plants on tomato. The effect of the different pathogen used (Sub plot) showed significant differences ($p < 0.05$) on overall fruit number of tomatoes. There was significant interaction ($p < 0.05$) effect on the main plot and sub-plot treatment on pathogen damage on tomato plants (Table 5.1).

Sweet pepper was shown to have been affected significantly ($p < 0.05$) by the application of biofumigation treatments. Purple *C. gynandra* treatment have shown to positively affect the overall yield, overall fruit number and total number of surviving plants when compared to the standard control rape and a non bio fumigant chemical control in sweet pepper plants (Table 5.3). However, no significant ($p < 0.05$) differences were found between bio fumigant treatments on the pathogen damage scores of sweet pepper of plants (Table 5.3). Tomato was shown to have not been affected by the application of main plot treatments and no significant ($p < 0.05$) differences were found in the overall yield, total number of fruits and pathogen damage except for total number of surviving plants (Table 5.3).

Table 5.1. Analysis of variance (*P* value) for the pesticidal activity of green and purple *C. gynandra*, rape (*B. napus*) and a non bio fumigant control.

		Sweet pepper				Tomato			
		Overall yield	Fruit number	Number of plants	Pathogen damage	Overall yield	Fruit number	Number of plants	Pa da
Source	D.F.	P. value	P. value	P. value	P. value	P. value	P. value	P. value	P.
Bio fumigant (B)	3	0.002	0.045	0.029	0.474	0.168	0.293	<0.001	0.
Pathogen (P)	1	0.249	0.981	0.182	0.058	0.212	0.036	0.069	0.
B X P	3	0.964	0.177	0.943	<0.001	0.766	0.661	0.111	0.

Table 5.2. The pesticidal activity of green and purple *C. gynandra*, rape (*B. napus*) and a non bio fumigant control on plants inoculated with *Rhizoctonia solani* and *Pythium ultimum*.

	Sweet pepper				Tomato		
Main plot	Overall yield	Overall fruit number	Number of plants	Pathogen damage	Overall yield	Overall fruit number	Number plants
Green C. gynandra	2939.00†^{b}	49.90^{b}	14.00^{bc}	3.50	17956.00	358.00	20.38^{a}
Non bio fumigant	5293.00^{a}	74.90^{a}	15.75^{a}	2.75	15070.00	309.00	18.00^{f}
Purple C. gynandra	5064.00^{a}	70.40^{a}	14.38^{ac}	3.00	24947.00	419.00	19.50^{bde}
Rape	5022.00^{a}	75.90^{a}	13.25^{c}	4.50	17492.00	414.00	20.25^{ac}
LSD	1415.00	19.37	1.53	NS	NS	NS	0.35
CV%	19.30	17.90	66.70	24.50	30.90	22.20	1.10

†The means followed by the same letter in a column are not significantly different from each other according to the LSD value. NS: Not significant

5.2 FIELD TRIAL DISCUSSION

The results in this study, showed that the application of *C. gynandra* plant tissue bio fumigants has an effect on overall yield, the overall fruit counts and the total number of plants of sweet pepper plots inoculated with *Rhizoctonia solani* and *Pythium ultimum*. The results in this study, also showed that the application of *C. gynandra* plant tissue bio fumigants has an effect on the total number of plants of tomato plots inoculated with *Rhizoctonia solani* and *Pythium ultimum*.The results obtained in this experiment could be as a result of the allelochemical effects of GSLs on the pathogens *Rhizoctonia solani* and *Pythium ultimum* inoculated as what has been reviewed by Brown and Morra (1997) in previous work. It is also reported that GSLs may greatly influence fungal growth and are suspected to be the major inhibitors of fungal activity (Sotelo et al., 2015).

In this study the biofumigation with the purple and green *C. gynandra* increased the yields of both tomato and sweet pepper in the trials and it was comparable to the chemical control These results supports the findings of (Handiseni et al., 2013)) and illustrates the potential of biofumigation for reducing *Rhizoctonia solani* and *Pythium ultimum* in the field. The findings indicate that field biofumigation of *Rhizoctonia solani* and *Pythium ultimum*, by incorporation of *C. gynandra* plant tissue. This represents a useful reduction in *Rhizoctonia solani* and *Pythium ultimum*, for Zimbabwean farmers. Moreover, the similar efficacy recorded in this paper to

that recorded by Watts et al., 2014 when reporting on fosthiazate nematicide for reduction of PCN population densities, suggests in-field biofumigation to be a strong candidate for replacement of granular nematicides in the event of their retraction from industry (Watts et al., 2014).

