

**ALLELOPATHIC INTERACTIVE EFFECTS OF RICE, CYMBOPOGON,  
DESMODIUM, MUCUNA AND MAIZE IN UGANDA**

**KAIIRA MOSES GODFREY B Sc. AGRICULTURE MAKERERE UNIVERSITY  
M Sc. AGRONOMY ACHARYA N.G. RANGA, AGRICULTURAL UNIVERSITY,  
RAJENDRANAGAR, HYDERABAD-500 030 INDIA**

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TECHNOLOGY**

**FACULTY OF AGRICULTURE  
UNIVERSITY OF NAIROBI**

**DECLARATION**

This thesis is my original work and has not been presented for an award of a degree in any other University.

Kaiira Moses Godfrey

Signedí í í í í í í í í í í í í í í . Dateí í í í í í í í í í í í í í í í

This thesis has been submitted with our approval as university supervisors

Prof. George. N. Cheminingowa

Department of Plant Science and Crop Protection, University of Nairobi

Signedí í í í í í í í í í í í í í í .Dateí í í í í í í í í í í í í í í í

Dr. Fredrick. O. Ayuke

Department of Land Resource Management and Agricultural Technology, University of Nairobi

Signedí í í í í í í í í í í í í í í .Dateí í í í í í í í í í í í í í í í

Dr. Yona Baguma

Principal Research Officer, National Agricultural Research Organization, Uganda.

Signedí í í í í í í í í í í í í í í .Dateí í í í í í í í í í í í í í í í

## **DEDICATION**

This thesis is dedicated to Jeremiah Bagiire Family

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## ABBREVIATIONS/ACRONYMNS

ABA	Absciscic acid
ADM	Additive Dose Model
AMDIS	Automated Mass spectral Deconvolution and Identification System
CR	Competitive Ratio
DNA	Deoxyribonucleic Acid
GA	Gilbberellic Acid
GS/GC	Gas Spectometry/ Gas Chromatograph
IAA	Indole Acetic Acid
LAI	Leaf Area Index
LER	Land Equivalent Ratio
LSR	Leaf, Stem, Root
MGT	Mean Germination Time
MS	Mass Spectrometry
MSM	Multiplicative Survival Model
NaCRRRI	National Crops Resources Research Institute
NERICA	New Rice Cultivars for Africa
NIST	National Institute of Science and Technology
PCA	Principal Compound Analysis
ROI	Returns on Investment
SGI	Seed Germination Index
TIC	Total Ion Chromatograms
UBOS	Uganda Bureau of Statistics
UNDP	United Nations Development Programme



## GENERAL ABSTRACT

Rice is a major food and cash crop under various mixed cropping systems in Uganda. Rice and some intercrops exhibit allelopathic properties and productivity is on the decline yet little is known about the causes of allelopathy, declining ecosystem productivity and the potential benefits from the ecosystems. The main objective of this study was to determine the compounds in *Oryza sativa*, *Cymbopogon nardus*, *Desmodium uncinatum*, *Mucuna pruriens* and *Zea mays* and their allelopathic interactive effects. Specific objectives were: (i) to identify the compounds in rice, cymbopogon, desmodium, mucuna and maize; (ii) to determine the allelopathic potential of compounds in rice, cymbopogon, desmodium, mucuna and maize; (iii) to determine the effects of allelopathy on striga, crop growth, nutrient uptake and productivity of rice based intercropping with cymbopogon, desmodium, mucuna and maize; (iv) to evaluate the allelopathic potential of rice, cymbopogon, desmodium, mucuna and maize mixed mulches on weeds and rice; (v) to assess the effects of plant powders and aqueous extracts from rice, cymbopogon, desmodium, mucuna and maize on weeds. Objective (i) was implemented between June and August 2013 at the National Crops Resources Research Institute (NaCRRI), Namulonge, Uganda. Plants were uprooted at 45 days after emergence (DAE) and samples analysed for organic compounds using the National institute of Science and Technology library. In objective (ii) pot screening, equal compartments agar, germination tests and donor to receiver plants in the same pot studies were conducted during 2013B. In objective (iii) a screen house experiment was done at NACRRI during 2013B and two field studies were conducted during 2014A. In the screen house two different crop species were planted in the same pot and as sole crops. In the field studies, maize, mucuna, desmodium and cymbopogon were tested in sole crop and intercrop systems. In objective (iv) on-station and on-farm experiments were conducted during 2015A. Cymbopogon, mucuna, rice and maize species were each planted and

uprooted at 55 DAE and combined in sets of three equal proportions of individual stover to give four types of mulches that were applied under 12 treatments at planting of rice. Objective (v) was conducted at NACRRI during 2013B and in the field during 2015A. Leaf, stem and root (LSR)) powders from rice, maize, cymbopogon, desmodium and mucuna were combined in equal proportions of three to make eight powdered mixtures that were applied to 72 pots in a screen house. Field studies were conducted on station and on farm with maize, mucuna, rice and cymbopogon mulches, Butanil herbicide, hand hoeing and roguing of weeds with a weedy check. Data were recorded on the compounds in blank forest soil (control), soils potted with cymbopogon, desmodium, rice, mucuna and maize crops. Data were also collected on compounds in cymbopogon, desmodium, rice, mucuna and maize stover. Radicle and plumule length were measured, germinated seeds counted and percent germination, mean germination time (MGT) and seed germination indices (SGI) calculated for desmodium, rice, mucuna and maize seedlings. Root lengths, plant height, dry biomass, width and length of leaves, number of weeds and plants per pot, plant nutrient uptake and reserves in the soil at harvest were established in potted experiments. Data were collected on maize green leaves, striga per 100 rice plants, total and filled panicles per plant, grains per plant, filled grains per panicle and grain yield of rice under field studies. Land equivalent ratio, competitive ratio, relative growth rates (RGR), gross returns, net returns and returns on investments (ROI) were calculated for different enterprises. Blank soil, cymbopogon, desmodium, rice, mucuna and maize produced 24, 7, 5, 11, 7 and 6 major compounds respectively. Cymbopogon, desmodium, rice, mucuna and maize predominantly produced 10, 6, 6, 6 and 9 compounds respectively from their stover. Maize, rice and mucuna leachates reduced the root lengths, height and biomass for weeds. *G. parviflora* root and stem growth reduced on application of root exudates. Mean germination time for test plants increased while the SGI reduced at 75% leachate concentrations. Growth of rice, mucuna and desmodium reduced when potted with mucuna and maize. Maize potted with

desmodium and cymbopogon recorded reduced RGR, NPK crop uptake and reserves in the soil at harvest unlike rice potted with mucuna, desmodium and cymbopogon. Conversely, the maize growth increased when potted with mucuna and desmodium and with 75% concentration of rice/ desmodium and rice/maize leachates. Striga weed counts reduced in rice intercropped with mucuna, desmodium and cymbopogon but increased with maize and thus, reduced rice tillering under the latter treatment with 63% of the weeds recorded on rice plants. Sole rice and rice intercropped with cymbopogon produced higher rice grain yields and partial land equivalent ratio (LER) for rice. The highest combined LER were recorded under rice + desmodium and rice + cymbopogon cropping systems. Rice + maize and rice + mucuna intercropping systems were least productive and mucuna was the most competitive crop followed by maize and cymbopogon. Butanil, hand hoeing twice or thrice and mulching rice with maize/mucuna based mulches increased rice growth, grain yields and ROI compared to rice/cymbopogon based mulches which most effectively controlled weeds including striga. Increasing LSR powder concentrations reduced the number of weeds and mean weight per weed. Rice/cymbopogon and maize/ mucuna LSR powdered mixtures and liquid bio-extracts reduced weed densities and weight while Butanil recorded the lowest weed densities and weight. Highest weed weight/m<sup>2</sup> and unit weed weight were under the weedy check. The highest striga count was recorded under Butanil, 3 and 2 hand hoeing (hh) and there were no striga under cymbopogon mulches and weedy check. Highest ROI were under 2hh, 3hh and maize/mucuna based mulches given 1 hand roguing of weeds. There is high potential to utilise allelopathy as a weed management technology for increased crop productivity. Further studies are recommended on the allelopathic potential of the compounds identified in the current study and in use of *C. nardus* and *M. pruriens* for *Striga hermonthica* weed control.

# CHAPTER 1

## INTRODUCTION

### 1.1 Background information

Farmers in the Uganda's South Eastern Agro-Ecological Zone (USEAZ) grow upland rice especially New Rice for Africa (NERICA). NERICA is an interspecific upland rice cultivar, developed as a cross between Asian (*Oryza sativa*) and African (*Oryza glaberrima*) traditionally cultivated rice species in Africa (Jones *et al.*, 1997). The Asian species is known for its high yielding potential while its African counterpart is known to be more resistant to local biotic and abiotic stresses. The resulting NERICA cultivars have been widely accepted by African subsistence rice farmers and have resulted in a consistent increase in rice production (Jamil *et al.*, 2011; Cissoko *et al.*, 2011).

Rice is a major food and cash crop under various mixed cropping systems, but it has been reported to exhibit allelopathic properties (Khanh *et al.*, 2007; Kato-Noguchi *et al.*, 2008a; Mennan *et al.*, 2012). Given the prevailing reduction in profitability of rice due to decline in productivity that is associated with drought, poor soil fertility coupled with increased incidence of noxious weeds (e.g. striga), pests and diseases [Uganda National Development Plan (UNDP), 2010], there is need for appropriate alternative weed control technologies, intercrops and companion crops to diversify and detoxify the fragile traditional agricultural ecosystems. Considering their significance and economic importance, cultivation of some selected crops is an option for improved livelihoods and basic service support in drylands. There is, however, need for a systematic approach towards cultivation of the crops to provide a consistent supply of rice and other crop products of high quality. *Mucuna pruriens*, *Cymbopogon nardus* (L.), *Desmodium uncinatum*, and *Zea mays* are some of the intercrops

and companion crops being grown in the upland rice cropping systems. Desmodium (Pickett *et al.*, 2010), cymbopogon (Prapaipit *et al.*, 2013), rice (Chuihua *et al.*, 2004), maize (Ayeni and Kayode, 2004) and mucuna (Soares *et al.*, 2014) have been reported to exhibit allelopathic properties. Allelopathy is defined as any direct or indirect harmful or beneficial effect by one plant (including micro-organisms) on another through production of chemical compounds that escape into the environment (Rice, 1984). These chemical compounds known as allelochemicals are secondary metabolites and can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms.

Rice is a major food crop in the drylands of eastern Uganda and has ranked second to maize among cereals grown for over a decade. The Uganda statistical abstracts report (2017) indicates that 218,000 metric tons (Mt) were harvested in the country from 75,086 Hectares (ha) in 2010, compared to 183,000 Mt, harvested from the same area in 2008. Despite the increased production, the productivity of rice kept on declining ( $2.90 \text{ Mt ha}^{-1}$ ) in 2010. This farm level productivity is below the attainable potential and was attributed to low applications of modern technologies (UNDP, 2010). Farmers in Eastern Uganda are growing NERICA rice as sole and in mixtures at subsistence levels, but are not aware of their allelopathic potentials. Productivity of rice in Eastern Uganda is possibly on the decline (UNDP, 2010) due to allelopathy. There is need to identify the allelopathic compounds in rice and common intercrops, determine the allelopathic potential of emerging upland rice ecosystems, identify and recommend those with high detoxification capacities and ability to control weeds using allelopathy in order to increase crop productivity and incomes.

The auto-toxification, intra-specific and residual effects of allelopathic cultivars, and the tolerance of weed populations upon repeated cultivation of allelopathic cultivars in the same

fields should be investigated. The generated information would not only be important in improving the integrated management practices for weeds, including the notorious striga, but also help plant breeders develop crop cultivars with resistance to biotic stresses. The information will marginally reduce the use of herbicides over time as a significant economic benefit to farmers and reduce the negative ecological impacts on the environment. Gealy and Yan (2012) noted that allelopathic substances, if present in crop varieties, may reduce the need for weed management, particularly herbicide use if conventional breeding methods are applied in the breeding of allelopathic cultivars. The researchers suggested that the technology should combine weed suppression ability with high yield potential, disease resistance, early maturity and quality traits.

## **1.2 Problem statement**

In Eastern Uganda the productivity of rice is on the decline despite efforts to increase the production levels. Rice plays an important role as both a food and cash crop in Uganda and the crop was ranked fourth among the cereal crops in 2010. The declines in crop yields in the region have been associated with factors such as poor soil fertility, use of unimproved seeds, poor extension services, pests and diseases. Rice plants secrete allelochemicals from their roots into the neighbouring environment over their entire life at phytotoxic levels. In the ecological zone, rice fields are characterised by stunted growth, even when fertilisers and agronomic practices are applied resulting into lower grain yields, regeneration failure of crops and weeds in fields where rice is threshed, replanting problems in paddy fields and detrimental effects on the growth of companion crops and crops following rice. The observed properties could be caused by allelopathy arising from metabolites released in rice root exudates or from decomposed rice stover. The causes of allelopathy, decline in rice ecosystems productivity and the potential benefits from allelopathic interactive effects of

upland rice and the emerging intercrops in Eastern Uganda are not known and need to be investigated.

### **1.3 Justification**

Upland rice cropping systems are characterised by mixed cropping and some allelopathic plants are emerging as key intercrops of rice. Intercropping of upland rice with some of these crops provides alternative sources of revenue given the high demand of these profitable products. In order to appraise this new alternative and possible interaction with upland rice, it must be considered that allelopathic effects are produced by upland rice, some intercrops and weeds. The interactions amongst these crops need to be defined as the bio-active allelopathic compounds and their effects are not known. Weed management studies in the various crops using various crop residues and bio extracts singly have been conducted (Cheema *et al.*, 2010b; Mahmood *et al.*, 2010; Prapaipit *et al.*, 2013; Namkeleja *et al.*, 2013; Wang *et al.*, 2013), but studies on use of mixed leaf, stem and root powders, mixed mulches and bio-extracts of rice, maize, mucuna, cymbopogon and desmodium crops for weed control are limited. There is need to profile the bio-active compounds in rice, cymbopogon, desmodium, mucuna and maize; determine their allelopathic potential, establish the effects of allelopathy on growth of striga and other weeds, crops nutrient uptake and rice crop productivity.

### **1.4 Objectives**

The main objective of this study was to establish the causes of declining rice productivity in Eastern Uganda and determine probable effects of allelopathy in rice, cymbopogon, desmodium, mucuna and maize. The specific objectives of the study were:

- (i) To identify the secondary metabolites in rice, cymbopogon, desmodium, mucuna and maize root leachates and plant stover.

(ii) To determine the allelopathic potential of the compounds in rice, cymbopogon, desmodium, mucuna and maize root leachates and plant stover.

(iii) To determine the effects of allelopathy on striga infestation, growth, nutrient uptake and productivity of rice intercropped with cymbopogon, desmodium, mucuna and maize.

(iv) To evaluate the allelopathic effects of rice, cymbopogon, desmodium, mucuna and maize mixed mulches on weeds and rice.

(v) To determine the allelopathic control potential of mixed plant powders and water extracts from rice, cymbopogon, desmodium, mucuna and maize on weed growth.

### **1.5 Hypotheses**

i. Rice, cymbopogon, desmodium, mucuna and maize produce a wide range of secondary metabolites in roots and stover.

ii. The metabolites produced in rice, cymbopogon, desmodium, mucuna and maize root exudates and stover exhibit allelopathic potential.

ii. Striga infestation, crop growth, nutrient uptake and productivity are reduced under rice based intercropping with cymbopogon, desmodium, mucuna and maize.

iv. Weed and rice growth are affected by the mixed allelochemicals in rice, cymbopogon, desmodium, mucuna and maize mulches.

v. Weeds are affected by mixed allelopathic plant powders and water extracts from rice, cymbopogon, desmodium, mucuna and maize.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Allelochemicals and allelopathy

##### 2.1.1 Allelochemicals

Allelochemicals also called allelopathins are a subset of secondary metabolites which are not required for metabolism of an allelopathic organism (Iqbar and Fry, 2012). Allelochemicals with negative allelopathic effects are an important part of plant defense against herbivory. Ferguson and Rathinasabapathi (2012), observed that the metabolites are released from plant parts (e.g. roots, rhizomes, leaves, stems, bark, flowers, fruits and seeds) by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. These compounds belong to numerous chemical groups including: triketones, benzoquinone, coumarins, flavonoids, terpenoids, strigolactones, phenolic acids, lignin, fatty acids and non-protein amino acids. A wide range of these biochemicals are synthesized during the shikimate pathway (Hussain *et al.*, 2011) or, in the case of essential oils, from the isoprenoid pathway. Li *et al.*, (2010) reported that allelochemicals can be classified according to their different structures and properties as namely: (i) water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes and ketones; (ii) simple lactones; (iii) long-chain fatty acids and polyacetylenes; (iv) quinones (benzoquinone, anthraquinone and complex quinones); (v) phenolics, flavonoids and tannins (vi) cinnamic acid and its derivatives; (vii) coumarins; (viii) steroids and terpenoids (sesquiterpenes, diterpenes, and triterpenes).

Phenolic and Terpenoid compounds are the most important and common plant allelochemicals. Phenolics consist of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Within the context of allelopathy, the term phenolic compounds is

generally thought of as containing a range of compounds that include simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins, and a few of the flavonoids (Zeng *et al.*, 2008). Phenolic compounds generally arise from the pentose-phosphate pathway. 4-Phosphate erythrose and phosphoenolpyruvic acid undergo condensation reactions with 7-phosphate altoheptulose, which generate phenolic compounds after a series of transformation steps in the shikimic and acetic acid (polyketide) metabolic pathways. Triterpenes are specific to the plant kingdom and like steroids, arise via squalene, from mevalonate. Terpenoids and steroids are formed by units of five carbons derived from 2-methylbutadiene ( $\text{CH}_2\text{C}(\text{CH}_3)\text{CHCH}_2$ , isoprene), although they can also contain other elements such as oxygen.

### **2.1.2 Allelopathy**

Allelopathy refers to interactions occurring in the natural environment that exert both inhibitory and stimulative effects by influencing the growth of individuals within or between plant species at a certain concentration (Putnam and Tang, 1986). In laboratory studies with plant extracts the isolated compounds from plant tissue, exudates or even synthetic compounds identical to natural ones are called allelopathins and the term  $\div$ phytotoxicity $\oslash$  is used to distinguish it from allelopathy.

## **2.2 Allelopathic potential of rice, cymbopogon, desmodium, maize and mucuna**

### **2.2.1 Allelochemicals and allelopathic potential of cymbopogon**

Khanuja *et al.*, (2005), revealed the presence of citral (a mixture of geranial and neral), geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, geraniol,  $\div$  - caryophyllene, methyl heptenone, geranyl acetate and geranyl formate in the essential oils of different species of cymbopogon with marked variations. The essential oil of *C. citratus* exhibited

allelopathic activity and affected seed germination and seedling growth of corn and barnyard grass. Prapaipit *et al.*, (2013), observed inhibitory effects of *C. nardus* on the shoot and root growth of cress, lettuce, rapeseed and Italian rye grass at concentrations of 0.03 g dry weight equivalent extract /ml. They further recorded higher sensitivity of roots for the test plants to the extracts than their shoots and the effects were attributed to allelopathic compounds.

