EFFECTS OF FRESH SHOOT BIOMASS OF (Chromolaena odorata) [L.] King and H. Robinson ON THE GERMINATION AND GROWTH OF (Abelmoschus esculentus) [L.] Moench.

BY

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CERTIFICATION

This is to certify that this project work was done by ANIBUEZE EBERE .Stephanie of the Department of Plant Science And Biotecnology, Federal University Oye -Ekiti. The report has been read and approved and has met the requirement for the award of Bachelor of Science (B.Sc) Degree in Plant Science and Biotechnology, Federal University Oye Ekiti state.

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DEDICATION

This research work is dedicated to:

The Almighty God for His grace, mercies, favour and protection over my life, and also for granting me the strength to complete my program in school.

My ever loving parents Dr and MRS Anibueze for their undying love and support not only from inception to completion of my studies in Federal University Oye Ekiti, but at all times, and for teaching me to live a purposeful life. Lastly, to my siblings for their love and encouragement.

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I am grateful to God for the grace he granted me to complete my mission in school, I would not have come this far on my own. Indeed He is the source of my wisdom, foundation of my knowledge, the giver of divine strength and the spring of illuminating inspirations. His guidance and protection throughout the period of my studies cannot be quantified, He is to be honoured.

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TABLE OF CONTENTS

Chapter three

Chapter 4

List of graphs

List of tables

ABSTRACT

This study was carried out in the Department Of Federal University Oye Ekiti, Ekiti State. Investigation was carried out on the effects of fresh shoot biomass of *Chromolaena odorata* on okra *Abelmoschus esculentus*. The growth variables considered include, radicle length, plumule length, number of roots, for the green house experiment the plant height, leaf area, stem girth, and pod weight were also observed. The leachate of *chromolaena odorata* resulted to a reduction in radicle length, plumule length and number of roots. The radicle length of okra treated with leachates of 15g/250ml were adversely affected, while the plumule length of 5g/250ml, 15g/250ml and 20g/250ml greatly decreased compared to the control. The highest decrease in number of roots was noticed in the 15g/250ml concentration. The control group had the highest plant height increased in a concentration dependent manner with exception of those treated with 200g, the leaf area of the control group was observed to be lower than all the treatment groups, but it increased in a concentration dependent manner. Also there was a concentration dependent increase in the steam girth of *A. esculentus*. The pod weight of the treated plants were lower *than the control. The group treated with 50g highest moisture content, the fat content was higher* in the control and group treated with 50g than the other treated group. The protein content of the treatment groups was observed to be significantly higher when compared to the control group. Crude fiber content of the treatment group was significantly higher than the treatment groups. The ash content of the control was significantly lower than the control groups, the carbohydrate content of the control group increased significantly compared to the treatment group with the lowest at 150g treated group. The study concluded that the leachate and FSB affected the seed germination, growth and the food content of *Abelmoschus esculentus*.

CHAPTER ONE

1.0 INTRODUCTION.

In the developing countries, the rural farmers usually collect the fallen leaves of siam weed and put them in the field soil for composting. In slash-and-burn agricultural system, this weed is also incorporated in the soil after burning the dried plants. But there are reports that the farmers face difficulty in crop establishment in the *Chromolaena odorata* ,treated fields (Koutika and Kamga 2006).

Recently, increased interest has been given to enhance the crop emergence under the slash-and burn agriculture in many countries (Koutika LS, 2002). According to Raimundo RLG *et al.*, (2007), *Chromolaena odorata* needs light to germinate and is suppressed when shaded by other plants. The plant grows on a wide range of solids, but not on flooded sites. Through symbiosis with vesicular-arbuscular mycorrhizae, it also can grow well on poor soils condition (Chakraborty, A. *et al.,* 2011). Under a *Chromolaena odorata* uncultivated soil, the soil structure improves and the pH and biological activity of poor soil increase. Wilson D, *et al.,* (2007) stated that *Chromolaena odorata can* tolerate mechanical injuries caused by slashing and burning, as it is able to form new shoots on the swollen part of the root. However, frequent injuries will reduce the plant's regenerative capacity (Fara *et al* 2007). *Chromolaena odorata* plant contains the allelochemicals which affect germination, growth, development, distribution and behaviour of neighbouring plants including weeds (Wink, 1987; Fujii et al, 2007; Hashem .A., *et al.,* 2011). Allelochemicals are defined as any direct or indirect harmful or beneficial effect by a plant including microorganisms on another through production of chemical compounds that escape

into environment (Rizvi, *et al.,* 1992). The chemical can be found in root, stem, leaves, flowers and fruits, and inhibit root growth, shoot growth, germination percentage as well as nutrients uptake (Marwat N, *et al* 2006; Ali N, *et al* 2011). The allelopathic effects are selective and vary with different trees since these plants will vary in the amount of original secondary metabolites and would release different amount of the phytotoxins.

Generally, leaves are the most powerful source of allelochemicals (Lawan, *et al.,* 2011). According to (Fara *et al.,* 2014) the phytochemical screening on the extract proved that there were presence of tannins, flavonoids, alkaloids, terpenoids, steroids and carbohydrates which aid in the inhibition on the roots and shoots elongation. By virtue of its strong inhibitory effect, the invasive weed *Chromolaena odorata* has the potential to interrupt regeneration processes of other species of plant by decreasing the germination and reducing early growth rates as well as has the potential of using it's allelopathic characters to suppress the growth of weeds. It can further be used as a tool to formulate new eco-friendly bioherbicides for weeds control in agro-ecosystems and natural ecosystems.

Chromolaena odorata has been ethnomedicinally used as a therapeutic agent for a variety of diseases. In a systematic review by Anup .K., *et al* (2011), the pharmacologic studies conducted on *Chromolaena odorata* indicate the immense potential of this plant in the treatment of conditions, such as wound healing, anti-inflammatory, analgesic, antipyretic, diuretic, antimicrobial, anti-mycobacterial and many more. Flavonoids, triterpenes, chalcones, steroids which were isolated from this plant may be responsible for its pharmacological activities. Also the isolation of phytoconstituents has been done on leaves, roots, flowers but no work has been done on the stem part. So this part has to be explored by the researchers.