Results in this study were contrary to the results by Soldevilla Martinez (2009) for the non-brassica cover crop rye, but the results on *C. gynandra* were very similar to those obtained for the brassicas. In our study the yield varied significantly between the non bio fumigant controls, non-plant residues and the green and purple *C. gynandra* plant tissue bio fumigants.The negative impact of *Brassica* tissues been investigated, and the few studies that were found show contradictory results (Sotelo et al., 2015). In the case of *R. solani*, other studies the results shows that the response varies with the bio fumigant being used (Bonanomi et al., 2007; Boydston and Hang, 1995; Soldevilla Martinez, 2009; Sotelo et al., 2014).

C. gynandra bio fumigant species effects were shown to vary significantly in sweet pepper yields, number of fruits and number of surviving plants (table 7.2). This suggests that the GSL concentration of the tested *C. gynandra* accessions have variable effects as bio fumigants on *Rhizoctonia solani* and *Pythium ultimum* activity (table 7.2). Overall the results of this study suggest that our candidate purple and green *C. gynandra* are effective in reducing *Rhizoctonia solani* and *Pythium ultimum* activity and this was comparable to our control under field conditions.

Green *C. gynandra* plant tissue as a treatment had low levels of glucosinolates (table 4.1) and it has been increasing yields comparatively with the chemical controls which are effective in soilborne disease suppression. This supports the experiment by Larkin and Griffin (2007), where they studied the control of soilborne potato diseases using brassica green manures with different levels of glucosinolates. The results indicated that disease reductions were not always associated with high production of glucosinolates. In the present study the mechanisms behind any suppressive effects of green manure *C. gynandra* crops were not investigated, but since the *C. gynandra* had specific suppressive effect, it can be concluded that *C. gynandra* glucosinolates had a suppressive factor in this case even at low levels (Soldevilla Martinez, 2009).

In this study inconsistencies of pathogen suppression may be due to low concentrations of ITCs released during glucosinolate hydrolysis. Such abiotic factors may also lead to reduced toxicity of ITCs during the fumigation process. Until the relationship between environmental factors, glucosinolate accumulation and hydrolysis and the resulting soil fumigation is fully understood it will be difficult to predict the full potential of pathogen suppression using biofumigation. There has been evidence produced that biofumigation can successfully be used to control several fungal pathogens (Bonanomi et al., 2007; Hollister et al., 2013; Hu et al., 2015; Kaur et al., 2011), and similarly in this study it has been observed green and purple C. gynandra can suppress the effects of *Rhizoctonia solani* and *Pythium ultimum* species.

The results from this study indicate that biofumigation using *C. gynandra* from the field trials was not consistent on the tomato trials. However, the use of different cultivars which possess glucosinolate profiles more resistant to the effects of abiotic factors, including moisture and temperature, may produce different results. *R. solani and P. ultimum* are known to be notoriously difficult to control using traditional control methods, and therefore the pathogen itself may possess attributes which prevent effective control.

The high volatility level of ITCs has often been highlighted as an aspect which may limit the efficiency of a biofumigation system (Gimsing and Kirkegaard, 2009). However the biofumigation principal works on the ‘mustard bomb’ effect, releasing a short blast of isothiocyanates at high concentrations which aims to kill soil borne pathogens and weeds within the soil. It is also hoped that this approach will limit any adverse effects on non-targeted soil fungi. However, investigating an incorporation method which will best seal ITCs into the soil, and limit their initial depletion will allow them to come into contact with increased numbers of pathogens within the soil.

The weed species in the field are primary source of future weed populations and this provides seed bank, a unique source for predictive management studies (Karavina and Mandumbu, 2012a). Species richness (S) (table 7.3) quantifies how many different types, weed species, are contained in the field under study, the number of different species in the corresponding list ranged from 4.62 to 6.38. Weed richness (S) is different from weed abundances as it is different from diversity. Species evenness refers to the proportion that each species comprises of the whole. The results above show that the diversity is high on all the quadrats sampled. The diversity index is a quantitative measure that reflects how many different weed species are there in the field under study and simultaneously it takes account of how evenly the weed species are distributed among the types.

The value of a diversity index increases both when the number of types of weeds increases and when evenness increases as shown on table 7.3 above. The Shannon-Wiener diversity index (H) was originally proposed to quantify the entropy (uncertain or information content) in strings of text. This method is used as the weed species are of interest to the farmer. Weed density studies are an important weed management strategy to consider in implementing a new cropping system. The effects of new planting systems such as green manuring (biofumigation) can be accurately determined by weed density studies as it will reveal weed trends in time (Karavina and Mandumbu, 2012b).