### **2.2.2 Allelochemicals and allelopathic potential of desmodium**

Hooper *et al.*, (2010), isolated three isoflavanones, 5,7,2,4-tetrahydroxy-6-(3-methylbut-2-enyl) isoflavanone (uncinane A), 4,5-dihydro-5,2,4-trihydroxy-5-isopropenylfuran-(2,3;7,6)-isoflavanone (uncinane B), and 4,5-dihydro-2-methoxy-5,4-dihydroxy-5-isopropenyl furano-(2,3;7,6)-isoflavanone (uncinane C) from the root exudates of *Desmodium uncinatum*. Hooper (2015) reported C-glycosylflavones as the major compounds in the root exudates of *Desmodium uncinatum*. Pickett *et al.* (2010) observed that although soil shading and addition of nitrogen fertilizer showed some benefits against *S. hermonthica* infestation, a putative allelopathic mechanism for *D. uncinatum* was observed when an aqueous solution from *D. uncinatum* plants was applied.

### **2.2.3 Allelochemicals and allelopathic potential of rice**

Kong *et al.* (2004b) isolated two compounds; flavone (5,7,4-trihydroxy-3,5-dimethoxyflavone) and a cyclohexenone (3-isopropyl-5-acetoxycyclohexene-2-one-1) from leaves of allelopathic rice accession PI 312777 using column chromatography. Kato-Noguchi (2011), identified two main inhibitory substances in rice exudates by spectral data as 3-hydroxy- $\beta$ -ionone and 9-hydroxy-4-megastigmen-3-one and reported that the inhibitory activity of a mixture of the two compounds was much higher than that of the sum of the individual inhibitory activities of two compounds, suggesting that the two compounds may

act synergistically to inhibit the growth of cress and barnyard grass. Kong *et al.* (2004a) reported momilactone A and B to contribute 61.4-86.6% of the observed growth inhibition of barnyard grass by rice seedlings. The remaining 14.4-38.6% of activity was presumed to be caused by other putative rice allelochemicals such as 3-isopropyl-5-acetoxycyclohexene-2-one-1 and 5,7,4-trihydroxy-3,5-dimethoxy-flavones. Kong *et al.* (2006) reported that Allelopathic rice P1312777 and Huagan-1 released momilactone B, 3-isopropyl-5-acetoxycyclohexane-2-one-1 and 5,7,4-trihydroxy-3,5-dimethoxyflavone into soil at phytotoxic levels. Kong *et al.* (2007) observed that rice roots release 5,4-dihydroxy-3,5-dimethoxy-7-O- $\beta$ -glucopyranosylflavone, momilactone B, 3-isopropyl-5-acetoxycyclohexene-2-one-1 and flavone O glycoside allelochemicals.

Kato-Noguchi *et al.* (2008a) reported that all rice cultivars contained momilactone A in the shoots and roots and concentrations differed among the cultivars. Momilactone A was also found in all culture solutions in which the rice seedlings were grown and the concentrations differed among the cultivars. Kato-Noguchi *et al.* (2008b) observed that momilactone B inhibited the growth of typical rice weeds like *Echinochloa crus-galli* and *E. colonum* at concentrations greater than 1  $\mu$ m. Kong *et al.* (2008) recorded reduced growth of *Echinochloa crus-galli* in paddy fields and attributed it to allelochemicals released by the roots of rice. Kato-Noguchi *et al.* (2008b) reported that rice crop secreted momilactone A and B into its rhizosphere throughout its entire life cycle and the secretion level increased and reached a peak at flowering. Kato-Noguchi (2012), similarly, reported that momilactone A caused only 0.8-2.2% inhibition and momilactone B caused 59-82% of the observed growth inhibition of *E. crus-galli* roots and shoots by rice seedlings.

#### **2.2.4 Allelochemicals and allelopathic potential of mucuna**

Vadivel and Pugalenth (2008) observed that the main (5%) phenolic compound of *Mucuna* seeds is L-DOPA. Soares *et al.* (2014), reported that L DOPA is exuded from the roots of mucuna, where its concentration can reach 1 ppm in water-culture solution and 50 ppm in the immediate vicinity of the roots and this concentration is high enough to reduce the growth of neighboring plants. Tomita *et al.* (2003), observed that the root growth of lettuce seedlings was inhibited by a volatile gas from *Mucuna pruriens* seedlings. Yoshiharu (2003), reported that incorporation of fresh leaves of *M. pruriens* into the soil (1.0% w/w in dry weight equivalent) reduced succeeding emergence of *Phaseolus vulgaris* up to 60%, and plant biomass up to 30% of the control. Nishihara *et al.* (2004), studied the effects of L-DOPA on different plant species and attributed it to an allelochemical. Nwaichi and Ayalogu (2010), observed that the growth indices measured on companion crops such as plant height, leaf area and dry weight, confirmed allelopathic suppression by mucuna.

#### **2.2.5 Allelochemicals and allelopathic potential of maize**

Kato-Noguchi (2010), identified three allelochemicals in the acetone extract obtained from the mesocotyls and coleoptiles of 5-day-old seedlings as 5-chloro-6-methoxy-2-benzoxazolinone (Cl-MBOA) which is a naturally occurring new benzoxazolinone, 6-methoxy-2-benzoxazolinone (MBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA). Ayeni and Kayode (2014), evaluated the allelopathic effect of the water extracts from *Sorghum bicolor* stem and maize (roots and tassel) on the germination and seedling growth of okra (*Abelmoschus esculentus* L. They reported that the extracts inhibited the germination of Okra seeds more in seeds treated with maize (roots and tassel) and the radicle and plumule lengths were retarded. The inhibition in radicle length and germination of Okra increased with increase in concentration of the extracts.

### **2.3 Properties of allelochemicals**

Bio-active compound properties are expressed by the mode of action of a chemical and can be broadly divided into a direct and an indirect action (Rizvi *et al.*, 1992). Effects through the alteration of soil properties, nutritional status and an altered population or activity of micro-organisms and nematodes represent the indirect action. The direct action involves the biochemical and physiological effects of allelochemicals on various important processes of plant growth and metabolism. The effects on growth and metabolism vary from changes in germination and mortality responses to the more subtle plastic responses such as a reduction in size, mass or number of organs. The existence of direct, versus indirect effects is yet to be proven (Rizvi *et al.*, 1992).

### **2.4 Allelochemical production and allelopathic inhibition**

Production of allelochemicals by plants varies with the environmental conditions and increases under stress. According to Rice (1984), a variety of environmental conditions influence the quantity of chemicals produced; some allelochemicals are influenced by the amount, intensity and duration of light with the greatest quantities being produced during exposure to ultraviolet and long-day photoperiods, and more allelochemicals are produced under conditions of mineral deficiency, drought stress and in cooler temperatures. The type and age of plant tissue during extraction is important since compounds are not uniformly distributed in plants. Plants' capacities to release allelopathic compounds vary between and within species. Kong *et al.* (2008), showed that the microbial community was affected by the allelochemical 5, 4-dihydroxy-3,5-dimethoxy-7-O- $\beta$ -glucopyranosylflavone exudated from allelopathic rice roots, through immediately hydrolyzing glucose with stimulation of soil bacteria and a glycone (5,7,4-trihydroxy-3,5-dimethoxyflavone) with inhibition of soil fungi.

Ferguson and Rathinasabapathi (2012), reported that allelopathic inhibition is complex and can involve the interaction of the different classes of chemicals with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds. They further noted that physiological and environmental stresses, pests and diseases, solar radiation, herbicides, less than optimal nutrient, moisture and temperature levels can also affect allelopathic weed suppression. Different plant parts, including flowers, leaves, leaf litter and mulch, stems, bark, roots, soil and soil leachates and their derived compounds have allelopathic activity that varies over a growing season. Allelopathic chemicals can also persist in soil affecting both neighboring plants as well as those planted in succession.

## **2.5 Sources and dynamics of allelochemicals**

Rice (1984), reported that allelochemicals are present in virtually all plant tissues including leaves, fruits, stems and roots. The leaves were reported to be the most consistent source, while roots were considered to contain fewer and less potent toxins. Aldrich (1984), similarly, observed that allelochemicals are more concentrated in the leaves, stem or roots rather than in the fruit or flowers. Rice (1984), further, reported four ways in which the chemicals are released namely volatilization, leaching, exudation and decomposition of plant residue. The vapor is absorbed through the stomata of the receiver plants, absorbed from condensate in dew or reach the soil and taken up by the roots. The water soluble phytotoxins may also be leached from roots and above ground parts or actively exudated from the living roots.

Allelopathy can act as an inducible defense mechanism mediated by recognition of root exudate components specific to other plant species found in the relevant ecosystem. Kato-Noguchi (2011), found that rice allelopathic activity due to levels of momilactone B,

increased in the presence of barnyard grass seedlings and barnyard grass root exudates and the increase was not due to nutrient competition between the two plant species. Kato-Noguchi (2011), presumed that rice plants detected components of the barnyard grass root exudates, which triggered increased production and secretion of momilactone B. Inderjit and Duke (2005), reported that the assessment of allelochemical dynamics in soil settings is limited by minute quantities of allelochemicals. There are two major sinks in the soil namely soil solids and water that determine the fate of allelochemicals and depending on the extraction method, quantification of a compound in soil may only be a reflection of the quantities that remain after one or more of these potential sinks have been more or less saturated. Phytotoxicity is largely a function of the concentrations of bio-available allelochemicals remaining in the soil environment.

Plants can develop extensive root systems and great surface area which is further increased in the case of mycorrhizal roots. In situations where the roots of competing plants come in direct contact with one another, it is difficult to determine if transfer of allelochemicals is soil-mediated or directly transferred through actual root-root contact. Putman and Tang (1986), observed that even though most plant tissues contain potential allelochemicals, only those released into the environment can inhibit the germination and growth of neighboring plant species and act as allelochemicals in natural ecosystems. The researchers also postulated that natural products found in root exudates are more likely to act as allelochemicals than those simply identified in plant tissues, emphasizing the need to couple measurement of phytotoxicity with relevant localizations.

Uren (2000), reported that roots of many weed and crop species contribute biologically active compounds known as exudates from living root systems into the environment and which



influence growth and establishment of crops and weed species. Many perennial wood and herbaceous plants have deep and extensive root/rhizome subterranean systems, which can produce prolific amounts of root exudates over long periods of time. Root exudates contribute many organic compounds to the rhizosphere. In addition to simple and complex sugars and growth regulators, root exudates contain different classes of primary and secondary compounds including amino acids, organic acids, phenolic acids, flavonoids, enzymes, fatty acids, nucleotides, tannins, steroids, terpenoids, alkaloids, polyacetylenes and vitamins (Rice 1984 and Uren, 2000). The amount of root exudates produced varies with the plant species, cultivar and age of plant, substrate and stress factors.

## **2.6 Effects of allelochemicals on physiological and biochemical processes in crops**

Several studies have been conducted in various crops but studies on the effects of allelopathy on physiological and biochemical processes in rice, cymbopogon, desmodium, mucuna and maize under field conditions are limited. The available literature and the related studies in other crops are hereby reviewed. El-Darier (2000), reported reduced N, P & K uptake at 5% concentration of eucalyptus in both beans and maize crops. In maize the uptake of K increased as N dropped while P remained constant. The eucalyptus leaf powder (5%) had significant inhibitory effects on uptake of N, P and K in beans (74, 50 & 66%) and maize (76, 68 & 53%). Geng *et al.*(2009), reported that a low concentration of dibutyl phthalate increased the absorption of N by tomato roots but decreased P and K absorption. A high concentration of the dibutyl phthalate inhibited the absorption of N, P and K, while, a low concentration of diphenylamine stimulated the absorption of N and K but inhibited the absorption of P and concluded that the effects of allelochemicals on the uptake of ions are closely related to allelochemical concentrations and classifications. Cheng and Cheng (2015), reported that allelochemicals can inhibit the activities of  $\text{Na}^+/\text{K}^+$ -ATPase involved in the

absorption and transport of ions at the cell plasma membrane, which suppresses the cellular absorption of  $K^+$ ,  $Na^+$ , or other ions.

Pawlowski *et al.* (2012), observed that volatile monoterpenes; eucalyptol and camphor of *Eucalyptus* widen and shorten root cells, besides inducing nuclear abnormalities and increasing vacuole numbers. Citral of *Cymbopogon citrates* has been reported by Chaimovitch *et al.* (2012), to cause disruption of microtubules in wheat and *Arabidopsis thaliana* L. roots. Burgos *et al.*, (2004), noted that the rye allelochemicals benzoxazolinone (BOA) and 2, 4-dihydroxy-1, 4(2H)-benzoxazin-3-one (DIBOA) significantly inhibited the regeneration of cucumber root cap cells and thus inhibited growth. Nishida *et al.* (2005), reported that the monoterpenoids (camphor, 1, 8-cineole, beta-pinene, alpha-pinene, and camphene) affected cell proliferation and DNA synthesis in plant meristems. Cai and Mu (2012), found that higher concentrations of the extracts of *Datura stramonium* L inhibited primary root elongation and lateral root development, decreased root hair length and density and inhibited cell division in root tips of soybean. Harun *et al.*, (2014), have shown that allelochemicals significantly inhibit the activity of antioxidant enzymes and increase free radical levels, resulting in greater membrane lipid peroxidation and membrane potential alteration. This diminishes the scavenging effect of activated oxygen and damages the whole membrane system of plants. Poonpaiboonpipat *et al.*, (2013), found that lemongrass (*Cymbopogon citratus*) essential oil, damages the membrane system of barnyard grass (*Echinochloa crus-galli* L.), causing lipid peroxidation and electrolyte leakage.

Allelochemicals can alter the contents of plant growth regulators or induce imbalances in various phyto-hormones, which inhibit the growth and development of plants with respect to seed germination and seedling growth. Liu and Hu (2001), found that the growth of wheat

seedlings was inhibited by the accumulation of indole-3-acetic acid (IAA) and Gibberellic acid (GA) with a simultaneous increase in Abscisic acid (ABA). An aqueous extract from rice was shown to significantly stimulate IAA oxidase activity in barnyard grass and reduce IAA levels, thereby damaging the growth regulation system and inhibiting wheat seedling growth (Lin *et al.*, 2001). Yang *et al.* (2005), observed that most phenolic allelochemicals stimulate IAA oxidase activity and inhibit the reaction of peroxidase (POD) with IAA, bound GA or IAA to influence endogenous hormone levels. Li *et al.*, (2010), reported that Phenolic allelochemicals can lead to increased cell membrane permeability. Consequently, cell contents spill and there is increased lipid peroxidation. Finally, there is slow growth or death of plant tissue. The researchers also observed that phenolic allelochemicals can also inhibit plants from absorbing nutrients from surroundings and affect the normal growth of plants.

The impact of phenolic allelochemicals on the respiration of plants has been shown to be weakened oxygen absorption capacity, while the impact on photosynthesis has mainly been to reduce the chlorophyll content and photosynthetic rate (Li *et al.*, 2010). Patterson (1981), reported that 10 - 30  $\mu\text{mol/L}$  caffeic acid, coumaric acid, ferulic acid, cinnamic acid, and vanillic acid could significantly inhibited the growth of soybean (*Glycine max*). Photosynthetic products and chlorophyll content of *G. ma x* were also strongly reduced.

Yu *et al.* (2003), reported that leaf transpiration, stomatal conductance and the intercellular  $\text{CO}_2$  concentrations decreased in incubated cucumber seedlings treated with solutions containing derivatives of benzoic and cinnamic acids. He and Lin (2001), reported that some phenolics (ferulic acid and cinnamic acid) inhibited protein synthesis. Phenolic allelochemicals from *O. sativa* were reported to inhibit amino acid transport and protein synthesis, and the subsequent growth of treated plants. All phenolics were reported to reduce integrity of DNA and RNA (Zing *et al.*, 2001; Ni, 2000) and in the vast majority of cases,

phenolic compounds appeared as a mixture and not as a single substance. Einhellig *et al.* (2004), concluded that the contribution made to allelopathy by phenolic compounds was probably never due to a single substance.

## **2.7 Germination of seeds**

A number of studies have been conducted in various crops but studies on the effects of allelopathy on growth and productivity of rice, cymbopogon, desmodium mucuna and maize under field conditions are limited. Monocotyledonous seeds have aleurone membrane of cells and in their germination, amylase enzyme catalyses the hydrolysis of starch and stored proteins into simple sugars and amino acids respectively using Gibberellic Acid (GA). Dicotyledonous seeds lack the aleurone membrane of cells and imbibition of water is more prolific than in monocotyledons. The DNA is photo-activated before GA can have its effect and the process is accomplished by formation of a chemical signal known as Pfr, a phytochrome pigment that absorbs red light (660 nm) energy, pro-activating the genes in DNA and effecting seed germination. When dicotyledonous seeds absorb far red light (730 nm) energy or exposed to darkness, the internal chemical structure is altered instantly and changed into Pr form of phytochrome and none of the seeds germinates.

## **2.8 Effects of allelochemicals on crop growth and productivity**

El-Darier (2000), reported that the leaf area index (LAI) and dry matter production of maize and beans significantly reduced at 5% concentration of Eucalyptus extracts in both crops. Tomita *et al.*, (2003), observed that the root growth of lettuce seedlings was inhibited by a volatile gas from *Mucuna pruriens* seedlings. Kong *et al.*, (2004b), reported that momilactone A and B contribute 61.4-86.6% of the observed growth inhibition of barnyard grass by rice seedlings. The remaining 14.4-38.6% of activity was presumed to be caused by other putative

rice allelochemicals namely 3-isopropyl-5-acetoxycyclohexene-2-one-1 and 5, 7, 4-trihydroxy-3,5-dimethoxy-flavones. Ram *et al.* (1998), reported higher productivity efficiency, land equivalent ratio (LER) of 1.88 for *Cymbopogon winterianus* intercropped with legumes. They recommended intercropping *C. winterianus* with winter legumes to achieve greater yield advantage and economise N fertiliser doses.

## **2.9 Economic benefits from intercropping systems**

Rajeswara (2003), observed maximum monetary returns from *Java citronella* intercropped with tomato or green gram. Intercropping *J. citronella* with red gram, horse gram and brinjal recorded reductions in biomass and essential oil yield. Jabbar *et al.* (2009), revealed that all intercropping systems gave substantially higher yield advantages over sole rice in terms of total rice grain yield equivalent (16-38%) and land equivalent ratio (25-75%). Economic benefits were achieved from the intercropped rice over sole rice with the highest benefits from rice + maize followed by rice + cowpea and + rice + bean compared to the minimum returns from the sole crop of rice. Jabbar *et al.*, (2010), in a dominance study reported that rice + maize, rice + cowpea and rice + pigeon pea intercropping systems were more profitable than growing rice alone and other intercropping systems. Rana *et al.* (2013), observed that the highest total rice grain yield equivalent (TRGYE), (6.88 Mt ha<sup>-1</sup>) was recorded for the treatment of intercropping *Sesbania* in two rows interval of rice and the minimum TRGYE (5.60 Mt ha<sup>-1</sup>) was for sole rice clearly indicating yield advantages of intercropping over monocropping of rice.