1.1.1 Statement of problem

Insufficient information on the impacts of *Chromolaena odorata* on the emergence and establishment of field crops in humid tropics especially has led to gross loss in farm produce annually. Therefore this study will help evaluate the effects of *Chromolaena odorata* extracts on seed germination and seedling growth of field crops, which might be helpful in designing appropriate management strategies for sustainable agriculture.

1.1.2Aim of study

The general aim of this study is to investigate the effects of *Chromolaena odorata* on okra *Abelmoschus esculentus.*

1.1.3 OBJECTIVES OF STUDY

This study was undertaken to evaluate the effects of *Chromolaena odorata* on

i. growth and germination of okra

ii. quantity of fruits produced in the presence of *Chromolaena odorata* on okra,

iii. to compare the food contents of the okra used in the study with the fruits produced in the presence of the Fresh Shoot Biomass of *Chromolaena odorata.*

CHAPTER TWO

LITERATURE REVIEW

2.1.1 Okra (*Abelmoschus esculentus*)

It is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. It is one of the oldest cultivated crops and presently grown in many countries and is widely distributed from Africa to Asia, southern Europe and America. It is usually consumed for its green tender fruits as a vegetable in a variety of ways. These fruits are rich in vitamins, calcium, potassium and other mineral matters, the mature okra seed is a good source of oil and protein has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid, which is essential for human nutrition. Its mature fruit and stems contain crude fibre, which is used in the paper industry (Sathish *et al.*, 2013),

According to (Lamont W., 1999) okra bast is a multicellular fiber and was analyzed, the estimated average chemical compositions of OBF(*Abelmoschus esculentus* variety) are 67.5 % a-cellulose,15.4 % hemicelluloses, 7.1 % lignin, 3.4 % pectic matter,3.9 % fatty and waxy matter and 2.7 % aqueous extract. It is clear that the main constituents of OBF are a-cellulose, hemicelluloses and lignin and the rest are very minor in proportion, so render a little influence to the structure of OBF. Therefore, the structure of a-cellulose, hemicelluloses and lignin and the mode of combinations that exist in between themselves are dominating the structure of OBF.

2.1.2 Uses of okra

Okra is used for Antispasmodic, Demulcent, Diaphoretic, Diuretic, Emollient, Stimulant, Vulnerary purposes. The roots are very rich in mucilage, having a strongly demulcent action, this mucilage can be used as a plasma replacement. An infusion of the roots is used in the treatment of syphilis, the juice of the roots is used externally in Nepal to treat cuts, wounds and boils and the leaves furnish an emollient poultice. A decoction of the immature capsules is demulcent, diuretic, emollient and it is used in the treatment of catarrhal infections, dysuria and gonorrhoea. The seeds are antispasmodic, cordial and stimulant, an infusion of the roasted seeds has sudorific properties, (Adetuyi .F. O., Sanjeet k., et al).

According to (Sathish *et al* 2013), paper fibre obtained from the stems is used as a substitute for jute. It is also used in making paper and textiles. Okra contains special fibre which takes sugar levels in blood under control, providing sugar quantity, acceptable for the bowels. Mucilage, found in okra, is responsible for washing away toxic substances and bad cholesterol, which loads the liver. However okra contains special fibre which takes sugar level in blood under control, providing sugar quantity acceptable for the bowels. The mucilage it contains is responsible for washing away toxic substances and bad cholesterol which loads the liver.

Also Purgative properties of okra are beneficial for bowel purification. Okra fibre contains valuable nutrient for intestine microorganisms, this ensures proper intestine functionality and

ensures recovery from psychological and mental conditions, like, depression and general weakness. Okra is an effective remedy for ulcers and joint healthiness, It guards the mucous membranes of the digestive system, by covering them with additional layer.

According to Indian researches, okra is a complex replacement for human blood plasma. In order to keep the valuable substances safe, it's necessary to cook okra as shortly as possible, processing it either with steam, or on low heat. (Siemonsma J.S. et al 2000; Akinyele B.O., et al 2007)

2.1.3 Diseases of okra

The most serious fungal diseases of okra in Africa are damping-off (*Macrophomina phaseolina*, Pythium*aphanidermatum*, and *Rhizoctonia solani*), vascular wilt (*Fusarium oxysporum*), Cercospora blight (*CercosporaAbelmoschus*, *Cercospora malayensis*) and powdery mildew (*Erysiphe cichoracearum*, *Oidium abelmoschi*). Okra mosaic virus (OkMV), transmitted by flea beetles (*Podagrica*), is wide spread in Africa but damage is much less important than that caused by okra leaf curl disease(OLCV), transmitted by whitefly (*Bemisia tabaci*). Whitefly is also the vector of yellow vein mosaic virus (BYVMV), a major cause of crop failure in Asia. These viruses can only be controlled through control of the vectors. Nematodes of the genus *Meloidogyne* constitute a major problem. Damage by nematodes is avoided by crop rotation (e.g. with cereals) and by large applications of organic manure.

Important pests are fruit and stem borers (*Earias* spp. And*Heliothis* spp., *Pectinophora gossypiella*), flea beetles(*Podagrica* spp.) and jassids (*Empoasca* ,spp.). Chemical control is hazardous because crop harvesting is frequent. Common okra is in general more seriously affected by diseases and pests than West African okra (Hochreutimer B.P.G.,:1924; Terell E.E 1974; Gopalan C.,2007).

2.2 *Chromolaena odorata* (L.) King & H. Rob.