6.1 Conclusion and Recommendations In vitro experiment

This study demonstrated that *Cleome gynandra* as a bio fumigant has a suppressive effect on the growth of soil pathogens (*Rhizoctonia solani AGI-1A, Rhizoctonia cerealis, Rhizoctonia solani, Pythium irregulare, Pythium ultimum, Pythium aphanidermatum, Phytophthora infestans, Phytophthora capsci* and *Phytophthora cinnamomi*). Green *C. gynandra* is more effective than Purple *C. gynandra*. Further research is essential to demonstrate if the suppressive effect on their growth can be achieved with pathogens under greenhouse and field conditions. Biofumigation has the potential to become a common management tool used in vegetable and cut flower cultivation as a part of overall integrated management strategy to control soil-borne pathogen.

6.2 Conclusions and Recommendations greenhouse pathogens experiment

In the present study both green and purple *C. gynandra* were found to be potent bio fumigants obtained from macerating whole plant of *C. gynandra* at full flowering stage and can be used as a green manure crop. *C. gynandra* when used as a green manure can act as bio-pesticide on the plant soilborne pathogens such as *Rhizoctonia solani* and *Pythium ultimum* and can be used as a bio fumigant. The application of *C. gynandra* reduced the incidence and severity of disease damage on both tomato and sweet pepper and it was comparable to the control Ortivar® and Uniform. Further toxicity of *C. gynandra* (if any) to the environment is to be studied.

6.3 Conclusions and Recommendations field experiment

Our results demonstrate that the application of *C. gynandra* plant tissue had a pesticidal effect and can suppress the development of the pathogens, *Rhizoctonia solani* and *Pythium ultimum,* species and weeds species in the field studied. The results also conclude that the effects between different *C. gynandra* bio fumigants, weed species and pathogen species can vary depending on the combination of *C. gynandra*, weed and pathogen species. However, the use of different *C. gynandra* species which possess more glucosinolate concentrations, more resistant to abiotic stresses, including moisture and temperature, may produce different results.

Further studies are required to explain the mechanisms behind the *C. gynandra* suppressive effect in the field and how it can be exploited in integrated crop management. A study looking into the incorporation techniques and the cost benefit analysis into the field will also help to improve the use, effectiveness and efficiency of the *C. gynandra* plant tissue as bio fumigants.

7. Acknowledgements

The authors acknowledge IFS grant awarded to Dr. M. Handiseni which funded the work. I would like to thank Syngenta Agro-Services Zimbabwe for the chemicals Ortivar and Uniform. The support of the Floriculture and Research Farm Section (Horticulture Research Centre), Weeds Research Unit (Agronomy Research Institute) and the Plant pathology section (University of Zimbabwe, Crop science Department) in establishment, management and data collection is appreciated. The Tobacco Research Board (Plant pathology) is also acknowledged for the pathogen specimens. Sincere appreciation also goes to the Department of Crop Science, University of Zimbabwe, the Department of Research and Specialist Services (DR&SS) and the Head of Horticulture Research Institute for the support.

8. REFERENCES

Alves, A.O., Santos, M.M.B., Santos, T.C.G., Souza, E.B., Mariano, R.L.R., 2014. Biofumigation with essential oils for managing bacterial wilt of sweet peppers. J. Plant Pathol. 96.

Angus, J.F., Gardner, P.A., Kirkegaard, J.A., Desmarchelier, J.M., 1994. Biofumigation: ITCs released from Brassica roots inhibit growth of the take-all fungus. Plant Soil 162, 107–112.

Bonanomi, G., Antignani, V., Pane, C., Scala, F., 2007. Suppression of soilborne fungal diseases with organic amendments. J. Plant Pathol. 311–324.

Boydston, R.A., Hang, A., 1995. Rapeseed (Brassica napus) green manure crop suppresses weeds in Potao (Solanum tuberosum). Weed Technol. 9, 669–675.

Brown, J., Morra, M.J., 2005. Glucosinolate-containing seed meals as a soil amendment to control plant pests. Natl. Renew. Energy Lab. 510, 8–17.

Brown, P.D., Morra, M.J., 1995. Glucosinolate-containing plant tissues as bioherbicides. J. Agric. Food Chem. 43, 3070–3074.

Edwards, S., Ploeg, A., 2014. Evaluation of 31 Potential Bio fumigant Brassicaceous Plants as Hosts for Three Meloiodogyne Species. J. Nematol. 46, 287–295.

Fahey, J.W., Zalcmann, A.T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56, 5–51.