## **2.10 Allelopathy verses competition**

In allelopathic interactions, some phytotoxic substances are released by donor plants into the environment that affect the growth of receiver plants, whereas in competitive interactions, a growth resource is removed from the environment by one plant so that the growth resources

available to other plants are reduced. It is important to distinguish allelopathy from competition in order to manipulate the allelopathic potential of a crop species. Leather and Einhellig (1988), reported that allelopathy and competition occur simultaneously in the field where crop plants often grow together. Wu *et al.*, (2000), however, observed that experiments can be conducted under controlled conditions to understand some particular aspects of allelopathy and several bioassays have been developed to separate allelopathy from competition.

### **2.11 Categories of allelopathy and allelopathic interactions**

Allelopathy is divided into two categories namely 'true allelopathy' and 'functional allelopathy' (Zimdahl, 2013). True allelopathy involves the direct release into the environment of allelochemicals in form of leaf leachates, volatiles or root exudates, seeds and flowers (Farooq *et al.* 2011). Allelochemicals may also be released from decomposed mulches as phyto-active secondary compounds by chemical or microbial metabolism, a phenomenon called functional allelopathy (Zimdahl, 2013). Several allelopathic interactions of agricultural importance have been reported including crop to crop, crop to weed, weed to crop, plant to insect and plant to pathogen (Zohaib *et al.*, 2016; Abbas *et al.*, 2017).

### **2.12 Effects of allelochemical concentrations and plant densities of donor and receiver species on allelopathy**

Olofsdotter *et al.* (2002) reported that phenolic acids occur in soils at concentrations below  $5\text{mg kg}^{-1}$  soil, the bio active threshold for allelopathic effects in rice. Wu *et al.* (2000), reported negative allelopathic effects of wheat seedlings on the root growth of rye grass as a receiver plant and the allelopathic effect was dependent upon seedling density of donor wheat plants. It is therefore important that the density of both donor and receiver species is taken

into account when designing bioassays. The researchers recommended densities of bioassay species similar to field situations. Two models; Additive Dose Model (ADM) and the Multiplicative Survival Model (MSM) by Morse (1978), are often employed in allelopathy research to study the joint action of chemicals in a mixture. The ADM assumes that chemicals in a mixture can replace each other on the basis of their biological exchange rate or their relative potency, and any departure of the effect of mixtures from the ADM is characterized by either reduced (antagonistic) or enhanced (synergistic) effects. While the ADM assumes the chemicals to have similar molecular targets in the receiver plant, the MSM assumes that chemicals have different molecular targets and exert their effects independent of each other in the receiver plant.

Liu *et al.*, (2003), reported that when bioassay techniques are used to study the effects of allelochemicals, the plant processes are stimulated at low allelochemical concentrations and inhibited as the concentrations increase. Blouin *et al.* (2004), similarly reported that when herbicides are applied in mixture and infestation by weeds is less than expected compared with when herbicides are applied alone, a synergistic effect exists and the inverse response was described as antagonistic. Liu *et al.* (2003), developed a highly flexible but simple empirical model to describe the general pattern for stimulation at sub toxic concentrations (hormesis) and used the model to analyze some experimental data from allelochemical effects. Bustos, *et al.* (2008), reported that intercropping sage, spearmint, basil and oregano with coffee stimulated the plagiotropic growth of *Coffea Arabica* plants most effectively in young production systems and was reported a promising approach for increasing yield and quality production in coffee farms. This could have been due to hormesis. Zhao-Hui *et al.* (2010), reported that chemicals in a mixture can replace each other on the basis of their

relative potency as antagonistic or synergistic in effects on the target. The researchers also reported allelochemicals to act in a mixture and not as single substances.

### **2.13 Effects of allelochemicals on weeds**

Mattice *et al.* (1998), reported that allelopathy of rice against weeds correlated with the amounts of phenolic acids released by living rice roots. Namkeleja *et al.* (2013), who investigated the effects of different concentrations of leaf and seed water extracts from *Argemone mexicana* (0, 25, 50, 75 & 100%) on the germination and growth parameters of two native weed species, observed that seed germination, root length, shoot length, seedling length, fresh weight and dry weight of the species were significantly reduced by leaf and seed extracts. Roots were more affected than shoots and leaf extract was more suppressive than seed extracts.

### **2.14 Effects of weeds on crop productivity**

The effects of weeds on rice are reduced yield and quality mostly due to competition for nutrients, water and sunlight and in upland direct seeded rice, yield reductions range between 35 and 45% (Labrada, 2003). Weeds also exacerbate pests and disease problems by serving as alternate hosts and reduce efficiency of harvesting. The researcher similarly reported that NERICA 4 was more tolerant to weed pressure than other varieties. The grain yield, number of spikelet and days to 50% flowering of NERICA varieties differed in plots weeded once and twice and the spikelet numbers reduced by 30% in the weedy check. The weed biomass was negatively correlated with grain yield, number of spikelets per plant and plant height. Weeding delayed the flowering of upland rice by 4-8 days compared to the weedy check.



## 2.15 Striga weed and its control

The parasitic seed plant of most importance in Africa is the genus *Striga* (Family Scrophulariaceae). Members of this genus are obligate annual hemi parasites; they are chlorophyllous, but require a host to complete their life cycle (Musselman, 1987). Although 30 or more species of *Striga* have been described, only 5 are presently of economic importance in Africa (Ramaiah, *et al.*, 1983). *Striga hermonthica* (Del.) is of the highest economical importance in Africa. *Striga hermonthica* is a noxious weed that results in significant yield losses in many rice growing areas. It is found to infect a wide range of cereal hosts including sorghum, maize and rice. Ramaiah, *et al.* (1983), reported that *Striga* is an obligate parasite that requires a host for survival and its seeds are found commonly in the soil. The germination of *striga* seeds is triggered by hormone host-derived signals like strigolactones. The growth is chemotropic (Chang, *et al.*, 1986). Once the *striga* germinates, it attaches to the roots of host plants and form specialized structures called haustoria. Using these structures, the parasite absorbs nutrients from the host leaving it severely stunted. *Striga* plants emerge from the ground, flower profusely, producing a large number of seeds that lay dormant in the ground awaiting the next host. The seeds spread easily by wind, water and soil via animal vectors. The chief means of dispersal, however, is through human interaction, machinery, tools and clothings.

Khan *et al.* (2003), observed that intercropping maize with *D. uncinatum* not only reduced stem borer colonization on maize but also reduced parasitisation of maize by *S. hermonthica*. They attributed it to a novel allelopathic effect of the root exudates of the intercrops. Jamil *et al.* (2011), studied the production of strigolactones with the objective of identifying pre-attachment *striga* resistance of NERICA cultivars and reported that the quantity and quality of strigolactones produced by NERICA cultivars varied and affected germination, attachment

and emergence rate of striga. They observed that cultivars that produced less strigolactones (NERICA 1) were less infected by striga than cultivars that produced more strigolactones (NERICA 7, 8, 11 & 14). Cissoko *et al.*, (2011), studied the ability of these cultivars to resist the parasite during attachment after its germination. They found that some of the cultivars (NERICA 7, 8, 9, 11 & 14) were highly susceptible to this stage while NERICA 1 and 10 showed considerable resistance.

### **2.16 Control of weeds using allelopathic mulches and liquid extracts**

Cheema *et al.* (2004), reported that sorghum surface mulch (10-15 Mt ha<sup>-1</sup>) applied at sowing controlled 26-37% weeds and increased maize yield by 36-40%. Cheema *et al.* (2010b), observed that a mixture of sorghum + sunflower (18 L ha<sup>-1</sup>) stover suppressed the density and dry weight of weeds by 50 and 49% respectively. The rice husks (18 L ha<sup>-1</sup>) suppressed the weed density and dry weight up to 46 and 49% respectively. The researchers noted that the mulches used should be selected carefully, not to include flowers and seeds that they may introduce more weed seeds into the field.

Studies involving the use of bio-extracts from *C. nardus*, *D. uncinatum*, *M. pruriens*, *O. sativa* and *Z. mays* are meagre but the available literature on similar studies with other crops is hereby reviewed. Cheema *et al.* (2004), indicated that Sorgaab foliar spray controlled 18-50% of the weeds and increased maize grain yield by 11-44% relative to hand weeding, chemical herbicides and sorghum mulch. Iqbar and Cheema (2008), reported 62-92% purple nutsedge control with 75-88% dry weight reduction from Sorgaab application in combination with reduced doses of S-metolachlor herbicide. The researchers further observed that purple nut sedge density and dry weight were suppressed by 78-95% and 83-95% when Sorgaab and brassica water extracts were respectively used in combination with reduced rate of

glyphosate. Cheema *et al.* (2010b), concluded that allelopathy can be utilized for reducing the dose of herbicide by 40-67% in combination with allelopathic crop water extracts and still obtain the same weed control. Cheema and Khaliq (2000), observed that soil incorporation of sorghum stover at 2, 4 and 6 Mg ha<sup>-1</sup> reduced weed dry weight by 42, 48 and 56 percent respectively.

Widespread herbicide resistance in weeds has created great interest in the development of herbicides with novel target sites, particularly herbicides developed from natural plant products (Duke *et al.*, 2000). The triketone herbicides, including mesotrione, 2-(4-mesyloxy-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone a patented allelochemistry bio-herbicide, were developed through the optimization of leptospermone, a natural plant product produced by the bottlebrush plant (*Callistemon citrinus* Stapf.) that exhibits herbicidal activity (Mitchell *et al.*, 2001). The researchers reported that the bio-herbicide has been used as an effective tank-mix partner with pre-emergence and post-emergence applications in maize for controlling broad leaved weeds and grasses. Heap (2007), reported no naturally occurring herbicide resistance to mesotrione or other triketone herbicides.

Danijela (2014) studied mesotrione, and reported high efficacy in the control of *A. retroflexus*, *Chenopodium* spp, *C. arvense*, *D. stramonium*, *S. nigrum*, *S. arvensis* and *X. Strumarium*. High efficacy was confirmed in *A. theophrasti*, *A. artemisifolia*, *B. convolvulus*, *L. serriola*, *P. aviculare* and *P. lapathifolium* only at higher (1.2 L ha<sup>-1</sup>) doses. The efficacy significantly improved for *E. crus-galli*, *H. trionum*, *S. glauca* and *S. halepense* in combination with terbuthylazine and S-metolachlor, while in the combination with nicosulfuron it only increased for the Johnson grass developed from rhizomes. The treatments had no control over

*C. arvensis* and *C. dactylon*. Application of mesotrione alone, or in combination with terbuthylazine, nicosulfuron and S-metolachlor, indicated good selectivity towards maize.

### **2.17 Allelopathic potential of weeds**

Hegazy and Farrag (2007), found that percent germination of *Beta vulgaris*, *Lycopersicon esculentum*, *Sonchus oleraceus* and *M. indicus* were inhibited by flavonoids, alkaloids, terpenoids and volatile oils from methanol, water and oil extracts of *Chenopodium ambrosioides* weed. Esfandiar *et al.*, (2012) observed that the radicle length and dry weight of millet and basil were more sensitive to bindweed allelochemical materials than plumule and dry weight and the effect increased at higher concentrations. Irena (2014) evaluated the effects of weed concentrations (1.25, 2.5, 5.0 & 10.0 %) of *Sorghum halepense*, *Convolvus arvensis* and *Cirsium arvense* on seed germination and early seedling growth of *Pisum sativum* (L) varieties and observed that weed extracts significantly reduced germination percentage, shoot and root length, shoot and root weight and seed vigor index of the tested *Pisum* species. Zohaib *et al.* (2016), reported that weeds interfere with crops through competition and allelopathy. The researchers further, observed that weeds produce allelochemicals that alter various physiological processes such as enzyme activity, protein synthesis, photosynthesis, respiration, cell division and enlargement, which ultimately leads to a significant reduction in crop yield. They concluded that allelopathic weeds represent a potential threat for crop plants and cause economic losses.

### **2.18 Weed control stimulation by allelopathic chemicals**

Kong *et al.* (2006), reported that allelochemicals involved in rice allelopathy from living and dead plants are substantially different and the concentrations of the allelochemicals released from allelopathic rice seedlings into the soil increased to over 3 folds when they were

surrounded with *Echinochloa crus-galli* or lepodomoide, an ionic compound the weed produces. The results imply that allelopathic rice seedlings can sense certain allelochemicals in the soil and respond by increased production of allelochemicals inhibitory to the *Echinochloa crus-galli* weed. Kong *et al.* (2004b) and Zhao *et al.* (2005), made similar observations.

## CHAPTER 3

### METABOLITES PRODUCED IN RICE, CYMBOPOGON, DESMODIUM, MUCUNA AND MAIZE ROOTS AND VEGETATIVE MATERIALS

#### 3.1 Abstract

Crop species produce biologically active chemicals in form of leachates, exudates, volatile gasses and as decomposed products. The compounds influence the growth of crops and weed species and belong to numerous chemical groups including: triketones, benzoquinone, coumarins, flavonoids, terpenoids, strigolactones, phenolic acids, lignin, fatty acids and non-protein amino acids. An experiment was conducted at the Uganda National Crop Resources Research Institute, Namulonge, between June and August 2013, to identify compounds in root exudates released into soil potted with *Cymbopogon nardus*, *Desmodium uncinatum*, *Oryza sativa*, (NERICA 1), *Mucuna pruriens* and *Zea mays* (LONGE 6H) at 45 days after planting. Twenty four organic compounds identified in the soil not planted with crops (control) included fifteen terpenoids, two alcohols, and one each of trihalomethanes, ethers, phenols, ketones, furans, alkanes and aldehydes. Five terpenoids, a phenol and an alkane were exudated via cymbopogon plant roots and desmodium plant roots released three terpenoids, one alkane and a furan. Rice crop produced eight terpenoids, two alkanes and one furan. Five terpenoids, one phenol and an alkane were released by mucuna crop while six terpenoids were found in maize crop root exudates. *Cymbopogon nardus* stover produced ten terpenoids and one ester while desmodium stover produced six terpenoids and three phenolic compounds. Rice stover released six terpenoids, three phenolic compounds and one ester while, two terpenoids and four phenolic compounds were identified in mucuna stover. Maize stover released five terpenoids and four phenolic compounds. The bio-compounds identified could be allelopathic and responsible for various properties of *C. nardus*, *D. uncinatum*, *O.*

*sativa*, (NERICA 1), *M. pruriens* and *Z. mays* (LONGE 6H) in natural and agricultural ecosystems.

**Key words:** allelopathic, cymbopogon, desmodium, exudates, maize, mucuna, phenolic, rice terpenoids.

### 3.2 Introduction

A wide range of biochemicals are synthesized in the shikimate pathway or, in the case of essential oils, in the isoprenoid pathway and are not required for metabolism of the allelopathic organisms (Hussain *et al.*, 2011). Allelochemicals are a subset of secondary metabolites released from plant parts by leaching, root exudation, volatilization and residue decomposition in both natural and agricultural systems. Allelochemicals cause a numbers of ecological and economic problems such as decline in crop yield due to soil sickness, regeneration failure and replant problems. Kong *et al.* (2007), observed flavone O glycoside, Momilactone B, 5,4-dihydroxy-3,5-dimethoxy-7-O-b-glucopyranosylflavone and 3-isopropyl- 5-acetoxy cyclohexene-2-one-1 in rice plants. Rimando and Duke (2003), identified azelaic acid; r-coumaric acid; 1 H-indole-3-carboxalde-hyde; 1 H-indole-3-carboxylic acid; 1 H-indole-5-carboxylic acid and 1, 2-benzenedicarboxylic acid and bis (2-ethylhexyl) ester in rice stover. Chuihua *et al.* (2004), identified 3-Isopropyl-5-acetoxycyclohexene-2-one-1, momilactone B and 5, 7, 4 $\phi$ -trihydroxy-3 $\phi$ , 5 $\phi$ -dimethoxyflavone as bio-active compounds from an allelopathic rice accession (PI312777).

Kato-Noguchi (2011), identified two main inhibitory substances from maize plant leaves by spectral data as 3-hydroxy- $\beta$ -ionone and 9-hydroxy-4-megastigmen-3-one. Nishihara *et al.* (2004) and Soares *et al.* (2014), reported L DOPA to be exudated from the roots of *Mucuna*

*pruriens*. Khanuja *et al.* (2005), revealed the presence of citral (a mixture of geranial and neral), geraniol, citronellol, citronellal, linalool, elemol, 1, 8-cineole, limonene, geraniol, -caryophyllene, methyl heptenone, geranyl acetate and geranyl formate in the essential oils of different species of *Cymbopogon*. Hooper (2010), reported C-glycosylflavones as the major compounds in the root exudates of *Desmodium uncinatum*. The objective of the study was therefore to identify secondary metabolites in *Cymbopogon nardus*, *Desmodium uncinatum*, *Mucuna pruriens*, *Oryza sativa* (NERICA 1) and *Zea mays* (LONGE 6H) root exudates and in the shoots that could be responsible for the allelopathic potential of the crops.

### **3.3 Materials and methods**

#### **3.3.1 Profiling of the bio-active compounds**

##### **3.3.1.1 Potting in the screen house**

A screen house study was conducted at the Uganda National Crops Resources Research Institute, Namulonge in Uganda. Plastic pots (radius, 7 cm; height; 26 cm) were filled with 3 kg of forest loam soil. The area received a total of 638 mm of the 1270 mm annual rainfall during the cropping season with minimum and maximum temperatures of 19 and 30 °C respectively. Five 4-day old pre-germinated seeds of *Oryza sativa* (NERICA 1), *Desmodium uncinatum*, *Zea mays* (LONGE 6H) and *Mucuna pruriens* seeds were planted in the pots. Since *Cymbopogon nardus* is propagated by vegetative means, five plantlets were uprooted, cleaned and immediately potted and placed in a screen house. One pot without any plant was maintained as a control and about 200 ml of tap water was applied to each pot every two days till harvest at 40 days after planting when the crops were near peak vegetative growth (Niemeyer *et al.*, 1998). Five plants were taken out of each of the plastic pots at harvest without disturbance and the plant roots were shaken gently to remove the rhizosphere soil.



Soil (100 g) was collected from the middle to the bottom of each pot as representative samples. The samples were oven dried at 80 °C for 12 hours to constant weight for compound analysis.