2.2.1 Description

Chromolaena odorata (independence leaf), this name was adopted in Nigeria when she got her independent and the plant was introduced into Nigeria in 1960 , also called *Osmia odorata* L. or *Euphatorium odoratum* L.), it belongs to the family *Asteraceae*. In taxonomical order of the plant, *Chromolaena* is the genus name while, *odorata* is the species name. Its common names include "Awolowo" in Yoruba language, in Igbo language," Obiraohu" in Bumaji-Boki, siam weed," triffi" weed in Hausa, bitter bush or jack in the bush (Okon and Amalu, 2003). It is a specie of flowering shrub in the sunflower family, it is a fast-growing perennial shrub, native to South America and Central America from Florida and Texas to Mexico and the Caribbean. It has been introduced into the tropical regions of Asia, Africa and the Pacific, and parts of Australia, Central and Western Africa, tropical America, India, Philippines, southern China, South Africa, eastern Indonesia, and Australia where it is an invasive weed (Raimundo R.L.G *et al,* 2007), this species has been considered as one of the most invasive weeds in world that threatens agriculture and the environment it is commonly called Siam Weed, Christmas Bush, and Common Floss Flower, it is sometimes grown as a medicinal and ornamental plant. *Chromolaena odorata* thrives in hot and humid areas, within latitudes 30o north and south and at an altitude of approximately 1000 m (Ambika, 1998). It proliferates in regions with rainfall greater than 200 mm per annum and where temperatures range from 20 °C to 37 °C 6 (Ambika & Jayachandra, 1990).

Chromolaena odorata is considered invasive weed of field crops in its introduced range, and has been reported to be the most problematic invasive species within protected rainforests in Africa.(Struhsaker .T.T., *et al* 2005).

It forms dense stands that prevent the establishment of other plant species. It is an aggressive competitor and may have allelopathic effects. It is also a nuisance weed in agricultural land and commercial plantations, it contains carcinogenic-pyrrolizidine alkaloids (Fu.P.P., *et al.,* 2002).

It can suppress crops and other plants by competing for nutrients and water, over-shading and allelopathy (Wilson, 2007). *Chromolaena odorata* leaves especially the young ones are toxic due to high levels of nitrate (Orapa .W., *et al* 2000).

According to Ambika, S.R., 2002, *Chromolaena odorata* can quickly establish and often form a dense scrambling thicket that grows through and smother plant crops, forestry, and native vegetation in most invaded countries. It invades a diverse range of habitats, including roadsides, agricultural fields, disturbed grasslands, and abandoned fields, causing great economic and biodiversity losses. The mechanisms underlying the invasion success of *Chromolaena odorata* are still not well understood. *Chromolaena odorata* is thought to depend on the combination of its high reproductive capacity (Koutika, *et al* 2010) high relative growth rate (Ramakrishnan *et al* 1989), capacity to inhibit native plant growth by light competition (Honu *et al.,* 2000), and accumulation of native soil pathogens (Mangla *et al.,* 2008). Previous studies have shown that *Chromolaena odorata* produces a variety of allelochemicals, including flavonoids, terpenoids, and alkaloids (Akinmoladun, A.C.).

Chromolaena odorata is a herbaceous perennial plant that forms dense tangled bushes 1.5-2.0m in height. It occasionally reaches its maximum height of 6m (as a climber on other plants). Its stems branch freely, with lateral branches developing in pairs from the auxiliary buds. The older stems are brown and woody near the base tips and young shoots are green and succulent. The root system is fibrous and does not penetrate beyond 20-30cm in most soils. The flower heads are borne in terminal corymbs of 20 to 60 heads on all stems and branches. The flowers are white

or pale bluish-lilac, and form masses covering the whole surface of the bush. Rachel (Cruttwell, *et al.,* 1996) *Chromolaena odorata* is a big bushy herb with long rambling (but not twining) branches; stems terete, pubescent; leaves opposite, flaccid-membranous, velvety-pubescent, deltoid-ovate, acute, 3-nerved, very coarsely toothed, each margin with 1-5 teeth, orentire in youngest leaves; base obtuse or subtruncate but shortly decurrent; petiole slender,1-1.5cm long; blade mostly 5-12cm long, 3-6cm wide, capitula in sub-corymbo seaxillary and terminal clusters; peduncles 1-3cm long, bracteate; bracts slender, 10-12mm long; involucre of about 4-5 series of bracts, pale with green nerves, acute, the lowest onesabout 2mm long, upper ones 8-9mm long, all acute, distally ciliate, flat, apprised except the extreme divergent tip; florets all alike (disc-florets), pale purple to dull off-white, the styles extending about 4mm beyond the apex of the involucres, spreading radiately; receptacle very narrow; florets about 20-30 or a few more, 10-12mm long; ovarian portion4mm long; corolla slender trumpet form; pappus of dull white hairs 5mm long; achenesglabrous. The seeds of Siam weed are small (3-5mm long, ~1mm wide, and weigh about 2.5mg seed-1.(Vanderwoude *et al.,* 2005).

(Onwugbuta-Enyi, 2001) have studied the allelopathic effects of *Chromolaena odorata* toxin on tomatoes (*Lycopersicum esculentum* Mill), and found significant growth reduction of the tomatoes with *Chromolaena odorata* leaf extract at concentrations as low as 1 g of fresh weight in 40 ml of water. Similarly, (Suwal *et al.*, 2002 have revealed that aqueous extracts of *Chromolaena odorata* may contain water-soluble allelochemicals that exert inhibitory effects on the germination and seedling growth of paddy and barnyard grass by Petri dish bioassays. Most of these findings showed that substances released by the parts of *Chromolaena odorata* remarkably influence the seed.

2.2.2 Ecology

Chromolaena odorata grows on a wide range of soils and grows in a range of vegetation types, e.g. forests (annual rainfall 1500mm), (Vanderwoude *et al.*, 2005). In arid areas, it is restricted to riverbanks and it will only become invasive in the frost-free areas of medium to arid woodland which are not water-stressed in the growing season. (Honu YAK *et al., 2000*; Vanderwoude, 2005) For good growth of Siam weed seedlings, the relative humidity should be in the range of 60 – 70%; at values higher than 80% the growth performance was poor (Vanderwoude *et al.* 2005). Experiments shows that Siam weed seedlings grows well at 30°C and even better on mulched soils at 25°C. In heavy shade, Siam weed will not seed. It has a negative relationship with tree canopy cover and appears to be most abundant on the edge of forested areas.(Phoebe Luwum 2002; Vanderwoude *et al* 2005).

According to Witkowski (2002) in north-eastern India, Siam weed is regarded as a nutrient-demanding early successional species (Ramakrishnan *et al* 1998). It takes advantage of the flush of soil that becomes available after a disturbance, such as fire or land clearing for agriculture, and exhibits relatively high foliar N, P and K contents (Ramakrishnan et al, 2005).