Fan, C.M., Xion, G.R., Ji, G.H., He, Y.Q., 2008. Potential Biofumigation Effects of Brassica oleracea var. caulorapa on Growth of Fungi. J Phytopathol. 156, 321–325. https://doi.org/10.1111/j.1439-0434.2007.01343.x

Gimsing, A.L., Kirkegaard, J.A., 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochem. Rev. 8, 299–310. https://doi.org/10.1007/s11101-008-9105-5
Handiseni, M., Brown, J., Zemetra, R., Mazzola, M., 2013. Effect of Brassicaceae seed meals with different glucosinolate profiles on Rhizoctonia root rot in wheat. Crop Prot. 48, 1–5. https://doi.org/10.1016/j.cropro.2013.01.006
Hanschen, F.S., Yim, B., Winkelmann, T., Smalla, K., Schreiner, M., 2015. Degradation of Bio fumigant Isothiocyanates and Allyl Glucosinolate in Soil and Their Effects on the Microbial Community Composition. PLoS ONE 10. https://doi.org/10.1371/journal.pone.0132931
Hollister, E.B., Hu, P., Wang, A.S., Hons, F.M., Gentry, T.J., 2013. Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities. FEMS Microbiol. Ecol. 83, 632–641. https://doi.org/10.1111/1574-6941.12020
Hu, P., Hollister, E.B., Somenahally, A.C., Hons, F.M., Gentry, T.J., 2015. Soil bacterial and fungal communities respond differently to various isothiocyanates added for biofumigation. Front. Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00729
Karavina, C., Mandumbu, R., 2012a. Biofumigation for crop protection: potential for adoption in Zimbabwe. J. Anim. Plant Sci. 14, 1996–2005.
Karavina, C., Mandumbu, R., 2012b. Phytoparasitic nematode management post-methyl bromide: Where to for Zimbabwe? J. Agric. Technol. 8, 1141–1160.
Kaur, R., Rampal, G., Vig, A.P., 2011. Evaluation of antifungal and antioxidative potential of hydrolytic products of glucosinolates from some members of Brassicaceae family. J Plant Breed Crop Sci 3, 218–228.
Lord, J., Lazzeri, L., Artkinson, H., Urwin, P., 2011. Biofumigation for control of potato cyst nematodes: Activity of brassica leaf extracts and green manures on Globodera pallida in vitro and in soil. J. Agric. Food Chem. 59, 7882–7890.
Matthiessen, J.N., Shackleton, M.A., 2005. Biofumigation: environmental impacts on the biological activity of diverse pure and plant-derived isothiocyanates. Pest Manag. Sci. 61, 1043–1051. https://doi.org/10.1002/ps.1086
Mhazo, M.L., 2011. The Effectiveness of Home Made Organic Pesticides Derived from Wild Plants (Solanum pindiriforme and Lippia javanica), Garlic (Allium sativum) and Tobacco (Nicotiana tobacum) on Aphid (Brevicoryne brassica) Mortality on Rape (Brassica napus) Plants [WWW Document].
Ngadze, E., 2014. In vitro and greenhouse evaluation of botanical extracts for antifungal activity against Phythopthora infestans. JBiopest 7, 199–204.
Prasad, P., Kumar, J., Pandey, S., 2015. Biofumigation: Success and Prospects in Soilborne Plant Disease Management. Int. J. Appl. Pure Sci. Agric. 1, 2394–5532.
Sarwar, M., Kirkegaard, J.A., 1998. Biofumigation potential of brassicas. II. Effect of environment and ontogeny on glucosinolate production and implications for screening. Plant Soil 201, 91–101.
Soldevilla Martinez, M., 2009. Influence of cover crops on the development of some soil-borne plant pathogens.
Sotelo, T., Lema, M., Soengas, P., Cartea, M.E., Velasco, P., 2015. In Vitro Activity of Glucosinolates and Their Degradation Products against Brassica-Pathogenic Bacteria and Fungi. Appl. Environ. Microbiol. 81, 432–440. https://doi.org/10.1128/AEM.03142-14
Sotelo, T., Soengas, P., Velasco, P., Rodríguez, V.M., Cartea, M.E., 2014. Identification of Metabolic QTLs and Candidate Genes for Glucosinolate Synthesis in Brassica oleracea Leaves, Seeds and Flower Buds. PLoS ONE 9, e91428. https://doi.org/10.1371/journal.pone.0091428
Vaughn, S.F., Isbell, T.A., Weisleder, D., Berhow, M.A., 2005. Bio fumigant compounds released by field Pennycress (Thlaspi arvense) seedmeal. J. Chem. Ecol. 31, 167–177. https://doi.org/10.1007/s10886-005-0982-4
Vincent, V., Thomas, R., 1962. An Agricultural Survey of Southern Rhodesia Part 1. Agro ecological survey. Gov. Print. 102–154.
Watts, W.D.J., Grove, I.G., Tomalin, G.R., Back, M.A., 2014. Field screening of bio fumigant species for the reduction of potato cyst nematodes (Globodera sp.). Asp. Appl. Biol. 126.