### **3.3.1.2 Extraction and analysis of potential organic compounds from soil and plants**

Organic compounds in soils were extracted using solid phase micro-extraction (SPME) and by solvent extraction. Prior to the extraction, the SPME fibre was preconditioned for one hour at 250 °C under a stream of helium inside the gas chromatograph (GC) injection port liner. The SPME fibre used was gauge 24, 1 cm long, coated with divinyl benzene/polydimethylsiloxane and with 65 µM film thickness. In a single manual injection, one gram of each soil sample was accurately weighed into a 10 ml air tight glass vial. The sample and control extractions were placed into a thermostat heated block at 60 °C for 1 hour with the fibre exposed to the headspace for the entire duration. The fibre was retracted and introduced into the injection port of the GC in splitless mode. One gram of soil sample was accurately weighed into a 50 ml extraction tube and extracted with 10 ml of hexane by shaking at 250 revolutions per minute (rpm) in an orbital shaker for one hour. Two milliliter extract was aliquoted into an Eppendorf tube and centrifuged at 5000 rpm for 10 minutes and 1 ml extract was aliquoted into a GC vial for injection. One hundred milligrams of freeze dried samples were accurately weighed into 2 ml Eppendorf and 1800 µL of hexane was added with two mini steel balls placed inside the Eppendorf. The samples were vigorously ground in a genogrinder for 10 minutes. The extract was centrifuged at 5000 rpm for 5 minutes and 200 µL of extract was diluted with 800 µL of hexane in a GC vial for injection.

### 3.3.1.3 Gas Chromatography and Mass spectrometry instrumental analysis conditions

Samples were subjected to analysis using a 7890A GC system (Agilent Technologies, USA) coupled to a 240 ion trap mass spectrometer detector (Agilent Technologies) using the Agilent 7693A automatic liquid sampler for solvent extracted samples. A VF5-MS (5% phenyl methylpolysiloxane), 30 m × 0.25 mm id, 0.25 μm film capillary column was used with the injector port set at 280 °C. Helium was used as carrier gas at a flow rate of 1 ml/min. The oven temperature was programmed to rise from 50 °C to 180 °C at 4 °C/min followed by an increase to 250 °C at 3 °C/min. The ion trap mass spectrometer parameters were as follow: scan range 506540 (m/z), ionization mode EI and transfer line temperature, manifold temperature and trap temperature of 250 °C, 100 °C and 150 °C, respectively. Chromatograms and spectra representing individual samples were analysed using the automated mass spectral deconvolution and identification system software (AMDIS, US). The identification of the individual compounds was performed by comparing each of the mass spectra with the database of NIST 11 (Gaithersburg, MD, USA) and Wiley 7N (John Wiley, NY, USA) and also by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values obtained in the NIST web book for the same capillary column stationery phase.

## 3.4 Results

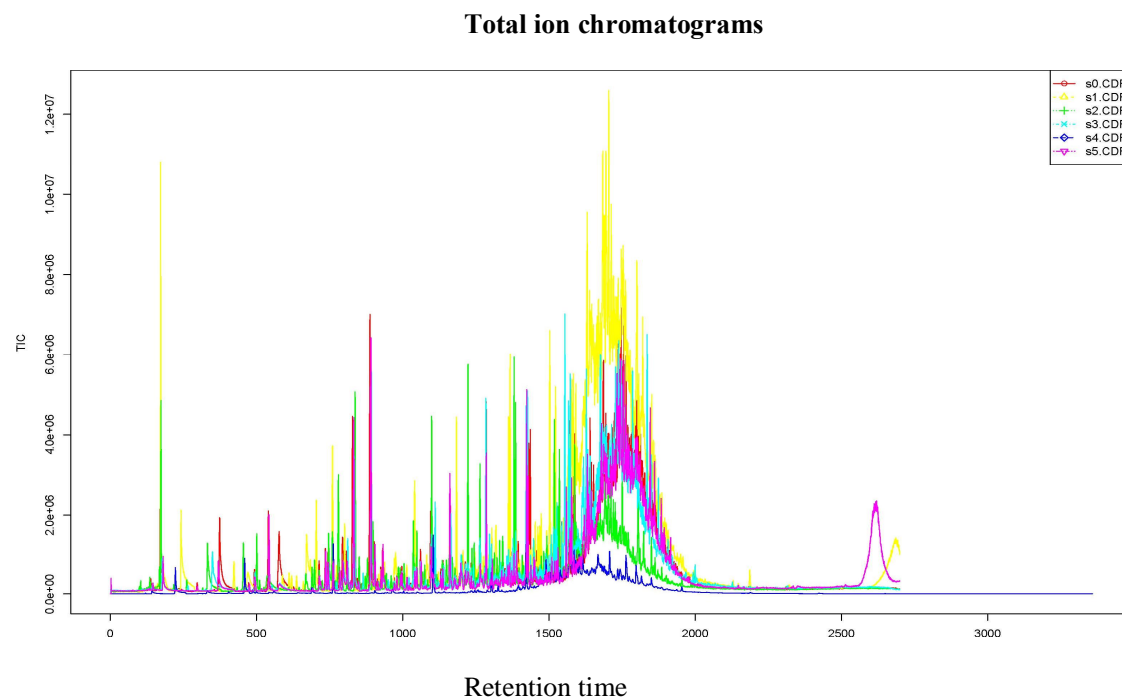
### 3.4.1 Total ion chromatograms for compounds in test soils overlayed on control

Graphical images of the total ion chromatograms generated from the solid phase micro-extraction (SPME) data files for compounds in soil treatment samples potted with *C. nardus* (S<sub>1</sub>), *D. uncinatum* (S<sub>2</sub>), *O. sativa* (S<sub>3</sub>) *M. pruriens* (S<sub>4</sub>) and *Z. mays* (S<sub>5</sub>) overlaid against the

control soil sample ( $S_0$ ) are presented (Figure 3.1). The compounds in  $S_0$  and treatment soils namely  $S_1$ ,  $S_2$ ,  $S_3$ ,  $S_4$  and  $S_5$  appeared similar with minor variations in the signal intensities of the total ion chromatograms (TIC) overlaid. The principal component analysis (PCA) showed no clustering of the sample spectra (Figure 3.2).

### 3.4.2 Analysis of secondary metabolites using XCMS online

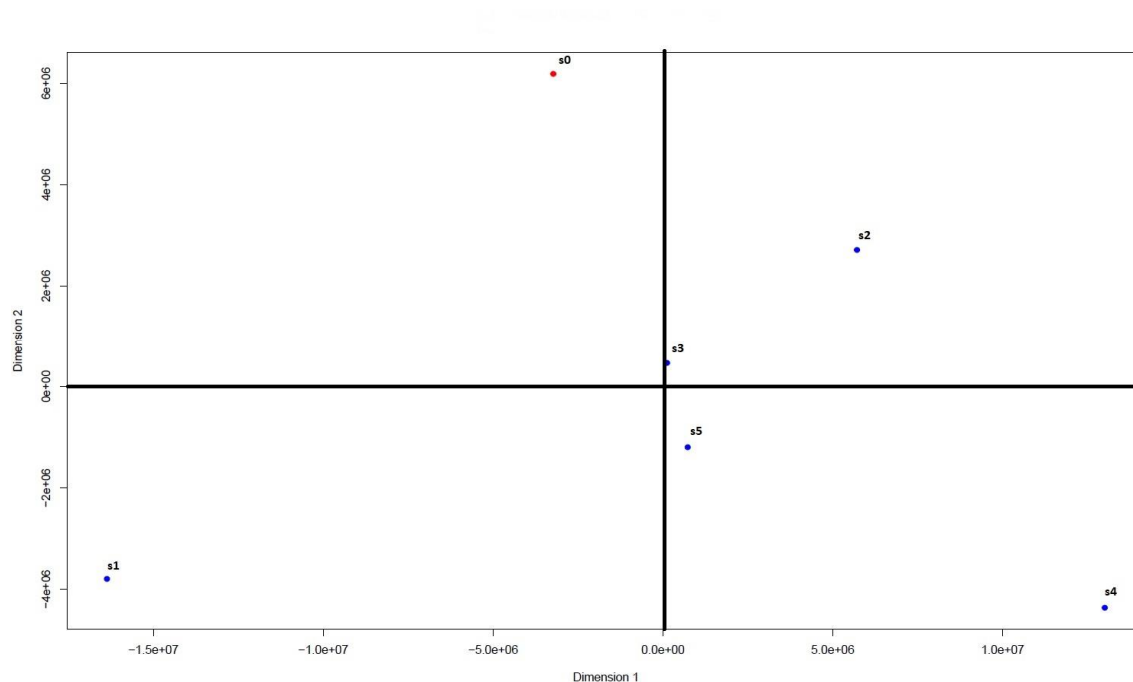
The generated principal component analysis plot for the control soil ( $S_0$ ) lay in quadrant 1, while the PCA for desmodium ( $S_2$ ) and rice ( $S_3$ ) were in quadrant 2, PCA for cymbopogon ( $S_1$ ) was positioned in quadrant 3 alone and the PCA for mucuna ( $S_4$ ) and maize ( $S_5$ ) were located in quadrant 4.



**Figure 3.1. Generated total ion chromatograms for secondary metabolites from cymbopogon ( $S_1$ ), desmodium ( $S_2$ ), NERICA 1 rice ( $S_3$ ), mucuna ( $S_4$ ) maize ( $S_5$ ) and control ( $S_0$ ) overlaid. TIC = Total Ion Chromatograms. CDF = Computable Document Format**

### 3.4.3 Secondary metabolites identified in the control and soil potted with test plants

The data on capillary column stationary phase retention time, relative match factors and the compounds identified in the control soil and soil potted with *C. nardus*, *D. uncinatum* and *O. sativa* (NERICA 1) *M. pruriens* and *Z. mays* (LONGE 6H) are indicated in Tables 3.1-3.6. Generally compounds with a positive log fold  $\geq 0.30$  were taken as dominant.



**Figure 3.2: PCA plot showing the clustering of the various compounds under study**

#### 3.4.3.1 Control

Twenty four metabolites were identified as the most probable compounds in the control treatment (Table 3.1). This was dominated by fifteen terpenoids namely Ethylbenzene, p-Dimethylbenzene, Vinyl benzene, o-Methyl styrene, m-Ethyl toluene, p-Ethyl toluene, 1,3,5-Trimethylbenzene, Isopropyl benzene, 1,2,3-Trimethylbenzene, o-Dichlorobenzene, 1,2,4-Trimethylbenzene, 1-Isopropyl-2-methylbenzene, L-Limonene, 3-Phenylpropene and 1-3-Diethylbenzene. Nine other compounds namely Trichloromethane, n-Butyl ether, oxime-

methoxy-phenyl, acetophenone, 2-n-Pentylfuran, 3,5-Dimethyloctane, n-Octanal, ethylhexanol and benzyl alcohol were also isolated from the control.

**Table 3. 1 Retention time, relative match factors for compounds in soil not planted with test crops (Control)**

<b>S/No.</b>	<b>Retention time (Min)</b>	<b>Relative match factor</b>	<b>Identified compound</b>	<b>Class of compound</b>
1	2.08	875	Trichloromethane	Trihalomethane
2	6.38	820	Ethylbenzene	Terpenoid
3	6.73	861	p-Dimethylbenzene	Terpenoid
4	7.14	867	n-Butyl ether	Ether
5	7.59	873	Vinyl benzene	Terpenoid
6	8.19	810	Oxime-, methoxy-phenyl	Phenol
7	9.98	795	o-Methyl styrene	Terpenoid
8	10.34	814	Acetophenone	Ketone
9	10.72	915	m-Ethyl toluene	Terpenoid
10	10.84	854	p-Ethyl toluene	Terpenoid
11	11.13	897	1,3,5-Trimethylbenzene	Terpenoid
12	11.50	887	Isopropyl benzene	Terpenoid
13	12.07	840	2-n-Pentylfuran	Furan
14	12.22	883	1,2,3-Trimethyl benzene	Terpenoid
15	12.47	866	3,5-Dimethyloctane	Alkane
16	12.68	854	n-Octanal	Aldehyde
17	13.13	919	o-Dichlorobenzene	Terpenoid
18	13.36	866	1,2,4-Trimethylbenzene	Terpenoid
19	13.52	888	1-Isopropyl-2-methylbenzene	Terpenoid
20	13.69	868	L-Limonene	Terpenoid
21	13.80	822	Ethylhexanol	Alcohol
22	13.90	789	3-Phenylpropene	Terpenoid
23	14.09	807	Benzyl Alcohol	Alcohol
24	14.42	822	1,3-Diethylbenzene	Terpenoid

Five compounds namely m-Ethyl toluene, o- methyl styrene, p-Ethyl toluene, 3,5-Dimethyloctane and benzyl alcohol isolated from the control treatment were not found in the treatment soils (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub>).

### 3.4.3.2 *Cymbopogon nardus*

Seven major compounds of which six were terpenoids were exudated into the soil by *C. nardus*. One compound namely 2-Ethylhexanol phenol, also identified in the control, was the only exudated major compound with positive log fold changes when overlayed with the control (Table 3.2). Tert-Amylbenzene, pentamethylbenzine, 1,2, Di-tert-butylbenzene and 2,3-Dimethylundecane were also released with high intensities while naphthalene and 1-Sec-Butyl-4-methylbenzene terpenoids were exudated with lower intensities (Log fold changes  $\leq$  0.30).

**Table 3.2 Retention time, relative match factors and positive log fold changes for compounds in *Cymbopogon nardus***

S/No	Retention time (Min)	Relative match factor	Positive log fold changes	Identified compound	Class of compound
1	13.80	859	0.70	2-Ethylhexanol	Phenol
2	18.48	896	0.37	tert-Amylbenzene	Terpenoid
3	18.98	892	0.26	1-Sec-butyl-4-methylbenzene	Terpenoid
4	19.41	860	0.17	Naphthalene	Terpenoid
5	19.70	876	0.53	Pentamethylbenzine	Terpenoid
6	21.29	921	1.80	1,2-Di-tert-butylbenzene	Terpenoid
7	18.83	867	0.39	2,3-Dimethylundecane	Alkane

### 3.4.3.3 *Desmodium uncinatum*

*Desmodium uncinatum* produced five dominant organic metabolites that included one furan named 2-n-Pentylfuran which had been identified in the control (Table 3.3). Three terpenoids namely tert-Amylbenzene, p-Ethyl toluene and 1-Sec-butyl-4-methylbenzene and an alkane identified as 2,3-Dimethylundecane were also released in the root exudates. All compounds were released at high intensities (Log fold changes > 0.30).

**Table 3.3 Retention time, relative match factors and positive log fold changes for compounds in *Desmodium uncinatum***

S/No	Retention time (Min)	Relative match factor	Positive log fold changes	Identified compound	Class of compound
1	10.84	876	0.36	p-Ethyltoluene	Terpenoid
2	12.07	874	0.87	2-n-Pentylfuran	Furan
3	18.48	882	0.74	tert-Amylbenzene	Terpenoid
4	18.83	867	0.42	2,3-Dimethylundecane	Alkane
5	18.98	901	0.65	1-Sec-butyl-4-methylbenzene	Terpenoid

### 3.4.3.4 *Oryza Sativa* (NERICA 1)

Eleven dominant compounds were released by rice in the root exudates. A furan namely 2-n-pentylfuran identified in rice had been isolated from the control treatment (Table 3.4). One alkane named 2,3-Dimethylundecane and six terpenoids identified as 1,2-Dimethyl-3-ethyl benzene, 1-Methyl-2-(2-propenyl)benzene, tert-Amylbenzene, 1-Sec-butyl-4methylbenzene, 1,3-Di-tert-butylbenzene and pentamethylbenzine were exudated dominantly. Compounds released with low intensities (Log folds  $\leq$  0.03) included two terpenoids namely 2-Ethyl-p-xylene and 1-methyl-3-propylbenzene and an alkane identified as 3,5-Dimethyloctane.

**Table 3.4 Retention time, relative match factors and positive log fold changes for compounds in *Oryza sativa* (NERICA 1)**

S/No	Retention time (Min)	Relative match factor	Positive log fold changes	Tentative compound	Class of compound
1	12.07	874	0.54	2-n-Pentylfuran	Furan
2	12.47	866	0.23	3,5-Dimethyloctane	Alkane
3	15.87	843	0.37	1,2-Dimethyl-3-ethylbenzene	Terpenoid
4	18.14	891	0.78	1-Methyl-2-(2-propenyl)benzene	Terpenoids
5	18.48	896	0.76	Tert-Amylbenzene	Terpenoids
6	16.423	896	0.90	2,3-Dimethylundecane	Alkane
7	18.98	892	1.30	1-Sec-butyl-4-methylbenzene	Terpenoids
8	14.84	842	0.08	2-Ethyl-p-xylene	Terpenoids
9	19.57	897	0.11	1-Methyl-3-propylbenzene	Terpenoids
10	19.70	876	0.83	Pentamethylbenzene	Terpenoids
11	21.29	869	0.34	1,3 Di-tertbutylbenzene	Terpenoid

#### 3.4.3.5 *Mucuna pruriens*

*Mucuna pruriens* released with high intensities seven organic compounds in its root exudates that included five terpenoids namely naphthalene, 1,2-Dimethyl-4-ethylbenzene, 1,3-Ditertiarybutylbenzene, 1-ethyl-3-methyl-benzene and 1,3-dichloro-benzene, an alkane called n-Tetradecane and a phenol called Dihydrocarveol (Table 3.5). All compounds were released at high intensities (Positive log folds > 0.30).



**Table 3.5 Retention time, relative match factors and positive log fold changes for compounds in *Mucuna pruriens***

S/No	Retention time (Min)	Relative match factor	Positive log fold changes	Identified compound	Class of compound
1	9.34	899	0.35	Dihydrocarveol	Phenol
2	8.00	895	0.38	Naphthalene	Terpenoid
3	12.45	899	0.52	1,2-Dimethyl-4-ethyl benzene; o-Xylene,	Terpenoid
4	15.06	875	0.46	n-Tetradecane	Alkane
5	16.71	872	0.31	1,3-Ditertiarybutylbenzene	Terpenoid
6	8.27	869	0.43	1-ethyl-3-methyl-benzene	Terpenoid
7	8.97	877	0.48	1,3-dichloro-benzene	Terpenoid

#### 3.4.3.6 *Zea mays* (LONGE 6H)

Six dominant compounds were identified in the soil potted with *Z. mays* that were apparently exudated by maize in the root exudates (Table 3.6). Three terpenoids namely m-Ethyl toluene; 1,2,4-Trimethylbenzene and 2-Ethyl-p-xylene and one furan called 2-n-Pentylfuran that had been identified in the control were exudated plus O-Dichlorobenzene and 1-Methyl-2-(2-propenyl) benzene. All the compounds were released with low intensities (Positive log folds  $\leq 0.30$ ) except 2-Ethyl-p-xylene.