2.2.3 Reproduction

Flowering and fruiting begins after plants are 1 year old (Binggeli P, *et al* 1998), the flowers are pollinated by insects. The small fruits mature in about a month. A second collection averaged 1,560,000 seeds/kg and gave 11 percent germination between 3 and 120 days after sowing. Germination is epigeal, the seeds are wind dispersed, and can be dispersed by animals (Binggeli P, 1999) Stems root whenever they come in contact with the ground.

2.2.4 Management

Individual stems last about 2 years and die back to or near the base and are replaced by new sprouts. Plants easily survive cutting and fire. The best current control method is mechanical or hand cutting followed by herbicide treatment. Partial control can be obtained.

Mechanical

Mechanical control has been routinely implemented for the management of *Chromolaena* (Ambika, 1998). Young plants can be easily hand-pulled. Hoes, machetes and bush knives are commonly used to eradicate small stands (Muniappan & Marutani, 1991). Weed control methods include labour intensive hand weeding, digging, uprooting and use of machinery such as brush cutters, mowers, tillers, ploughs and other tractor drawn equipment (Muniappan & Marutani, 1991). In timber plantations, manual slashing is usually performed annually (Erasmus, 1988). In conservation areas, mature stands are first slashed followed by uprooting. However, the use of motorised brush cutters and tractor-drawn mowers can be restricted by the accessibility of the terrain and the prevalence of desirable plants.

In general, slashing and burning outside the farm measures are taken to curtail the growth of the weed in agricultural land and prevent its spread. Ploughing to a depth of 20 cm or more is known to successfully limit weed infestation and this may account for its absence in mechanical holdings (M'Boob, 1991).

Chemical

Most chemical control studies on *Chromolaena* were conducted in the Philippines, Indonesia, India, West and South Africa. These include 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), glyphosate, 2,4,5 trichlorophenoxy-acetic acid (2,4,5-T),

trichlopyr, 3,6 dichloropicolinic acid (3,6-D), paraquat and paraquat-based mixtures with diuron, atrazine, terbuthylazine, ioxynil and fluorodifen (M'Boob, 1991).

2.2.5 Economic importance of *Chromolaena odorata*

Chromolaena odorata is an ornamental plant, It is mainly used by the tribal people for the treatment of cuts and wounds. It is also used for the treatment of Amenorrhea, Amygdalitis, Bite(Leech) Hunan, Cataplasm, Catarrh, Cold, Decongestant Diabetes, Diarrhea, fever, Gargle Hemostat, Hoarseness Inflammation Laryngitis Brutus, Leptospirosis, Pertussis, RheumatismVermifuge (Eze E. A 2013).

Chromolaena odorata forms dense stands preventing establishment of other species, both due to competition and allelopathic effects. When dry, *Chromolaena odorata* becomes a fuel which may promote wild bushfires (PIER 2003). *Chromolaena odorata* may also cause skin complaints and asthma in allergy-prone people. It is a major weed in plantations and croplands, including plantations of rubber, oil palm, forestry and coffee plants.

2.2.6 Chemical constituents of *Chromolaena odorata*

According to (Anup Kumar Chakraborty *et al* 2011) thirty-three components were identified from the volatile oil of *Chromolaena odorata*. Terpenoid compounds are major components of the volatile oil. The main terpenic componentsare trans-caryophyllene(16.22%), cadinene (15.53%), copaene(11.32%), caryophyllene oxide(9.42%), germacrene-D(4.86%) and humulene (4.23%) (Ling Bing).

(Yuan Jing-quan *et al*, 2005) suggested that the constituents of the Choloro-form soluble and petroleum ether-soluble portions in the 95% ethanol extract were isolated and purified by means of chromatography. Ten compounds were isolated and identified as odoratin, dillenetin, pectolinarigenin, quercetin-7, 4'-dimethyl ether, kaempferol 4'-methyl ether, isosakuranetin,

acacetin, dotriacontanic acid, â-sitosterol, daucossterol. The purified compound, 4', 5, 6, 7-tetramethoxyflavone, is an active ingredient isolated from Eupatorium odoratum. (Triratana T, *et al*, 1991). Isosakuranetin and a new chalcone, odoratin, have been isolated from the leaves of *Eupatorium odoratum*. The structure of odoratin has been shown to be 2'-hydroxy-4, 4', 5', 6'-tetramethoxy chalcone.

According to (Apichart Suksamrarn *et al*) Four flavanones isosakuranetin (5,7-dihydroxy-4'-methoxyflavanone), persicogenin (5,3'-dihydroxy-7,4'-dimethoxyflavanone),5,6,7,4' tetramethoxy flavanoneand 4'-hydroxy-5,6,7-trimethoxyflavanone,twochalcones, 2'-hydroxy-4,4', 5', 6' tetramethoxychalcone and 4,2'-dihydroxy-4', 5',6'-trimethoxychalcone(6), and two flavones, acacetin (5,7-dihydroxy-4'-methoxyflavone) and luteolin(5,7,3',4'-tetrahydroxyflavone) were isolated and identified from the flowers of *Chromolaena odorata.*

Phytochemical studies by (Sajan *et al* 2005) on the petroleum ether extract of the roots of *Eupatoriumodoratum* have resulted in the isolation of a novel triterpene, 3ß -hydroxy-28-carboxyolean-12-ene along with seven known compounds – poriferasterol, octadecane, butyrospermol acetate, bis(2- ethylhexyl) phthalate, chrysophanol, physcion and palmitic acid. Novel compound 3ß -hydroxy-28-carboxyolean-12-ene is designated as eupatoric acid. The cytotoxicity of all the isolated compounds except palmitic acid was studied using a lethality test against *Artemia salina* (brine shrimp).

An anionic peroxidase isoenzyme having a pI of 3.5 was purified from Eupatoriumodoratum. The molecular weight of the enzyme was identified as 55 kD. The specific activity of the crude extract was increased to 647 U/mg from 62 U/mg by ammonium sulfate precipitation. The enzyme was 114-fold purified by ion exchange chromatography and had a specific activity of 7094 lU/mg. The specificity constant(kcat/Km) of the isozyme was 8.75 × 105 s-1 M-1 with

ABTS and 6.9 × 105 s-1 M-1with H2O2 as substrates. The enzyme was found to be very stable at room temperature (30 ± 2 °C) and retained more than 90% activity even after a period of 2 months and was stable for more than 6 months at 4 ± 1 °C without any additive, stabilizer or preservative. The activation energy for inactivation (Ea) of the isozyme was120.14 kJ mol-1 and the half-life was found to be around 34 h at 50 °C. The purified Eupatorium peroxidase has an optimum pH of 4.5 and optimum temperature of 55 °C. This isozyme was stable in metal ionic solutions and showed increased activity in the presence of Hg2+, K+ and Ca2+. The enzyme can also be used as a low cost time temperature indicator strip for which the preliminary works have already been.

Recently, increased interest has been given to enhance the crop emergence under the slash-and-burn agriculture in many countries (Koutika LS, 2002) Thus studies evaluating the effects of siam weed extracts on seed germination and seedling growth of field crops might be helpful in designing appropriate management strategies for sustainable agriculture.

CHAPTER THREE

MATERIALS AND METHODS

This experiment was carried out in the laboratory of the Department of Plant Science and Biotechnology, Faculty Of Science, Federal University Oye Ekiti , Ekiti State Nigeria. Materials used for this study include; fresh shoot of *chromolaena odorata*, okra seeds (*Abelmoschus esculentus*), weighing balance, mortar, distilled water, conical flask, measuring cylinder, paper tape, Cleaned and dried Petri dishes, whattman No 1 filter paper, syringe, measuring bottles.

Fresh shoot of *chromolaena odorta* was collected within the University environment. The fresh leaves were blended with mortar without adding water.

About 5g, 10g, 15g and 20g each of *chromolaena odorata* and was weighed into plastic bottles each containing 250ml of distilled water for 24 hours. After about 24 hours, each sample was filtred. 2ml of the leachate was used to water the seeds of okra that was placed in Petri dishes containing double layered whatman No 1 filter paper. Each treatment had 5 replicates which contained 4 seeds of okra (*Abelmoschus esculentus*). Control experiment was set up by using distilled water and all the experiments were kept at room temperature. The radicle length, plumule length, and the number of secondary roots were observed, also the germination percentage was calculated based on the number of germinating seedlings.

SCREEN HOUSE EXPERIMENT

Seeds of okra (*Abelmecious esculentus), shoot,stems and leaves of chromolaena odorata,* nylon pots, weighing balance, 2mm sieve, 50cl measuring cup, loamy soil, mortar and pistil, white plastic spoons, permanent marker tape rule, weighing balance.

Loamy top soil was collected within federal university oye Ekiti environment in the absence of Chromolaena odorata so as not to interfere with the calculated result of the experiment. Fresh shoot, stems and leaves of *Chromolaena odorata was* collected within the university environment, it was crushed into smaller bits using mortar and pistil. 50g 100g, 150g and 200g of crushed Chromolaena was incorporated into 4 planting pots containing g of loamy top soil respectively then a control experiment was setup 0g Chromolaena odorata. The setup was wet using tap water from the screen house and allowed to sit for about 24 hours after which 3 seeds each of okro was planted in all the pots. After two weeks, two weaker plants was removed from each pot leaving the plant with more vigour.

The following measurements were taken at 8weeks after planting

- Plant height
- steam guart
- leaf area
- pod weight

The number of fruits was recorded, the seeds were weighed and their weights recorded, after which they were air dried and blended separately for proximate analysis.

3.3 DETERMINATION OF PROXIMATE COMPOSITION OF THE SEEDS

The proximate parameters such as moisture, ash, crude fiber, crude fiber, protein and carbohydrate contents of the samples were carried out as follows;

(i) . Determination of moisture content

Drying method is main method of estimating the moisture content of foods in which the percentage weight loss of water was estimated; usually after removal by heating under standardised conditions of oven drying at 105°C (the oven used was DHG-9023A model, made by B. BRAN Scientific and Instrument Company England). This method is considered to be reliable method provided that there is no chemical decomposition of the sample (A.O.A.C., 2006).

Cleaned and dried Petri dishes were weighed by using OHUS Adventure analytical balance and respective weight was recorded (W_1). 3.0 g of the sample was weighed into the dishes spreading as much as possible. The Petri dish and sample were weighed and recorded as W_2. The petri dishes with the samples were transferred into the thermosetting oven maintained at 105°C, and dried for about three hours. It was later transferred in to the desiccators for effective cooling and then reweighed. This process was performed repeatedly until a constant weight (W_3) was obtained (A.O.A.C., 2006). The loss in weight during drying in percentage was taken to be the percentage moisture content.

$$\% \text{ Moisture content} = \frac{Loss\ in\ weight}{Weight\ of\ sample} \times 100$$

$$\% \text{ Moisture content} = \frac{W2 - W3}{3} \times 100$$

Where 3 represent weight of sample

W_1 = Weight of empty evaporating dish

W_2 = Weight of empty evaporating dish + sample

W_3 = Constant weight, evaporating dish and dried sample.

(ii) Determination of crude fat

The crude fat was determined by Soxhlet extraction system. A previously dried filter paper was weighed as (W_1). 2.5g of the sample was added in the filter paper, weighed as (W_2). This was tightened very well with white thread and transferred into a thimble. A 500ml round bottom flask was filled up to two-third of its capacity with n-hexane. The soxhlet extractor was then fitted with a reflux condenser and the heat source of the extractor was adjusted so that the solvent boils gently and it was left to siphon for 8 hours, after which the paper was removed. The filter paper and defatted samples were dried in the oven at 50°C for about 30 minutes. The sample was allowed to cool down in dessicators and weighed as (W_3). The percentage fat content was calculated thus:-

$$\% \text{ Crude fat} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where,

W_1 = weight of the filter paper

W_2 = weight of the filter paper and the sample

W_3 = weight of the defatted sample and the filter paper

(iii) Determination of total ash

Clean flat bottom crucibles were placed in muffle furnace for about 15 minutes at 350°C, the crucibles were removed, allowed to cool in desiccators, properly labelled with lead pencil and each was weighed as (W_1). 1g of the sample was added to each labelled crucibles and samples were then transferred into the muffle furnace to ash at 550°C for 4 hours. After complete ashing i.e when the samples become whitish in colour, the crucibles were allowed to cool in a desiccator and reweighed as (W_3). Percentage ash was calculated and the ash used for mineral analysis.