**Table 3.6 Retention time, relative match factors and positive log fold changes for compounds in *Zea mays* (LONGE 6H)**

S/No	Retention time (Min)	Relative match factor	Positive log fold changes	Identified compound	Class of compound
1	10.72	866	0.29	m-Ethyltoluene	Terpenoid
2	12.07	894	0.17	2-n-Pentylfuran	Furan
3	13.13	897	0.11	o-Dichlorobenzene	Terpenoid
4	13.36	854	0.16	1,2,4-Trimethylbenzene	Terpenoid
5	14.84	842	0.31	2-Ethyl-p-xylene	Terpenoid
6	18.14	891	0.07	1-Methyl-2-(2-propenyl)benzene	Terpenoid

### 3.7 Comparison of compounds in root exudates of potted plants

Terpenoids and Phenols were the most common compounds identified in the root exudates of test plants. Cymbopogon, rice and desmodium each released two terpenoids namely Tert-Amylbenzene and 1-Sec-butyl-4-methylbenzene, and an alkane identified as 2,3-Dimethylundecane in root exudates (Table 3.7). Rice, desmodium and maize released 2-n-Pentylfuran as a common furan in the root exudates and one terpenoid named Pentamethylbenzine was only exudated in the roots of cymbopogon and rice crops. Naphthalene terpenoid was similarly exudated by mucuna and cymbopogon crops only while rice and maize produced 1-Methyl-2-(2-propenyl) benzene terpenoid in their root exudates.

**Table 3.7 Compounds exudated by cymbopogon, rice, desmodium, maize and mucuna in roots**

<b>Cymbopogon</b>	<b>Rice</b>	<b>Desmodium</b>	<b>Maize</b>	<b>Mucuna</b>
2,3-Dimethylundecane	2,3-Dimethylundecane	2,3-Dimethylundecane	o-Dichlorobenzene	Naphthalene
1-Sec-butyl-4-methylbenzene	1-Sec-butyl-4-methylbenzene	1-Sec-butyl-4-methylbenzene	1,2,4-Trimethylbenzene	1-ethyl-3-methyl-benzene
Tert-Amylbenzene	Tert-Amylbenzene	Tert-Amylbenzene	m-Ethyltoluene	Dihydrocarveol
2-Ethyl-hexanol-1	2-n-Pentylfuran	2-Pentylfuran	2-n-Pentylfuran	n-Tetradecane
Pentamethylbenzine	Pentamethylbenzine	p-Ethyltoluene	1-Methyl-2-(2-propenyl)benzene	1,3-Ditertiarybutylbenzene
1,2,Di-tert-butylbenzene	1,3,Di-tert-butylbenzene	2-Ethyl-p-xylene	2-Ethyl-p-xylene	1,3-dichlorobenzene
Naphthalene	1-Methyl-2-(2-propenyl)benzene 1-Methyl-3-propylbenzene 3,5-Dimethyloctane 1,2-Dimethyl-3-ethyl benzene 2-Ethyl-3-propylbenzene 2-Ethyl-p-xylene		1,2,3-Trimethylbenzene	1,2-Dimethyl-4-ethyl benzene;

A terpenoid identified as 1,2,Di-tert-butylbenzene and a phenol called 2-Ethyl-hexanol-1 were only produced by *C. nardus*. *Oryza sativa* (NERICA 1) was the sole producer of 1,3,Di-tert-butylbenzene, 3,5-Dimethyloctane, 1-Methyl-3-propylbenzene and 1,2-Dimethyl-3-ethyl benzene. Desmodium alone released p-Ethyltoluene and 2-Ethyl-p-xylene terpenoids. Maize crop exclusively exudated five terpenoids identified as o-Dichlorobenzene, 1,2,4-

Trimethylbenzene, m-Ethyltoluene, 2-Ethyl-p-xylene and 1,2,3-Trimethyl benzene. Terpenoids namely 1,2-Dimethyl-4-ethyl benzene; 1-ethyl-3-methyl-benzene, 1,3-dichloro-benzene, 1,3-Ditertiarybutylbenzene, a phenol called Dihydrocarveol and alkane called n-Tetradecane were entirely exudated by mucuna crop.

### 3.4.4 Bio-active compounds identified in vegetative material of the test plants

#### 3.4.4.1 *Cymbopogon nardus*

Retention time, relative match factors and compounds identified in *Cymbopogon nardus* stalk are indicated in Table 3.8.

**Table 3.8 Retention time, relative match factors and compounds in *Cymbopogon nardus* stover.**

S/No	Retention time (Min)	Relative match factor	Identified compound	Class of compound
1	13.59	854	Citronellal	Terpenoid
2	16.20	859	Citronellyl butyrate	Ester
3	16.57	847	-Citral	Terpenoid
4	16.99	870	cis-Geraniol	Terpenoid
5	20.24	874	trans-Carane	Terpenoid
6	20.42	888	Eugenol	Terpenoid
7	21.17	875	Geraniol acetate	Terpenoid
8	21.60	862	-Elemen	Terpenoid
9	22.57	892	Caryophyllene	Terpenoid
10	24.51	854	-Gurjunene	Terpenoid
11	25.48	895	-Cadinene	Terpenoid

Ten terpenoids namely Citronellal, -Citral, cis-Geraniol, trans-Carane, Eugenol, Geraniol acetate, -Elemen, Caryophyllene, -Gurjunene and -Cadinene and one ester named Citronellyl butyrate were identified.

#### 3.4.4.2 *Desmodium uncinatum*

Nine organic compounds were profiled from the *Desmodium uncinatum* stover (Table 3.9). They included six terpenoids namely Butylated Hydroxytoluene, 1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene, 1,4-Eicosadiene, 1-Ethyl-2-(1-phenylethyl)benzene, 3,4-Diethyl-1,1'-biphenyl and 2,2'-Diethylbiphenyl plus three phenols namely 2,5-Di-tert-butylphenol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol.

**Table 3.9 Retention time, relative match factors and compounds in *Desmodium uncinatum* stover**

S/No	Retention time (Min)	Relative match factor	Identified compound	Class of compound
1	25.12	849	Butylated Hydroxytoluene	Terpenoid
2	25.33	867	2,5-Di-tert-butylphenol	Phenol
3	29.57	845	3,4-Diethyl-1,1'-biphenyl	Terpenoid
4	30.15	863	1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene	Terpenoid
5	30.45	842	2,2'-Diethylbiphenyl	Terpenoid
6	31.02	785	1-Ethyl-2-(1-phenylethyl)benzene	Terpenoid
7	34.42	860	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phenol
8	35.09	759	(9Z)-9-Icosen-1-ol	Phenol
0	35.59	778	1,4-Eicosadiene	Terpenoid

### 3.4.4.3 *Oryza sativa* (NERICA 1)

Rice stover produced six terpenoids (Table 3.10) namely Butylated Hydroxytoluene; 3,4-Diethyl-1,1'-biphenyl, 1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene, 2,2'-Diethylbiphenyl, 1-Ethyl-2-(1-phenylethyl) benzene and 1,4-Eicosadiene. Three phenols identified as 2,5-di-tert-butyl- Phenol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and (9Z)-9-Icosen-1-ol and an ester called Hexadecanoic acid were also identified in the rice stover.

**Table 3.10 Retention time, relative match factors and compounds in *Oryza sativa* stover**

S/no	Retention time (Min)	Relative match factor	Identified compound	Class of compound
1	25.13	849	Butylated Hydroxytoluene	Terpenoid
2	25.32	845	2,5-di-tert-butyl- Phenol	Phenol
3	29.56	862	3,4-Diethyl-1,1'-biphenyl	Terpenoid
4	30.15	832	1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene	Terpenoid
5	30.45	865	2,2'-Diethylbiphenyl	Terpenoid
6	31.01	792	1-Ethyl-2-(1-phenylethyl)benzene	Terpenoid
7	34.40	874	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phenol
8	35.07	803	(9Z)-9-Icosen-1-ol	Phenol
9	35.60	782	1,4-Eicosadiene	Terpenoid
10	36.91	845	Hexadecanoic acid	Ester

#### 3.4.4.4 *Mucuna pruriens*

Compounds identified in the *M. pruriens* stover (Table 3.11) included two terpenoids named Butylated Hydroxytoluene and 1,4-Eicosadiene besides four Phenols named 2,5-di-tert-butyl-Phenol, Hexa-hydro-farnesol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, (9Z)-9-Icosen-1-ol and (9Z)-9-Icosen-1-ol.

**Table 3.11 Retention time, relative match factors and compounds in *Mucuna pruriens* stover**

S/No	Retention time (min)	Relative match factor	Identified compound	Class of compound
1	25.13	856	Butylated Hydroxytoluene	Terpenoid
2	25.32	841	2,5-di-tert-butyl- Phenol	Phenol
3	26.24	786	Hexa-hydro-farnesol	Phenol
4	34.41	856	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phenol
5	35.10	796	(9Z)-9-Icosen-1-ol	Phenol
6	35.60	814	1,4-Eicosadiene	Terpenoid

#### 3.4.4.5 *Zea mays* (LONGE 6H)

Maize (LONGE 6H) stover released five terpenoids (Table 3.12) namely Ionene, Butylated Hydroxytoluene, 3,4-Diethyl-1,1'-biphenyl, 1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene, 1,4-Eicosadiene and four phenols called Falcarinol, 2,5-Di-tert-butylphenol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and (9Z)-9-Icosen-1-ol.

**Table 3.12 Retention time, relative match factors and compounds in *Zea mays* stover**

S/No	Retention time (Min)	Relative match factor	Identified compound	Class of compound
1	20.51	876	Falcarinol	Phenol
2	20.54	904	Ionene	Terpenoid
3	25.13	903	Butylated Hydroxytoluene	Terpenoid
4	25.32	896	2,5-Di-tert-butylphenol	Phenol
5	29.56	842	3,4-Diethyl-1,1'-biphenyl	Terpenoid
6	30.15	831	1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene	Terpenoid
7	34.41	856	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phenol
8	35.09	759	(9Z)-9-Icosen-1-ol	Phenol
9	35.58	812	1,4-Eicosadiene	Terpenoid

#### 3.4.4.6 Comparison of compounds identified in plant materials

Compounds identified in the stover of cymbopogon, desmodium, rice, maize and mucuna plants are shown in Table 3.13. The majority were Phenols and Terpenoids. Rice, desmodium, maize and mucuna plant materials produced three compounds in common identified as 1,4-Eicosadiene; 2,5-di-tert-butylphenol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol. Butylated Hydroxytoluene and (9Z)-9-Icosen-1-ol bio-compounds were extracted from rice, desmodium and maize plant stover and 1,2,3-Trimethyl-4-[(1E)-1-propenyl] naphthalene terpenoid was identified only from the stover of rice, desmodium and maize. Three compounds namely 2,2'-Diethylbiphenyl; 1-Ethyl-2-(1-phenylethyl) benzene and 3,4-Diethyl-1,1'-biphenyl were produced only by rice and desmodium stover. Rice



exclusively released Hexadecanoic acid ester as mucuna produced exclusively Hexa-hydro-farnesol phenol.

**Table 3.13 Compounds in cymbopogon, rice, desmodium, mucuna and maize stover**

S/No	<i>Cymbopogon nardus</i>	<i>Oryza sativa</i> (NERICA 1)	<i>Desmodium uncinatum</i>	<i>Mucuna pruriens</i>	<i>Zea mays</i> (LONGE 6H)
1	Citronellal	1,4-Eicosadiene	1,4-Eicosadiene	1,4-Eicosadiene	1,4-Eicosadiene
2	Citronellyl butyrate	2,5-di-tert-butyl- Phenol	2,5-Di-tert-butylphenol	2,5-di-tert-butylphenol	2,5-Di-tert-butylphenol
3	-Citral	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	cis-Geraniol	Butylated hydroxytoluene	Butylated hydroxytoluene	Butylated hydroxytoluene	Ionene
5	trans-Carane	(9Z)-9-Icosen-1-ol	(9Z)-9-Icosen-1-ol	(9Z)-9-Icosen-1-ol	(9Z)-9-Icosen-1-ol
6	Eugenol	1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene	1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene	Hexa-hydro-farnesol	1,2,3-Trimethyl-4-[(1E)-1-propenyl]naphthalene
7	Geraniol acetate	2, 2'-Diethylbiphenyl	2, 2'-Diethylbiphenyl		Falcarinol
8	-Elemen	1-Ethyl-2-(1-phenylethyl)benzene	1-Ethyl-2-(1-phenylethyl)benzene		3,4-Diethyl-1,1'-biphenyl
9	Caryophyllene	3,4-Diethyl-1,1'-biphenyl	3,4-Diethyl-1,1'-biphenyl		Butylated hydroxytoluene
10	-Gurjunene	Hexadecanoic acid ester			
11	-Cadinene				

The compounds identified as (9Z)-9-Icosen-1-ol; Butylated Hydroxytoluene and 3,4-Diethyl-1,1'-biphenyl were only found in the maize stover. Ten terpenoids namely Citronellal, -

Citral, cis-Geraniol, trans-Carane, Eugenol, Geraniol acetate,  $\alpha$ -Elemen, Caryophyllene,  $\alpha$ -Gurjunene,  $\alpha$ -Cadinene and one ester called Citronellyl butyrate were only identified in cymbopogon stover. Cymbopogon had a unique profile of secondary metabolites.

### **3.5. Discussion**

#### **3.5.1 Compounds exudated into the soil**

The compounds in  $S_0$  and treatment soils namely  $S_1$ ,  $S_2$ ,  $S_3$ ,  $S_4$  and  $S_5$  appeared similar with minor variations in the signal intensities of the total ion chromatograms (TIC) overlaid. This signified that some compounds in the test samples were also found in the control. Twenty four compounds were identified in the soil not planted with a test plant (Control) and the clustering of the PCA plots for the compounds in the control treatment ( $S_0$ ) solely lay in the first quadrant. This signified that compounds in the control differed highly from sample treatments ( $S_1$ - $S_5$ ). This is supported by the observation that only five out of the twenty four compounds were identified for the five test crops under the NIST library. It is presumed that the bio-compounds identified in the control treatment had possibly been either exudated by forest plants or were deposited by decomposing plant materials. Rice (1984) and Uren (2000), reported that plant root exudates contain different classes of primary and secondary compounds.

Seven compounds were exudated by *C. nardus* but only one of these compounds (2-Ethylhexanol phenol) was found in the control treatment. The PCA plot for cymbopogon solely lay in the third quadrant, clearly indicating the lower levels of similarity with compounds in the other test plants. There are no similar compounds reported in the available

literature. Desmodium and rice exudated five and eleven compounds in the roots respectively. The PCA plots for desmodium and rice were close and in the second quadrant, signifying a close association between their compounds. Two similar terpenoids identified as 1-Sec-butyl-4 methylbenzene, and Tert-Amylbenzene as well as 2-n-Pentylfuran and 2,3-Dimethylundecane alkane were produced by desmodium and rice crops. The high positive folds for the furan that had been isolated in soil without test plants signified its increased production by both *D. uncinatum* and *O. sativa* and hence the possible reason for the PCA positioning for desmodium and rice. Contrary to the current study, Hooper (2010), reported C-glycosylflavones as the major compounds in the root exudates of *D. uncinatum*. Several researchers have reported different compounds released by various cultivars of rice. Kong *et al.* (2007) reported that Momilactone B, 5,4-dihydroxy-3,5-dimethoxy-7-O- $\beta$ -glucopyranosylflavone; 3-isopropyl-5-acetoxycyclohexene-2-one-1 and a flavone, O-glycoside were released by rice roots. Kato-Noguchi *et al.* (2008a), reported that rice secreted momilactone A and B into its rhizosphere. Kato-Noguchi (2011) identified 3-hydroxy- $\beta$ -ionone and 9-hydroxy-4-megastigmen-3-one compounds in rice root exudates. Kim and Shin (1998), noted that allelopathy is influenced more by genetics than the environment Jansen *et al.* (2001), reported that allelopathy is quantitatively inherited. Four main effect quantitative trait loci located on three chromosomes were identified, which collectively explained 35% of the total phenotypic variation of the allelopathic activity in the population.

Mucuna and maize PCA were in the fourth quarter. The crops did not release a common compound in the root exudates but their common clustering may be attributed to the dominance of terpenoids in both crops. Mucuna and maize released 5 and 6 terpenoids respectively, but mucuna clustered distantly from the control. Rice (S<sub>3</sub>) and maize (S<sub>5</sub>) clustered closely and were in close proximity to S<sub>0</sub> in PCA. This may be attributed to the

common presence of 2-n-pentylfuran in the three treatments. Despite cymbopogon and rice clustering in different quadrants, the PCA were close possibly due to the commonly exudated 2,3-Dimethylundecane, 1-Sec-butyl-4-methylbenzene; tert-Amylbenzene, and Pentamethylbenzine compounds. Seven significant compounds dominated by terpenoids were exudated by *M. pruriens*, but there were no similar compounds reported in literature. Nishihara *et al.* (2004) and Soares *et al.* (2014) reported L DOPA to be dominantly exudated from the roots of *M. pruriens*. Six terpenoids were identified in the soil potted with *Zea mays* crop and none of the compounds had been isolated in the soil without a test plant (control). Kato-Noguchi (2011), identified 3 different allelochemicals namely 5-chloro-6-methoxy-2-benzoxazolinone, 6-methoxy-2-benzoxazolinone and 2,4-dihydroxy-1,4-benzoxazin-3-one from the mesocotyls and coleoptiles of rice seedlings.

The potential effects of some of the bio-active compounds profiled in the test crops have been reported by Nishida *et al.* (2005) on cell proliferation and DNA synthesis in plant meristems. Kato-Noguchi (2011) and Poonpaiboonpipat *et al.* (2013) have reported that allelochemicals from rice and cymbopogon inhibit weed growth. Ayeni and Kayode (2014), recorded inhibited seed germination by compounds in maize. Reduced growth of component crops and subsequent plants were observed by Pickett *et al.*, (2010) and Soares *et al.* (2014), in desmodium and mucuna crops, respectively. Narwal *et al.* (2005), reported that accumulation of allelochemicals in the soil suppressed seed germination and plant growth decreased the volume of primary roots and increased secondary roots, reduced uptake of water and nutrients and subsequently caused chlorosis, ultimately resulting in the death of plants. Cheng and Cheng (2015), reported that allelochemicals inhibit the absorption and transport of ions at the cell plasma membrane in various crops. Chaimovitsh *et al.* (2012), reported citral of cymbopogon to cause disruption of microtubules in wheat and *Arabidopsis thaliana* L. roots.