$$\% \text{ Ash Content } = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

$$\% \text{ Ash Content } = \frac{W3-W1}{W2-W1} \times 100$$

Where,

W_1 = weight of empty crucible,

W_2 = weight of the crucible and sample,

W_3 = weight of the crucible and ash sample

(iv) Determination of Crude Protein (Using Kjeldhal Method)

The stages involved are;

(1) Digestion Stage

In this stage, 1g of the sample was weighed into a Kjedhal flask and 10ml of $H_2SO_4$with Kjedhal catalyst added. The weight is taken to be W_1. This was then heated on a heater until it was digested. The flask was rotated at intervals until the digest was clear (light green) and the heating was continued after that to ascertain complete digestion. This was allowed to cool and the digested sample was made to 50ml (V_1). The sulphuric acid action result in complete digestion of organic matter and the conversion of nitrogen into ammonium salt (ammonium sulphate).

$$2NH_3(aq) + H_2SO_4(aq) \xrightarrow{\text{catalyst}} (NH_4)_2SO_4(aq)$$

The digested sample was then diluted with 50ml distilled water after which 25ml was pipetted into a clean distilled flask and neutralized with 50ml 40% sodium hydroxide.

$$(NH_4)_2SO_4(aq) + 2NaOH(aq) \longrightarrow Na_2SO_4(aq) + 2NH_3(g) + 2H_2O(l)$$

(2) Distillation Stage

In this stage, the digested ammonia was trapped into 5ml 2% boric acid that is contained in a receiving flask in which 4 drops of mixed indicator (0.198g bromocresol green plus 0.132g methyl red in 200ml alcohol) has been added.

$$NH_3(g) + H_3BO_3(aq) \longrightarrow NH_4^+(aq) + H_2BO_3^-(aq)$$

(3) Titration Stage

The titration stage which is the last stage involves titrating the distillate against 0.01M HCl until the colour changes from bluish to pink/red.

$$(NH_3)_3BO_3(aq) + 3HCl(aq) \longrightarrow 3NH_4Cl(aq) + H_3BO_3(aq)$$

$$\% \text{ Nitrogen} = \frac{(T-B)\times 14 \times 0.01 \times V1}{Weight\ of\ sample \times V2} \times 100$$

where T = the titre value

B = blank

V_1 = volume of digest

V_2 = volume of digest used

% Crude Protein = % Nitrogen × 6.25

Crude Protein

The amount of crude protein contained in seeds is obtained by multiplying the nitrogen content of the food by 6.25. The factor 6.25 owes its origin to the assumption that all food protein contains 16% nitrogen, and that all nitrogen in a food is present as protein.

(v) Determination of crude fibre.

Crude fibre is the remaining organic component when the defatted sample has been successfully treated with diluted acid (H_2SO_4) and dilute base (NaOH). Crude fibre is the indigestible portion of any main food. It is known that fibre consists of cellulose, which can be digested to considerable extents by both ruminants and non-ruminants (Pearson, 1976). The determination of fibre content in plant tissue provides a distinction between the most digestible carbohydrates.

Preparation of reagents:-

- 1.25% H_2SO_4:- this was prepared by measuring 6.25ml of concentrated H_2SO_4 with the aid of measuring cylinder, and pour in 500ml volumetric flask that has about 200ml distilled water, properly mixed and make up to the mark with more distilled water labelled.
- 1.25% NaOH:- 6.25g of NaOH pellets was weighed with Ohus analytical balance and dissolved water in a beaker and transferred to 500 ml volumetric flask, then make up to the mark with distilled water labelled.
- HCl:- measuring cylinder was used to measure 10 ml concentrated HCl into 100ml volumetric flask which already contain distilled water, mixed and make up to mark with distilled water and labelled.

About 3.0 g (W_1) of defatted sample was weighed into 500 ml conical flask, 200ml of 1.25% of H_2SO_4 was added to the sample in the conical flask, placed on heating mantle and bring to boiling within 2 minutes, then allowed to boil gently for 30 minutes. The mixture was filtered through Whatman filter paper, in Buchner funnel and rinsed well with hot distilled water. The sample was scrapped back into the flask with spatula, placed on a heating mantle and 200ml of 1.25% NaOH was added then allowed to boil for few minutes and boiled gently for 30 minutes. It was filtered through Whatman filter paper, in Buchner funnel and rinsed well with hot distilled water for four times and once with 10% HCl to neutralize the NaOH remaining in the sample then rinsed with hot distilled water for four times and twice with ethanol. The residue was scrapped into a crucible and weighed (W_2), dried in a thermosetting drying oven at 105^0C, ashed at 550^0C in a muffle furnace, cooled in a desiccator and reweighed (W_3).

$$(\%)\ \text{Crude fibre} = \frac{weight\ loss}{weight\ of\ sample} \times 100$$

$$(\%)\ \text{Crude fibre} = \frac{W2-W3}{W2-W1} \times 100 \qquad \ldots\ldots\ldots\ldots (5)$$

Where,

W_1 = weight of empty crucible,

W_2 = weight of the crucible and sample,

W_3 = weight of the crucible and ash sample

(vi) Determination of carbohydrate content

Carbohydrate is the most abundant constituent of plants and animals. The most common approach for the determination of carbohydrate content of food is the difference between the total predominant content in percentages (ash, crude protein, fat, crude fibre, moisture) and one hundred (A.O.A.C., 1990).

% Carbohydrate = 100 – (% ash + % crude protein + % fat + % crude fibre + % moisture).