### 3.5.2 Compounds identified in plant materials

Ten terpenoids and one ester were identified only in cymbopogon stover. Khanuja *et al.* (2005), revealed the presence of six compounds namely citral, geraniol, elemol, 1 - caryophyllene, geranyl acetate and citronellal in the essential oils of species of cymbopogon. In the current study the same compounds were identified. In addition desmodium stover released six terpenoids and three phenols but none of it had been identified in this plant as per available literature. Rice stover produced six terpenoids, three phenolic compounds and one ester. Five carboxylic acids and an aldehyde were identified by Rimando and Duke (2003), from rice stover while Kong *et al.* (2004b), isolated a flavone and one cyclohexenone from leaves of an allelopathic rice accession. Mucuna stover produced two terpenoids and four phenols and maize stover released five terpenoids and four phenolic compounds. There is scarce literature on related or similar compounds from mucuna and maize vegetative materials. This may be attributed to genetic variations and environment influences of test materials.

Alkanes, terpenoids, furans, alcohols and phenols were exudated into the soil by the test plants. Terpenoids and phenolic compounds were the principal compounds identified in the stover materials together with an ester profiled in cymbopogon and rice stover. The results demonstrate that some of the compounds may have allelopathic properties and could be responsible for a number of ecological and economic problems in natural and agricultural ecosystems such as declines in crop yield due to soil sickness, plant regeneration failure and replant problems. The compounds may also be responsible for some of the beneficial weed control and other effects reported under these crops. The compounds released by the various crops had not been previously found and this could be due to the variety differences which have been reported to determine allelopathy.

## CHAPTER 4

### ALLELOPATHIC POTENTIAL OF CYMBOPOGON, RICE, DESMODIUM, MUCUNA AND MAIZE

#### 4.1 Abstract

Allelochemicals cause variable yield differences under various ecosystems worldwide. Studies were conducted at the National Crops Resources Research Institute, Namulonge, in Uganda to investigate allelopathic effects of *Desmodium uncinatum*, *Oryza sativa*, *Mucuna pruriens* and *Zea mays* crops. Studies involved pot screening, equal compartments agar, germination tests and growth of two plants in the same pot in a completely randomised block design. Pot screening results indicated significant ( $P \leq 0.05$ ) reductions in root lengths (49-63%), height (48-66%) and biomass (63-75%) for treated *Ageratum conyzoides*, *Bidens pilosa* and *Gallinsoga parviflora* weeds relative to the controls. Under equal compartment agar study, *G. parviflora* root and stem growth reduced (20-41% & 19-42%) with maize, rice and mucuna leachates. Mean germination time (MGT) for mucuna, desmodium, rice and maize seeds increased at 75% rice/ desmodium (0.08-0.45%) and rice/mucuna (0.20-0.5%) leachates relative to the control. Mucuna, desmodium, rice and maize seeds mean germination time (MGT) significantly ( $P \leq 0.05$ ) increased by 0.4-2.1 and 0.4-2.3 days at 75 percent leachate concentrations of rice /desmodium and rice/mucuna leachate concentrations respectively over the control. The MGT for desmodium, rice and maize seeds, similarly, increased by 0.6-2.8 days at 75% rice + maize leachate concentration. The seed germination indices (SGI) for mucuna, desmodium and rice seed significantly ( $P \leq 0.05$ ) reduced by 15-30%, 24-28% and 24-28% respectively with increases (25-75%) in rice/desmodium, rice/mucuna and rice/maize leachate concentrations. On the contrary, increases in concentrations of rice/desmodium and rice/maize leachates from 25 to 75% significantly

( $P \leq 0.05$ ) increased the seed germination index (SGI) for maize seeds to highest values by 25% and 119% respectively. Maize seeds given tap water produced the lowest SGI of 3.6 under the two treatments. Root length for rice reduced by 30 to 46% due to potting with mucuna, maize and desmodium but potting maize with mucuna and desmodium increased maize leaf length by 15 to 24%. Mucuna leaf width reduced by 32% when potted with maize while potting desmodium with maize and mucuna reduced the desmodium root length, leaf number, leaf length, leaf width and plant height by 49 to 64%. The observed allelopathic properties give ecosystems with plants of differing growth rates due to allelopathic influences on seed germination, growth and development. Strategic management of sole, mixed and succession crops under allelopathic ecosystems is crucial.

**Keywords:** allelochemicals, *Bidens pilosa*, *Desmodium uncinatum*, leachates, mean germination time, *Mucuna pruriens*, *Oryza sativa*, pot screening, *Zea mays*

## 4.2 Introduction

The phytotoxicity of bio-active compounds in allelopathic plants under various ecosystems affects the productivity and lifespan of plant species. In agricultural systems, allelopathy can cause interference between crop species and between crop and weeds species, thereby affecting the economic outcomes of plant production. Several allelopathic interactions of agricultural importance have been reported including crop to crop, crop to weed, weed to crop, plant to insect and plant to pathogen (Zohaib *et al.*, 2016; Abbas *et al.*, 2017). Problems of poor establishment and stunted growth of crops, due to allelopathic auto-toxification, resulting from growing the same crop on the same piece of land in succeeding years and due to allelopathic influences of weeds are common in managed agricultural ecosystems. Inhibitory effects on germination and establishment of crops and weeds caused by crop residues and foliar sprays have been reported by Cheema *et al.* (2010b). Danijela

(2014), reported mesotrione, a triketone herbicides to have high efficacy in the control of several weeds. Zohaib *et al.* (2016), reported that weeds produce allelochemicals that alter various physiological processes such as enzyme activity, protein synthesis, photosynthesis, respiration, cell division and enlargement, which ultimately leads to a significant reduction in crop yield. Allelopathic crop species are therefore a potential resource for production of bio-degradable weed control technologies.

The properties of allelochemicals are expressed by the mode of action of a chemical and can be broadly divided into a direct and an indirect action. The direct action involves the biochemical physiological effects of allelochemicals on various important processes of plant growth and development which vary from changes in germination and mortality to responses such as reduction in size, mass or number of organs. These are secondary expressions of primary effects on metabolic processes. Effects through the alteration of soil properties, nutritional status, population or activity of micro-organisms and nematodes represent the indirect action (Rizvi *et al.*, 1992). The authors further noted that allelochemicals are more concentrated in the leaves, stem or roots than in the fruits or flowers. Allelochemicals are released in four ways namely volatilization, leaching, exudation and decomposition of plant residue (Rice, 1984). The properties of the allelochemicals could be used to manipulate biosynthesis of putative compounds for weed management.

Aqueous methanol extracts of rice plants were found to inhibit the germination and growth of *Echinochloa Crus-galli* (Chung *et al.*, 2002). Kong *et al.* (2008), observed reduced growth of *Echinochloa crus-galli* in paddy fields and attributed it to allelochemicals released by the roots of rice. Ayeni and Kayode (2014), reported that the water extracts from *Sorghum bicolor* stem and maize inhibited the germination of Okra seeds. Nwaichi and Ayalogu



(2010), reported that the plant height, leaf area and dry weight measured as growth indices on companion crops confirmed allelopathic suppression by mucuna. Soares *et al.* (2014), reported that L-DOPA from the roots of mucuna was high enough to reduce the growth of neighboring plants. Pickett *et al.* (2010), observed that although soil shading and addition of nitrogen fertilizer showed some benefits against *S. hermonthica* infestation, a putative allelopathic mechanism for *D. uncinatum* existed when an aqueous solution from *D. uncinatum* plants was applied. Properties of bioactive compounds in maize, rice, desmodium and mucuna have not been established. The objective of the study was, therefore, to determine the allelopathic potential of *Desmodium uncinatum*, *Oryza sativa*, *Mucuna pruriens* and *Zea mays* crops.

### **4.3 Materials and methods**

Four studies were conducted at the Uganda National Crops Resources Research Institute (NaCRRI), Namulonge, during 2013A, to determine the allelopathic potential of bio-active compounds in test plants using four approaches namely pot screening for allelopathy on weeds (Dayan *et al.*, 2009), equal compartments agar (Hilt *et al.*, 2012), germination tests (Hagan *et al.*, 2013) and donor to receiver plants in the same pot (Dayan *et al.*, 2009).

#### **4.3.1 Pot screening**

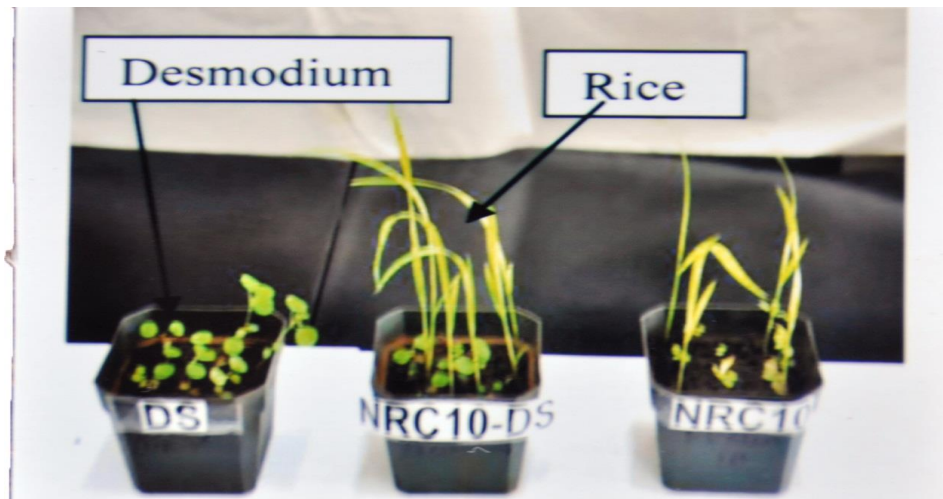
##### **4.3.1.1 Treatments and experimental design**

*Desmodium uncinatum*, *Mucuna pruriens* and *Zea mays* were screened for their allelopathic potential against rice genotypes in three subsequent stages. In the first preliminary un-replicated screening stage, five 4 days old pre-germinated seeds of each of the six upland rice genotypes namely NERICA 1, NERICA 4, NERICA 10, NEMCHE 1, NEMCHE 2 and NEMCHE 3 were potted with five, 4-day old pre-germinated seeds each of desmodium,

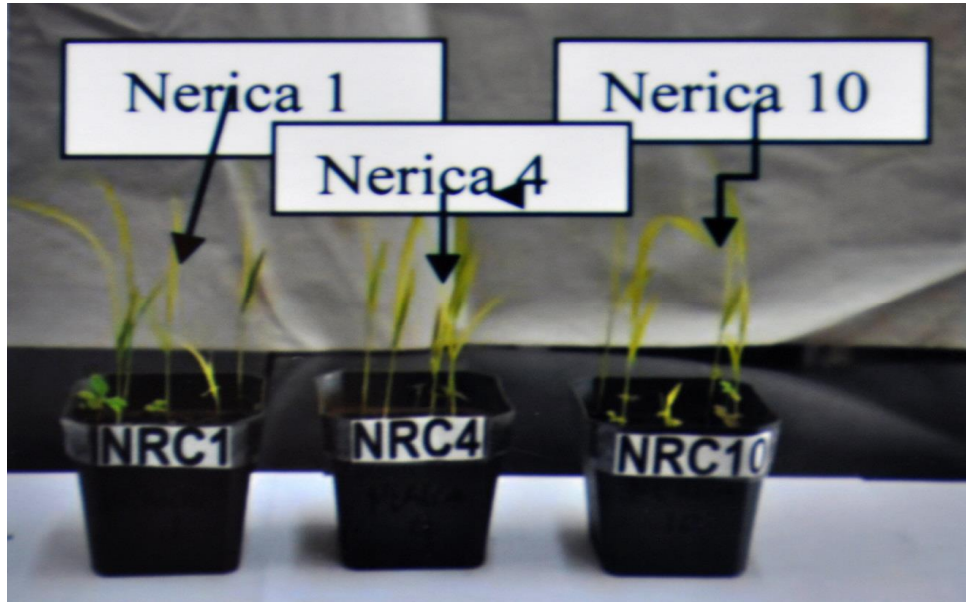
mucuna and three maize genotypes namely LONGE 6H, M17 and M25 in a completely randomised design. The 10 x 10 cm top diameter pots contained 300 g of sandy-loam soil (USDA classification system) as indicated in plates 4.1-4.4.



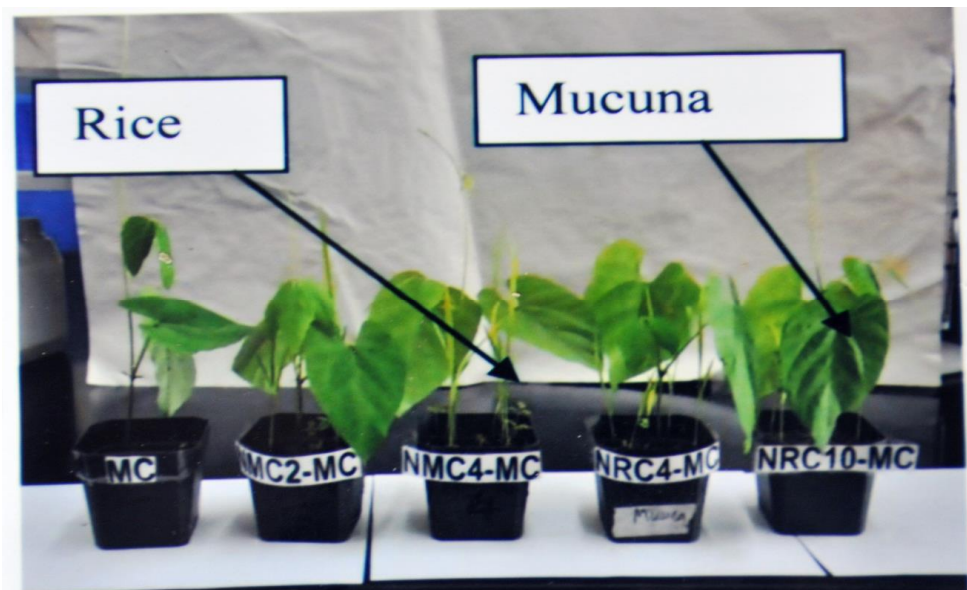
**Plate 4. 1** Pot screening of NEMCHE (1-3) rice with maize (LONGE 6H, M17 & M25) genotypes at stage 1 of Pot screening study



**Plate 4.2** Pot screening of NERICA (NRC) 1, 4 & 10 rice genotypes with Desmodium (DS) at stage 1 of Pot screening study



**Plates 4.3** Pot screening of NERICA (NRC) 1, 4 & 10 rice genotypes at stage 1 of Pot screening study



**Plate 4.4** screening of NEMCHE and NERICA rice with mucuna (MC) at stage 1 of Pot screening study

Each of the crops was also planted alone as controls in pots of similar dimensions. An initial watering of 80 ml followed by 120 ml of tap water was applied to each pot every two days (Dayan *et al.*, 2009). At stage two, one rice and maize genotype was selected for stage 3, blocked against non-uniform lighting with three replicates.

At stage three, NERICA 1, maize LONGE 6H, mucuna and desmodium were used to demonstrate their allelopathic potential on three receiver common rice weeds namely *Gallinsoga parviflora*, *Bidens pilosa* and *Ageratum conyzoides* (Plate 4.5). Pots measuring 10 x 10 cm top diameter, containing 300 g of sandy-loam soil and arranged in a randomised complete block design, blocked against non-uniform lighting environment and replicated thrice were planted separately with pre-germinated seeds to raise rice (three plants), maize (one plant), mucuna (one plant) and desmodium (five plants) per pot. The pots received 100 ml of tap water initially and were leached with an additional 200 ml of water on day 4, 6, 8 and 10, respectively, and the leachates were collected for subsequent use. Ten milliliter portions of the leachates collected on each of the days were applied to the soil in pots with ten receiver weed seeds under three replicates. Weeds receiving tap water at the same time intervals were used as controls (Dayan *et al.*, 2009).

#### **4.3.1.2 Data collection**

The crop root length, plant height and dry biomass were measured on the 11<sup>th</sup> day after planting on uprooted crop samples at stages 1 and 2. At stage three, leaves were counted, plant height and length of the longest roots on uprooted samples were measured weekly for three consecutive weeks on each of the rice and maize. The crops and weed samples were oven dried at 80 °C for 12 hours till constant weight. The shoot dry biomass was measured using an electronic balance.

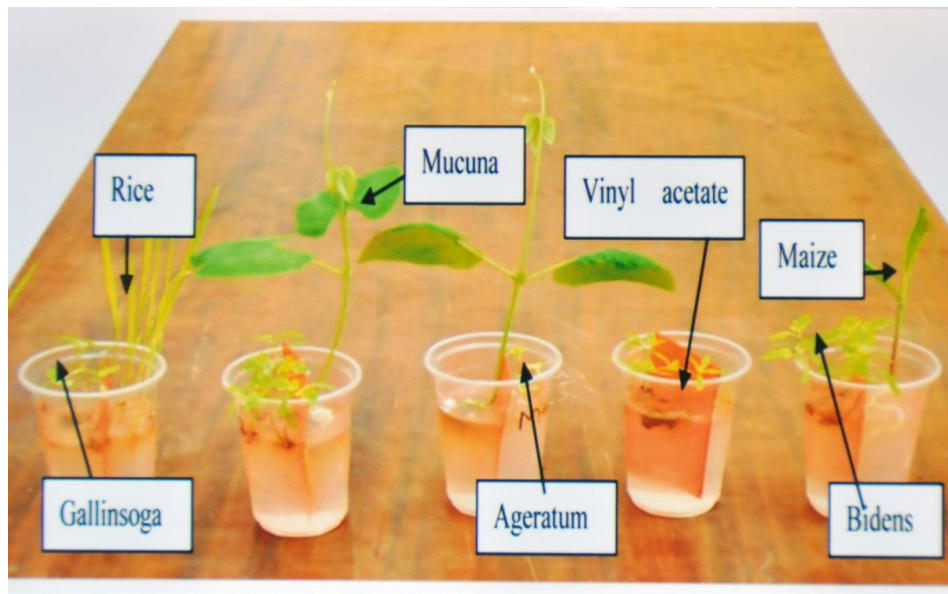
### 4.3.2 Equal compartment agar method

#### 4.3.2.1 Treatments and experimental design

In the equal compartment agar experiment (Hilt *et al.*, 2012) under a completely randomised design, a piece of semi-permeable vinyl acetate fibre was inserted across the centre and down the middle of a glass beaker pre-filled with 0.1% standard nutrient agar solution (Plate 4.6). The fibre divided the beaker into two equal compartments, with the lower edge of the membrane kept 1 cm above the agar surface. The composition for the nutrient solution at full strength in mol m<sup>-3</sup> was NO<sub>3</sub><sup>-</sup>, 4.5; SO<sub>4</sub><sup>2-</sup>, 2.0; [H<sub>2</sub>PO<sub>4</sub><sup>-</sup> + HPO<sub>4</sub><sup>2-</sup>] 0.08; Ca<sup>2+</sup>, 2.0; NH<sub>4</sub><sup>+</sup>, 0.5; Mg<sup>2+</sup>, 0.4; K<sup>+</sup>, 3.3. Five pre-germinated donor seeds of each of the screened allelopathic plants namely rice, desmodium and maize. Besides, three pre-germinated mucuna seeds were uniformly selected and separately sown on the agar surface in one-half of a glass beaker. The beakers were kept under a controlled growth cabinet in the laboratory. After the growth of the seedlings for seven days, ten pre-germinated seeds of each receiver weed species namely *Gallinsoga*, *Bidens* and *Ageratum* were sown in the second half of the agar surface. This allowed movement of molecules between donor and receiver plants, but did not allow root to root contact. The beaker was placed back in the growth cabinet for seven more days when the weeds were harvested.