(vii)Energy content

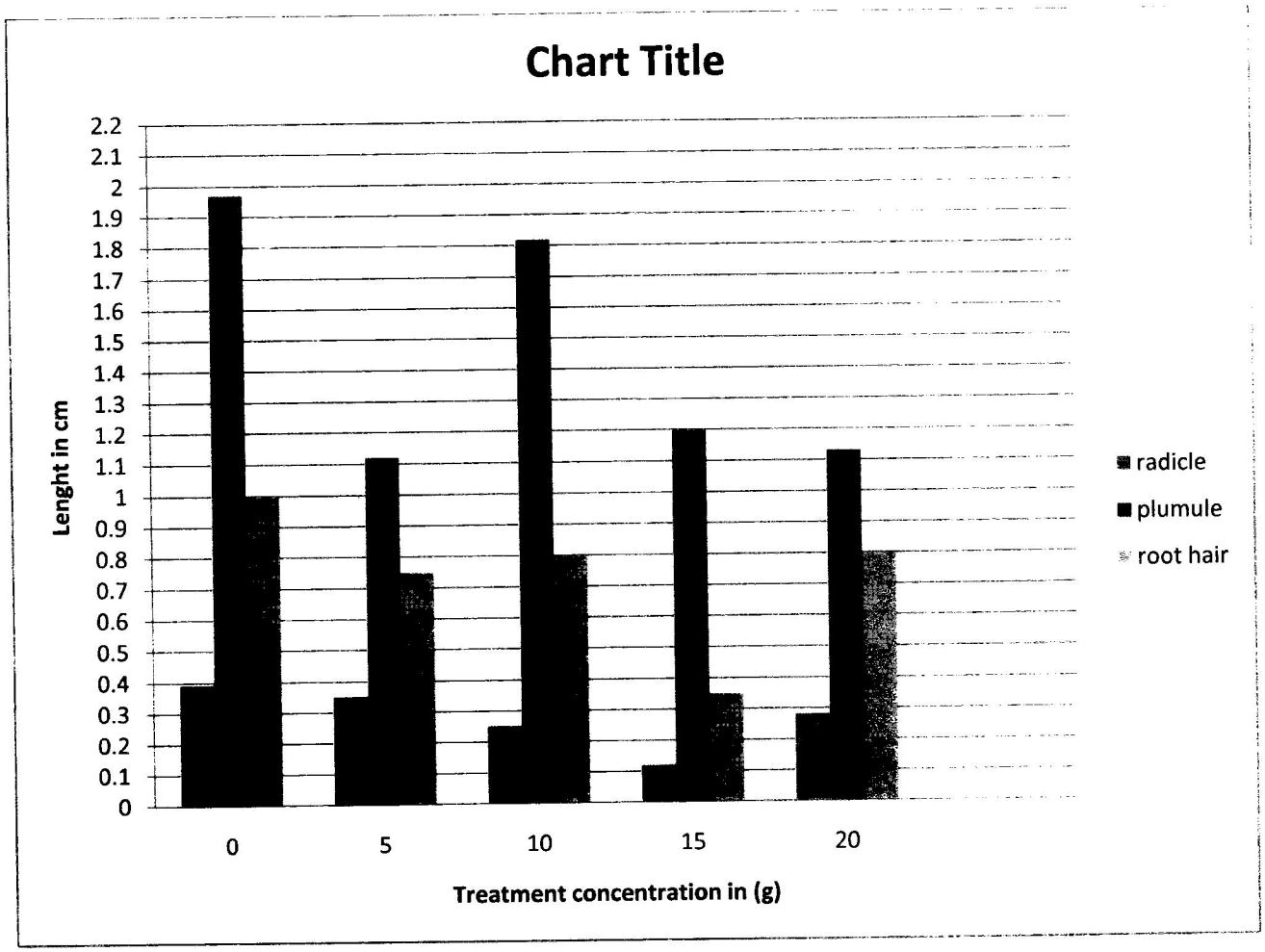
The gross food energy was estimatedusing metabolizable energy formula:

Energy = (% Protein × 17) + (% fat × 37) + (% carbohydrate × 17) KJ/100g(Edem et al, 1990

CHAPTER FOUR

Results and Discussion

Graph 4.1



Graph 4.1 Effects of varying concentrations of leachates of *chromolaena odorata* on radicle length, plumule length and number of roots of *Abelmoschus esculentus.*

From the result, it was observed that the radicle length of the control group was higher than the group treated with different concentrations 5g/250ml, 10g/250ml, n20g/250 of leachates obtained *chromolaena odorata*. The group treated with 15g/250ml had the lowest radicle length.

Also the plumule length was significantly higher in the control and the groups treated with 10g/250ml but the groups treated with 5g/250ml, 15g/250ml, 20g/250ml of *Chromolaena odorata* was negatively affected, the control had the highest plumule length.

The leachate of *Chromolaena odorata* had the highest negative effect on the root of plants treated with 15g/250ml , while it had a uniform effect on 5g/250ml and 20g/250ml. The control had the highest number of roots followed by plants with 10g/250ml.

Table 4.1: Effect of concentrations of fresh shoot biomass of *Chromolaena odorata* on different parts of *Abelmoschus esculentus*

Concentration (g/ml)	Plant Height	Leaf Area	Stem Girth	Pod Weight
0	7.53 ± 0.78^{a}	11.77 ± 0.89^{a}	0.80 ± 0.06^{a}	25.81 ± 5.56^{a}
50	7.77 ± 0.37^{a}	15.12 ± 2.05^{ab}	0.87 ± 0.09^{a}	20.06 ± 3.47^{a}
100	8.43 ± 0.26^{a}	16.44 ± 2.22^{ab}	1.00 ± 0.06^{a}	20.08 ± 3.03^{a}
150	8.97 ± 0.79^{a}	16.97 ± 1.54^{ab}	1.00 ± 0.06^{a}	20.94 ± 1.35^{a}
200	7.73 ± 1.27^{a}	19.63 ± 1.61^{b}	1.00 ± 0.10^{a}	22.19 ± 1.72^{a}

Table 4.2 shows the effect of varying concentrations of fresh shoot of biomass of *C. odorata* on different parts of *Abelmoschus esculentus*.

From the result of this study, it was observed that the plant height were higher in the treated groups when compared to the control group. There was no significant ($p > 0.05$) difference in the height of *Abelmoschus esculentus* plant following treatment with different concentrations of *Chromolaena odorata* fresh shoot biomass. However, the plant height increased in a concentration dependent manner with exception of those treated with 200 g/ml.