**Plate 4.5** Leachates from donor maize, rice, mucuna and desmodium produce to receiver weeds at stage 3 of pot screening study.



**Plate 4.6** Crops and weeds on equal compartment agar

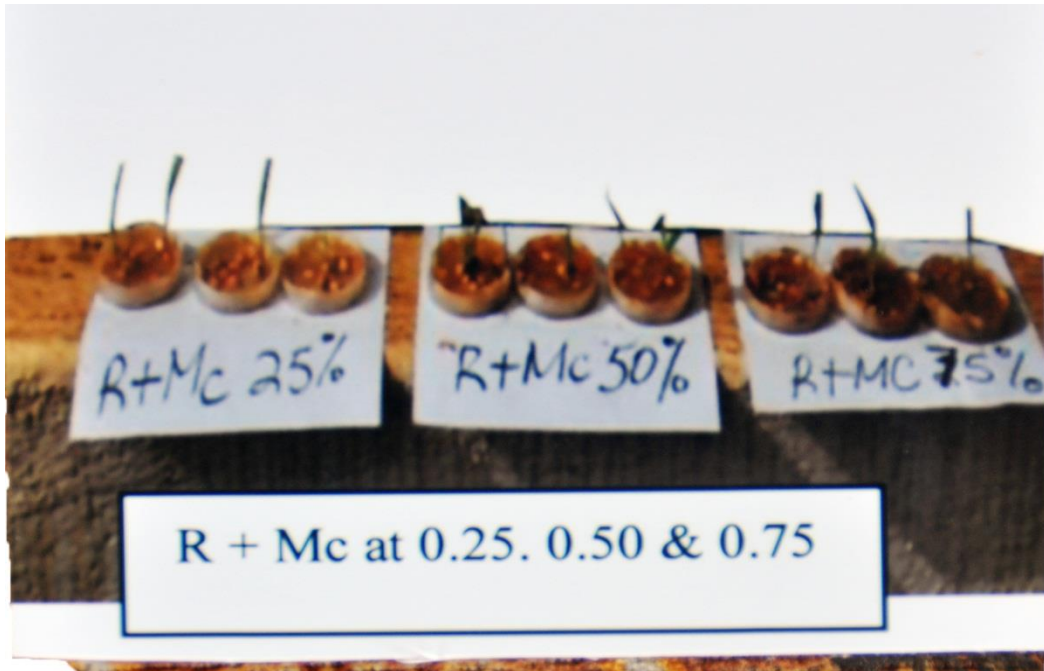
### 4.3.3 Germination test

#### 4.3.3.1 Experimental design and treatments

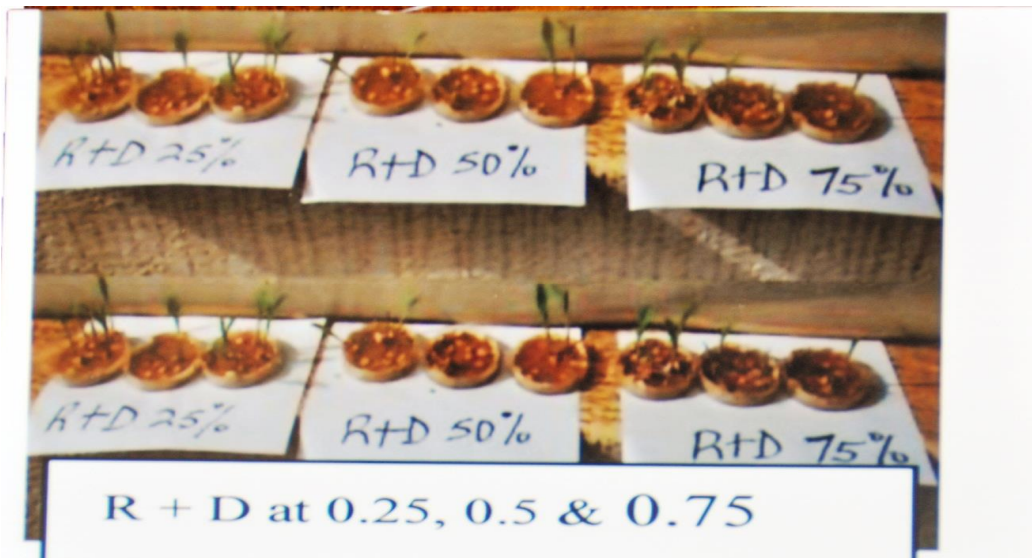
The experiment was conducted at NaCRRRI laboratories in a screen house to determine the allelopathic effects of bioactive compounds on germination of test plants. The stored leachates (100%) from maize, mucuna, rice and desmodium (In pot screening experiment) were constituted to 25, 50 and 75% concentrations and used in this study with a control treatment given tap water (Plates 4.7-4.9). Ten seeds each of test crops namely rice, desmodium, mucuna and maize that had been earlier pre-tested for germination (95-98% germination) were washed with tap water and put in petri-dishes on Whatman No.1 filter paper in four sets replicated three times under laboratory conditions in a completely randomised design. About 10 ml of the different leachate concentrations and tap water were added daily to the sets of petri-dishes for nine consecutive days as described by Hagan *et al.* (2013).



**Plate 4.7 Germination of maize seeds under rice/maize (RMz) mixed root leachate at 0.25, 0.5 and 0.75 concentrations**

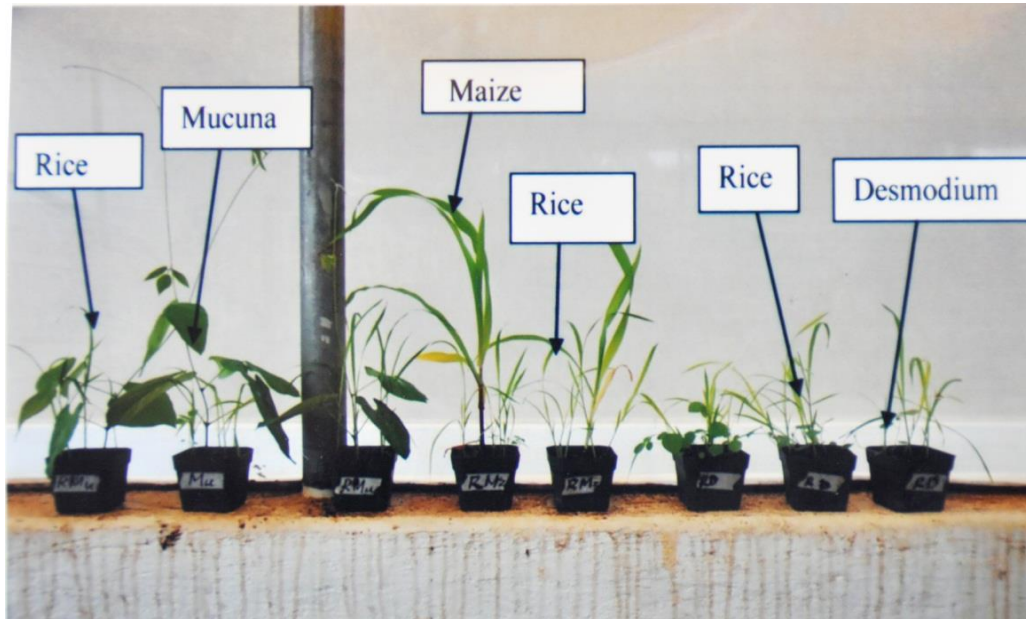


**Plate 4.8 Germination of maize seeds under rice/mucuna (R+Mc) mixed root leachates at 0.25, 0.5 and 0.75 concentrations**



**Plate 4.9 Germination of maize seeds under rice/desmodium (RD) mixed root leachates at 0.25, 0.5 and 0.75 concentration.**





**Plate 4.10 Donor and receiver plants in the same pot.**

#### **4.3.3.2 Data collection**

The radicle and plumule lengths, percentage germination (G%) and mean germination time (MGT), taken as time from imbibition to radicle emergence, were determined for ten successive days. The harvested plant samples were oven dried at 80 °C for 12 hours to constant weight and dry weight was measured on an electronic balance. The seed germination percentage was calculated using the formulae:  $G\% = (a / b) 100$ ; Where  $a$ , was the number of germinated seeds and  $b$  the total number of seeds in the treatment. Mean germination time (MGT) was calculated using the formula:  $MGT = (n \times d)/N$ , where,  $n$  was the number of seeds which germinated after each period in days ( $d$ ) and  $N$  was the total number of seeds that germinated at the end of the experiment (AOSA, 1983). The seed germination index (SGI) was also calculated as described by AOSA (1983) using the formula:  $SGI = (\text{number of germinated seeds}/\text{number of days at first count}) + \dots + (\text{number of germinated seeds}/\text{number of days at last count})$ .

#### **4.3.4 Donor to receiver plants in the same pot test**

##### **4.3.4.1 Experimental design and treatments**

In the donor rice to receiver plants in the same pot, plastic pots measuring 10 cm x 10 cm top diameter containing 300 g of sandy-loam soil were prepared in the screen house under a completely randomised design. Pre-germinated seedlings of each of the receiver plants namely maize (one seedling), mucuna (one seedling) and desmodium (four seedlings) were planted separately with rice (four seedlings) in the same pot as a donor to demonstrate its allelopathic potential on the receiver plants (Plate 4.10). The pre-germinated seedlings were also planted as sole maize (one seedling), mucuna (one seedling), desmodium (four seedlings) and rice (four seedlings) as described by Dayan *et al.* (2009). Each pot received an initial watering of 80 followed by 120 ml of tap water every two days for three consecutive weeks and no fertilizers were applied (Dayan *et al.*, 2009). The crops were uprooted at 21 days after planting.

##### **4.3.4.2 Data collection**

Data was collected on root length, leaf number, leaf length, leaf width, plant height and dry biomass for rice, mucuna, maize and desmodium harvested crop samples.

##### **4.3.4.4 Data analysis**

Collected data were subjected to analysis of variance (ANOVA) using Genstat statistical package (13<sup>th</sup> edition 2013). The significant differences between treatment means were separated using Fischer's least significant difference (LSD) test at  $P \leq 0.05$ .

## 4.4 Results

### 4.4.1 Effects of crop root leachates on growth and development of weeds

Mucuna, desmodium, maize and rice leachates reduced the root length (49-63%), plant height (48-66%) and plant biomass (63-75%) of *Ageratum conyzoides* weeds in the pot screening study (Table 4.1). Desmodium leachate caused the highest percentage reduction in *Bidens pilosa* weed root length (69%), plant height (30%) and total biomass (44%). Mucuna, rice and maize leachates, similarly, significantly ( $P \leq 0.05$ ) reduced *B. pilosa* root length and plant height. Mucuna, desmodium, rice and maize leachates reduced *G. parviflora* weed root length (21-39%) and plant height (20-42%).

**Table 4. 1 Growth of weeds under applied leachates from rice, mucuna, desmodium and maize crops.**

Receiver weeds									
Leachate	<i>Ageratum conyzoides</i>			<i>Bidens pilosa</i>			<i>Gallinsoga parviflora</i>		
	RL (cm)	PH (cm)	Bio (g)	RL (cm)	PH (cm)	Bio (g)	RL (cm)	PH (cm)	Bio (g)
Tap water	3.15a	5.54a	0.08a	4.36a	9.57a	0.27a	3.23a	5.05a	0.12a
Rice	1.38b	1.89d	0.02b	2.33c	7.10c	0.27a	3.28a	3.97b	0.05a
Mucuna	1.61b	2.88b	0.02b	3.69b	7.16c	0.26a	2.60b	2.93d	0.04a
Desmodium	1.51b	3.03b	0.03b	1.35d	6.73d	0.25a	2.05c	3.85c	0.09a
Maize	1.22b	2.50c	0.02b	2.45c	8.28b	0.27a	1.98d	4.03b	0.31a
<i>P</i> -value	<0.005	<0.001	<0.001	<0.001	<0.001	0.31	<0.001	<0.001	0.46
LSD (P=0.05)	0.90	0.22	0.02	0.10	0.12	NS	0.06	0.11	NS
CV (%)	6.3	3.9	3.0	2.0	0.8	7.2	1.2	1.5	4.0

Values with different letters in a column are significantly different at P = 0.05, NS = Not significant. RL = root length, PH = Plant height. Bio = Biomass.

#### 4.4.2 Donor plants and weeds on equal compartment agar

Maize leachates reduced *Gallinsoga* root length by 39 percent but the rice leachate had no effect on its root length. Tap water produced the tallest *Gallinsoga* weed. Maize root exudates reduced the *G. parviflora* weed root length and stem height by 41 and 20%, respectively, under the equal compartment agar study (Table 4.2). Rice exudates reduced *Gallinsoga* weed root and stem lengths by 28%. Mucuna exudates reduced *Gallinsoga* root length and stem height by 32 and 31% respectively relative to the control. *Gallinsoga* weeds did not influence the growth parameters of maize, rice and mucuna.

**Table 4.2 Growth of *Gallinsoga parviflora*, *Mucuna pruriens* *Zea mays* and *Oryza sativa* on equal compartment agar**

<b><i>Gallinsoga parviflora</i></b>			
<b>Plant + weed on Agar</b>	<b>Root length (cm)</b>	<b>Plant height (cm)</b>	<b>Biomass (g)</b>
Control (Sole gallinsoga)	3.00a	2.80a	0.02a
Maize/Gallinsoga	1.80c	2.30b	0.02a
Rice/Gallinsoga	2.20b	2.00c	0.02a
Mucuna/Gallinsoga	2.00b	2.00d	0.01a
P-value	<0.001	<0.001	0.06
LSD (PÖ0.05)	0.24	0.05	NS
CV (%)	2.80	3.00	3.80
<b><i>Mucuna pruriens</i></b>			
Control (sole mucuna)	28.00a	5.50a	4.60a
Mucuna/Gallinsoga	28.90a	5.30a	4.60a
P-value	0.08	0.18	0.12
LSD (PÖ0.05)	NS	NS	NS
CV (%)	3.30	2.60	1.70
<b>Maize (LONGE 6H)</b>			
Control (Sole maize)	7.20a	9.10a	1.80a
Maize/Gallinsoga	6.90a	9.20a	1.80a
P-value	0.76	0.30	0.39
LSD (PÖ0.05)	NS	NS	NS
CV (%)	17.80	2.10	7.0
<b>Rice (NERICA 1)</b>			
Control (sole rice)	5.50a	4.60a	0.10a
Rice/Gallinsoga	5.50a	4.80a	0.20a
P-value	0.96	0.07	0.06
LSD (PÖ0.05)	NS	NS	NS
CV (%)	4.70	3.90	3.20

Values with different letters in a column are significantly different at  $P \leq 0.05$ , NS = Not significant.

#### 4.4.3 Effects of concentration and type of leachate on seed germination

The percent seed germinations for *M. pruriens*, *D. uncinatum*, rice (NERICA 1) and LONGE 6H maize seeds under screen house conditions were not influenced by concentrations of leachates (Table 4.3).

**Table 4.3 Percent seed germination for mucuna, desmodium, rice and maize as influenced by leachate concentrations**

Percent seed germination												
Leachate type	Rice/Desmodium				Rice/Mucuna				Rice/Maize			
Concentration	Mc	Des	Rice	Mz	Mc	Des	Rice	Mz	Mc	Des	Rice	Mz
75%	80	100	50	100	100	100	10	90	90	90	20	100
50%	100	80	60	90	90	90	70	90	70	100	60	100
25%	70	90	80	100	100	100	60	100	100	90	70	100
0% (Control)	100	100	50	100	100	100	50	100	10	100	50	100
P-value	0.16	0.24	0.67	0.14	0.99	0.29	0.02	0.21	0.24	0.16	0.34	0.15
LSD ( $P \leq 0.05$ )	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	9	7.8	15	25	23	31	23	19	23	17	21	22

Values with different letters in a column are significantly different at  $P \leq 0.05$ . Mc = Mucuna, Des = Desmodium, Mz = Maize.

##### 4.4.3.1 Effects of types of leachates on mean germination time

The MGT for mucuna, desmodium, rice and maize seeds significantly ( $P \leq 0.05$ ) increased by 0.4-2.1 days and 0.4-2.3 days when rice/desmodium and rice/mucuna leachate concentrations increased from 25% to 75% respectively relative to the control (Table 4.4). The MGT for desmodium, rice and maize seeds, similarly, increased by 0.6-2.8 days when the rice/maize leachate concentration increased from 25% to 75%.

**Table 4.4 Mean germination time for mucuna, desmodium, rice and maize as influenced by leachate concentrations.**

<b>Mixed Leachates</b>												
	<b>Rice/Desmodium</b>				<b>Rice/Mucuna</b>				<b>Rice/Maize</b>			
	<b>Mean germination time (days)</b>											
<b>Leachate Concentration</b>	<b>Mc</b>	<b>Des</b>	<b>Rice</b>	<b>Mz</b>	<b>Mc</b>	<b>Des</b>	<b>Rice</b>	<b>Mz</b>	<b>Mc</b>	<b>Des</b>	<b>Rice</b>	<b>Mz</b>
75%	4.90a	7.60a	6.80a	7.70a	5.90a	8.00a	6.90a	7.90a	4.90a	7.80a	7.70a	6.90a
50%	4.80b	7.10b	4.90b	7.60b	5.00b	7.90a	4.70b	7.50b	4.90a	7.50b	7.60b	4.90b
25%	4.60c	7.30b	4.90b	7.20c	4.90c	7.40b	4.90b	7.10c	4.80a	7.30b	7.20c	4.70c
0% (Control)	4.50d	6.70c	4.70c	7.10d	4.90c	7.60b	4.60b	5.80d	4.70b	7.00c	7.10d	4.10d
P-value	<0.001	0.012	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (P≤0.05)	0.04	0.49	0.08	0.05	0.05	0.37	0.49	0.04	0.03	0.27	0.05	0.15
CV (%)	0.5	0.4	0.3	0.3	4.0	5.0	4.9	5.2	22.4	30.8	23.5	16.8

Values with different letters in a column are significantly different at  $P \leq 0.05$ . Mc = Mucuna, Des = Desmodium, Mz = Maize

#### **4.4.3.2 Effects of concentration of leachates on seed germination indices**

The mucuna, desmodium and rice seed germination indices (SGI) significantly ( $P \leq 0.05$ ) reduced (15-30%, 24-28% & 3-26%), with increased (25-75%) concentrations of rice/desmodium, rice/mucuna and rice/maize leachates. In contrast, increased concentrations (25-75%) of rice/desmodium and rice/maize leachates significantly ( $P \leq 0.05$ ) increased the SGI for maize seeds by 4 and 18 percent respectively (Table 4.5). In contrast, maize seeds given tap water as controls for rice/desmodium and rice/maize treatments produced the lowest SGI (3.6).