The leaf area of the control group was observed to be significantly ($p < 0.05$) lower than all the treatment groups. The leaf area also increased in a concentration dependent manner. The group treated with 200 g/ml had the highest value.

In the same manner, there was a concentration dependent increase in the stem girth of *Abelmoschus esculentus* after treatment with fresh shoot of biomass of *C. odorata*. However, there was no significant difference in the stem girth of the control and the treated groups. The control group had the lowest value.

It was observed from the result that the pod weight of plant treated with *C. odorata* fresh shoot biomass were lower than the control (with no treatment). The pod weight of the treatment groups are also concentration dependent.

Table 4.2: Effect of concentrations of fresh shoot biomass of *C. odorata* on the proximate analysis of *Abelmoschus esculentus* dried fruit

Concentration (g/ml)	Moisture Content	Fat Content	Protein Content	Crude fibre Content	Ash Content	Carbohydrate Content
0	12.08 ± 0.02^{d}	6.19 ± 0.02^{a}	15.72 ± 0.09^{a}	11.16 ± 0.06^{a}	6.27 ± 0.06^{a}	48.59 ± 0.05^{a}
50	13.07 ± 0.05^{e}	6.11 ± 0.01^{a}	16.72 ± 0.22^{b}	10.84 ± 0.14^{b}	6.12 ± 0.02^{b}	47.14 ± 0.12^{c}
100	10.77 ± 0.17^{b}	5.21 ± 0.01^{b}	20.27 ± 0.09^{c}	9.64 ± 0.04^{c}	5.93 ± 0.03^{c}	48.19 ± 0.14^{b}
150	11.15 ± 0.05^{c}	5.51 ± 0.09^{c}	23.28 ± 0.16^{e}	9.69 ± 0.04^{c}	6.09 ± 0.03^{b}	44.29 ± 0.03^{d}
200	9.81 ± 0.06^{a}	5.74 ± 0.09^{d}	21.46 ± 0.15^{d}	9.46 ± 0.04^{c}	6.13 ± 0.02^{b}	47.42 ± 0.07^{c}

Table 4.3 shows the effect of varying concentrations of fresh shoot of biomass of *Chromolaena odorata* on proximate analysis of *Abelmoschus esculentus*.

It was observed from this study that the moisture content of the control group was significantly ($p < 0.05$) higher than the group treated with different concentrations (100 g/ml, 150g/ml and 200 g/ml) of *Chromolaena odorata* fresh shoot biomass. The group treated with 50 g/ml had the highest moisture content.

There was no significant difference between the fat content of the control and the group that received 50 g/ml. However, the fat content of the control was significantly higher than the groups treated with 100 g/ml, 150g/ml and 200 g/ml of *Chromolaena odorata* fresh shoot biomass.

The protein content of the treatment groups were observed to be significantly ($p < 0.05$) higher when compared to the control group. The group that received 150 g/ml treatment had the highest protein content.

The crude fibre content of control group significantly ($p < 0.05$) increased when compare to the treatment groups. There was no significant difference in the crude fibre content of the groups treated with 100 g/ml, 150g/ml and 200g/ml of *Chromolaena odorata* fresh shoot biomass.

The ash content of treatment groups were significantly lower than the control. The group treated with 100 g/ml of *Chromolaena odorata* fresh shoot biomass had the lowest ash content. There was no significant difference in the ash contents of groups treated with 50 g/ml, 150g/ml and 200 g/ml of *Chromolaena odorata* fresh shoot biomass.

The carbohydrate content of the control group increased significantly when compared to the treatment groups. The group treated with 150 g/ml of *Chromolaena odorata* fresh shoot biomass had the lowest carbohydrate content.

Discussion

In this study it was observed that the radicle plumule and number of roots were lower in the treatment groups compared to the control groups with highest effect on the treatment with 15g/250ml of *Chromolaena odorata*, this is in accordance with Suwal et al. (2010) who also noticed phytotoxic suppressive action of water extract of Chromolaena odorata on germination and seedling growth of rice and barnyard grass.

The leachate showed a reduction in radical length, plumule length and number of root. The radical length of concentration 15g/250ml was greatly affected, while the plumule length of 5g/ml, 15g/ml and 20g/ml greatly decreased compared to the control. The highest decrease in number of roots was noticed in the 15g/250ml concentration, this is in line with, Onwugbuta-Enyi, (2001) who has studied the effects of *Chromolaena odorata* on tomatoes (*Lycopersicum esculentum* Mill), and found significant growth reduction of the tomatoes with *Chromolaena odorata* leaf extract at concentrations as low as 1 g of fresh weight in 40 ml of water.

Heidarzade *et al.*, 2012 suggested that the inhibition of germination and seedling growth is attributed to the allelochemicals present in the weed tissues.

The plant height increased in a concentration dependent manner with exception of those treated with 200g, the leaf area of the control group was observed to be lower than all the treatment groups, but it increased in a concentration dependent manner. Also there was a concentration dependent increase in the stem girth of *Abelmoschus esculentus.* The pod weight of the treated plants were lower than the control. The group treated with 50g highest moisture content, the fat content was higher in the control and group treated with 50g than the other treated group.

The protein content of the treatment groups was observed to be significantly higher when compared to the control group.

Crude fiber content of the treatment group was significantly higher than the treatment groups. The ash content of the control was significantly lower than the control groups, the carbohydrate content of the control group increased significantly compared to the treatment group with the lowest at 150g/ml treated group. The study concluded that the lichate and FSB affected the seed germination, growth and the food content of *Abelmoschus esculentus.*

According to (Inderjit *et al.*, 2001), allelopathy is an interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth .

Conclusion

This study showed that aqueous extracts of *Chromolaena odorata* can suppress the seed germination as well as root and shoot length growths of okra as observed in this study. *Chromolaena odorata* had significant effects on seed germination, seedling growth, and the food content and growth of *Abelmoschus esculenthus* and could possibly explain the mechanism of visible allelopathic effect observed in *Chromolaena odorata* in the previous studies. These effects of *Chromolaena odorata* on *Abelmoschus esculentus* could be as a result of the allelochemicals it contains.

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