**Table 4.3 Seed germination indices for mucuna, desmodium, rice and maize under mixed leachates**

Leachate Concentration	Mixed leachates											
	Rice/Desmodium				Rice/Mucuna				Rice/Maize			
	Mc	Des	Rice	Mz	Mc	Des	Rice	Mz	Mc	Des	Rice	Mz
75%	5.00d	6.60c	5.70d	4.50a	7.60b	5.70d	5.70b	2.50c	6.80d	3.20c	5.10c	7.90a
50%	6.10c	7.00b	7.50c	4.50a	7.60b	7.00c	7.90a	4.30b	7.10c	4.40b	5.20c	7.80b
25%	7.20b	7.00b	7.60b	4.30b	7.00b	7.50a	7.90a	4.40a	7.30b	4.30b	5.30b	6.70c
0% (control)	7.70a	7.60a	7.70a	3.60c	7.70a	7.30b	7.90a	4.40a	7.70a	6.30a	7.70a	3.60d
P-value	<0.001	0.001	<0.001	<0.01	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD ( $P \leq 0.05$ )	0.64	0.05	0.05	0.03	0.05	0.07	0.05	0.04	0.06	0.1	0.11	0.06
CV (%)	0.5	0.5	0.3	0.5	0.4	0.4	0.5	0.5	0.4	0.6	0.9	0.5

Values with different letters in a column are significantly different at  $P \leq 0.05$ , Mc = Mucuna, Des = Desmodium, Mz = Maize

#### 4.4.3.3 Effects of leachate concentrations on maize growth and biomass

Increases in rice/mucuna and rice/desmodium leachate concentrations (25-75%) had no effect on maize root length and dry biomass (Table 4.6). Increasing the rice + maize leachate concentration from 25 to 75% significantly ( $P \leq 0.05$ ) increased the maize root length by 36% and the plant biomass by 33%. Root lengths and biomass of maize plants subjected to 25% rice + maize leachate concentration and tap water were similar. Higher maize biomass of 3.60 g was recorded at 75% rice/maize leachate concentration. Maize plant height was not influenced by the types of leachates and changes in their concentrations.



**Table 4.4 Growth of maize as influenced by concentrations of rice, mucuna, desmodium and maize mixed leachates**

<b>Leachate &amp; concentration</b>	<b>Root length (cm)</b>	<b>Plant height (cm)</b>	<b>Biomass (g)</b>
Rice/Mucuna (75%)	6.30b	3.80a	1.10c
Rice/Mucuna (50%)	7.20b	3.80a	1.00c
Rice/Mucuna (25%)	8.20b	3.50a	1.10c
Rice/Desmodium (75%)	6.20b	3.70a	1.00c
Rice/Desmodium (50%)	6.10b	3.30a	1.20c
Rice/Desmodium (25%)	8.80b	4.40a	1.30c
Rice/Maize (75%)	13.50a	3.40a	3.60a
Rice/Maize (50%)	10.90a	3.70a	2.60b
Rice/Maize (25%)	8.60b	4.20a	2.40b
Tap water (0%)	8.70b	4.80a	2.40b
P-value	0.004	0.59	<0.001
LSD (P<0.05)	3.35	NS	0.67
CV (%)	23.3	22.8	27.6

Values with different letters in a column are significantly different at  $P \leq 0.05$ ; NS = not significant

#### **4.4.3.4 Effects of potting rice, maize, mucuna and desmodium on crop growth**

Root length for rice reduced by 30 to 46% due to potting with mucuna, maize and desmodium and rice plant height reduced by 32% when potted with desmodium (Table 4.7).

Potting maize with mucuna and desmodium increased maize leaf length by 15 to 24%.

Mucuna leaf width reduced by 32% when potted with maize and potting desmodium with maize and mucuna reduced the desmodium plant height, root length, leaf number, leaf length and leaf width by 49 to 64%.

**Table 4.5 Growth of rice, maize, desmodium and mucuna as donor-receiver plants in the same pot**

<b>Rice (NERICA 1)</b>	<b>RL (cm)</b>	<b>LN (leaves)</b>	<b>LL (cm)</b>	<b>LW (cm)</b>	<b>PH (cm)</b>	<b>Biomass (g)</b>
Control	9.00a	5.70a	27.00a	0.60a	17.70a	0.20a
Rice/Desmodium	4.80c	4.30a	28.00a	0.60a	12.00b	0.10a
Rice/Maize	5.00b	5.50a	31.50a	0.50a	17.30a	0.20a
Rice/Mucuna	6.30b	4.30a	38.30a	0.60a	22.00a	0.30a
P-value	<0.001	0.45	0.56	0.46	0.004	0.68
LSD (P ≤ 0.005)	2.00	NS	NS	NS	6.56	NS
CV (%)	8.6	5.6	6.5	7.8	6.0	6.7

<b>Maize (LONGE 6H)</b>	<b>RL (cm)</b>	<b>LN (leaves)</b>	<b>LL (cm)</b>	<b>LW (cm)</b>	<b>PH (cm)</b>	<b>Biomass (g)</b>
Control	13.00a	8.67a	64.30b	3.40a	40.70a	4.10a
Rice/Maize	14.67a	8.00a	71.00b	3.30a	46.00a	5.00a
Mucuna/Maize	14.33a	8.67a	84.30a	4.00a	53.70a	5.00a
Desmodium/Maize	14.33a	7.67a	75.70a	3.60a	46.30a	6.00a
P -value	0.63	0.56	0.004	0.45	0.74	0.52
LSD (P ≤ 0.005)	NS	NS	11.97	NS	NS	NS
CV (%)	23.1	15.3	4.5	13.4	16.3	18.2

<b><i>M. Pruriens</i></b>	<b>RL (cm)</b>	<b>LN (leaves)</b>	<b>LL (cm)</b>	<b>LW (cm)</b>	<b>PH (cm)</b>	<b>Biomass (g)</b>
Control	22.50a	22.50a	15.50a	8.80a	230.00a	5.60a
Rice/Mucuna	18.70a	15.70a	14.30a	8.10a	222.00a	5.20a
Maize/Mucuna	13.30a	17.30a	14.00b	6.00b	226.00a	2.80a
Mucuna/Desmodium	22.00a	18.30a	15.70a	9.20a	253.00a	3.30a
P-value	0.06	0.43	0.72	0.002	0.67	0.56
LSD (P ≤ 0.005)	NS	NS	NS	2.23	NS	NS
CV (%)	3.3	18.2	4.5	12.2	12.6	15.3

<b><i>D. Uncinatum</i></b>	<b>RL(cm)</b>	<b>LN (leaves)</b>	<b>LL(cm)</b>	<b>LW(cm)</b>	<b>PH(cm)</b>	<b>Biomass(g)</b>
Control	3.20a	5.30a	2.80a	2.80a	12.70a	0.02a
Rice/Desmodium	2.70a	4.70a	3.20a	3.10a	13.70a	0.03a
Maize/Desmodium	1.70b	2.70b	1.00b	1.00b	6.30b	0.01a
Mucuna/Desmodium	1.30b	3.30b	1.60b	1.50b	8.30b	0.01a
P-value	0.002	0.002	0.004	0.004	0.003	0.46
LSD (P≤0.05)	1.40	1.76	1.13	1.20	4.48	NS
CV (%)	5.4	5.6	12.1	6.2	5.3	5.6

Values with different letters in a column are different at  $P \leq 0.05$ , RL = Root length, LN = Leaf number, LW = leaf width, PH = Plant height

## 4.5 Discussion

### 4.5.1 Effects of leachates on weed growth and development

Leachates of mucuna, desmodium, maize and rice under the pot screening study and exudates of mucuna, maize and rice under equal compartment agar reduced weed growth and development. Rice, mucuna and desmodium growth parameters reduced when potted as two component crops. The reduced weed and crop growth may be attributed to allelopathic inhibitory influence of putative bio-compounds. Hooper *et al.* (2015), reported root exudates of *D. uncinatum* to inhibit weed growth. Kato-Noguchi (2011), reported that rice allelopathic activity increased in the presence of barnyard grass seedlings and their root leachates. Leachates from weeds possibly stimulated the release of allelochemicals from the weeds to the crops. In chapter 3 of this thesis, three terpenoids, an alkane and a furan were observed in desmodium plants root exudates. Rice crop produced eight terpenoids, two alkanes and a furan while mucuna crop exudated five terpenoids, one phenol and an alkane and six terpenoids were released via maize root exudates. Namkeleja *et al.* (2013) reported that leaf and seed extracts reduced the seed germination, root and shoot length, fresh weight and dry weight of weed species. Allelopathic interactions have been reported to affect physiological crop processes such as nutrient uptake and root apical tissue development that support plant growth. Silva and Rezende (2016), reported that application of phenolics obtained from jack bean (*Canavalia ensiformis*) controlled *E. sonchifolia* and *S. spinosa* weeds within 15 and 30 days after application respectively. Burgos *et al.* (2004) and Nishida *et al.* (2005), reported allelochemicals to significantly inhibit the regeneration of root cap cells, cell proliferation and DNA synthesis in plant meristems and inhibiting growth. Results are also supported by Zhao-Hui (2010) and Cheng and Cheng (2015), who observed reductions in absorption and transport of ions, cell division, change of cell ultrastructure and interference with the normal

growth and development of plants by phenolic acids. Mohammadali and Ayoub (2015), indicated highest inhibitory effects on chlorophyll, leaf area, root growth and nitrogen content by rice root exudates.

Crop root leachates controlled weed parts with different efficacy and the roots were more susceptible than the shoots. The weed control may be attributed to the modes of action, concentration and efficacy of the different bioactive compounds identified in root exudates of desmodium, rice, mucuna and maize. Esfandiar *et al.* (2012) and Ali *et al.* (2015), observed higher effects of increasing extract concentrations on germination percentage of crops with the roots of crop species being more affected than the shoots. The research findings from this study suggest high possibilities of developing bioherbicides from the bio-active compounds in mucuna, desmodium, maize and rice. Datta and Saxena (2001), reported that allelopathic crop species are a potential resource with phytotoxic properties against a broad range of weeds.

#### **4.5.2 Effects of leachate types and concentration on germination and growth of crops**

Mean germination time (MGT) for mucuna, desmodium, rice and maize seeds significantly increased with increases in rice/desmodium and rice/ mucuna leachate concentrations relative to the control. Mean germination time for desmodium, rice and maize seeds, similarly, increased at 75 percent rice/maize leachate concentration. Higher concentrations of rice /desmodium, rice/mucuna and rice/maize leachates reduced the seed germination indices for mucuna, desmodium and rice. Relative inhibitory effects on weed growth by leachates were higher under rice/mucuna mixed leachates than under rice/desmodium leachate and lowest under rice/maize mixed leachate. Delayed seed germination with increases in leachate

concentrations from different mixtures may be attributed to interactive effects of molecules in the identified compounds on processes that affect seed germination such as seed respiration. In chapter 3 of this thesis, rice and desmodium exudated in common; two terpenoids, one alkane and a furan and this possibly gave higher additive and synergistic effects on the molecules in form of inhibitory effects on seed germination. Rice/ mucuna and rice/ maize crop leachates had only one common terpenoid (1,3-Di-tert-butylbenzene) and one 2-n Pentylfuran amongst the 12, 7 and 7 compounds released in the root exudates of rice, mucuna and maize respectively. Thus, the lower efficacy by mixed leachates of rice, mucuna and maize may be attributed to antagonistic molecular effects. Synergy by molecules with similar targets was reported by Blouin *et al.* (2004), Zhao-Hui *et al.* (2010) and Farooq *et al.* (2011).

#### **4.5.3 Effects of leachate concentrations and potting on growth of crops**

Rice crop root length reduced when rice was potted with mucuna, maize and desmodium. Rice plant height reduced when potted with desmodium. However, potting maize with mucuna and desmodium increased maize leaf length. Mucuna leaf width reduced when potted with maize while potting desmodium with maize and mucuna reduced the desmodium plant height, root length, leaf number, leaf length and leaf width. The inhibitory and enhanced crop growth effects may be attributed to possible inhibition or support of physiological processes by some of the compounds identified in the root exudates under chapter 3 of this thesis. Nishihara *et al.* (2005) and Feng *et al.* (2010), reported a correlation between radicle growth inhibition, seedling development and the concentration of root diffused allelochemicals. Ali *et al.*, (2015), observed decreased germination percentage of barley and wheat with increasing extract concentrations and roots of both species were more affected than the shoots.

Increased rice/ maize leachate concentrations and potting maize with mucuna and desmodium increased the maize seed germination indices, growth and development. This may be attributed to hormesis common with allelochemicals. Hormesis is the stimulation of growth at sub optimal levels of allelochemicals common in auxin herbicides which mimic the growth hormone auxin but which are lethal at higher doses (Farooq *et al.*, (2011). Application of allelopathic water extracts were reported to boost crop growth by Iqbal (2014) and Abbas *et al.*, (2017). Glyphosate has been reported to cause 30 percent increased growth in both soybean and maize by Velini *et al.* (2008). Hormesis was also reported by Cedergreen and Olen (2010) when glyphosate promoted crop growth. Kaya *et al.* (2009) attributed hormesis to enhancement of physiological processes such as photosynthesis, respiration, increased gaseous exchange and stabilization of the plants photosynthetic pigment system. Belz and Duke (2014), remarked that the studies with herbicides are not done in sufficient detail to measure hormesis. Therefore, in many experiments hormesis has not been reported. Delayed seed germination due to concentrations of the bio-active compounds as observed causes late crop establishment and reduces the competitive advantage of crops resulting into formation of vegetation age structure and low productivity levels. Herbicide hormesis lacks wide practical application and its complete understanding is needed to exploit potential benefits and to minimize potential harmful effects in crop production.

Root leachates from *M. pruriens*, *D. uncinatum*, *Z. mays* and *O. sativa* reduced the growth of *A. conyzoides*, *G. parviflora* and *B. pilosa* weeds in the same pot. Increased concentrations of rice, desmodium and mucuna mixed leachates increased the mean germination time but reduced the seed germination indices (SGI) for mucuna, desmodium and rice seeds. The maize dry biomass and the SGI, conversely, increased at higher rice/desmodium and rice/maize leachate concentrations. Potting rice with mucuna, maize and desmodium reduced

rice root length while the width of mucuna leaves reduced when potted with maize. Potting desmodium with maize and mucuna reduced desmodium growth parameters. The maize growth improved with increases in rice/mucuna leachate concentrations and maize leaf length increased when potted with mucuna and desmodium due to hormesis. The observed growth changes were attributed to influences of allelochemicals in maize, rice, mucuna and desmodium on weeds and give potential for the development of bio-herbicides and strategic management of the study crops.

## CHAPTER 5

### PRODUCTIVITY OF RICE INTERCROPPED WITH CYMBOPOGON, DESMODIUM, MUCUNA AND MAIZE

#### 5.1 Abstract

Allelochemicals influence growth and development of component crops under intercropping systems and subsequent crops. A screen house experiment was conducted at the Uganda National Crops Resources Research Institute, Namulonge, during 2013B. Two field experiments were conducted at Ikulwe research station and on-farm in Pallisa district during 2014A under a randomised complete block design to determine the effects of rice based intercropping systems on *Striga hermonthica* infestation, nutrient uptake and grain yield. Results of a screen house study showed that rice potted with cymbopogon or mucuna, increased in plant height by 79%. However, rice plants potted with cymbopogon, mucuna, desmodium and maize reduced the rice root length by 30-47%. Relative to sole rice, high N, P and K nutrient uptake of 43-72% was recorded for rice potted with cymbopogon. Increased N and K nutrient uptakes were recorded by rice potted with mucuna (70 & 871%) and desmodium (66 & 75%) and the lowest uptake was under rice potted with maize crop. Potting maize crop with mucuna, cymbopogon and desmodium, increased the maize leaf length by 17 to 31%. Mucuna, desmodium and cymbopogon plants enhanced the nutrient uptake and relative growth rates (RGR) of rice but maize reduced the parameters. Increased RGR for maize was recorded when the crop was potted with desmodium and cymbopogon. The lowest nutrient uptake and RGRs for maize were with mucuna and rice component crops. Striga weed counts per hundred rice plants reduced (36-64 weeds) when intercropped with mucuna, desmodium and cymbopogon while rice intercropped with maize produced higher striga (110 weeds) which reduced the rice tillers by 65%. Rice grain yield was significantly higher under



sole rice both on station (1,111kg ha<sup>-1</sup>) and on-farm (631kg ha<sup>-1</sup>). Cymbopogon enhanced rice grain yield (1103 kg ha<sup>-1</sup>) to similar level with sole rice on station, but, a lower yield of 510 kg ha<sup>-1</sup> was observed under intercropping with cymbopogon on-farm. Partial Land Equivalent Ratios (LER) of rice intercropped with cymbopogon, desmodium, maize and mucuna reduced (0.66-0.99) and the partial LER for the intercrops, similarly, reduced (0.33-0.85) due to intercropping. Combined LER were highest under rice + desmodium and rice + cymbopogon (1.68 & 1.56) intercropping systems. Combined LER values for rice intercropped with maize or mucuna were lowest and similar to sole rice (1.00). Mucuna had the highest competitive ability followed by cymbopogon and maize and desmodium recorded the lowest competitive index.

**Keywords:** competitive index, cymbopogon, desmodium, land equivalent ratio, nutrient uptake, relative growth rates, rice, striga.

## 5.2 Introduction

Intercropping is a system of growing two or more crops, simultaneously, on the same land with special spatial arrangement. The advantages that accrue from the system were critically analysed by Willey (1979a). Upland rice growing became popular in the early 21<sup>st</sup> century with the introduction of the New Rice for Africa (NERICA) varieties. Rice plays an important role as both a food and cash crop in Uganda. The crop was ranked fourth among the cereal crops after maize, finger millet and sorghum and occupied a total of 138,000 hectares of land with an estimated output of 181,000 tons (Statistics, U.B.O.S., 2010). The Uganda statistical abstracts report (2017), indicated that 218,000 metric tons (Mt) were harvested in the country from 75,086 hectares in 2010, compared to 183,000 Mt in 2008. As much as production increased, the productivity has kept on declining with the average yield

being 2.9 Mt per hectare in 2010. This farm level productivity is below the attainable potential and was attributed to low applications of modern technologies (UNDP 2010).

The availability and adoption of appropriate cost-effective technologies by farmers is one strategy to overcome the limitations to higher on farm productivity. One such technology is the adoption of intercropping systems. Intercropping systems have been reported to smother weeds, improve soil fertility and nutrient uptake by component crops, reduce pests and diseases, increase the interception of solar radiation, uptake of soil water and nutrients by the base and intercrops at different strata above and below the ground surface and subsequently increase the productivity of the cropping systems.

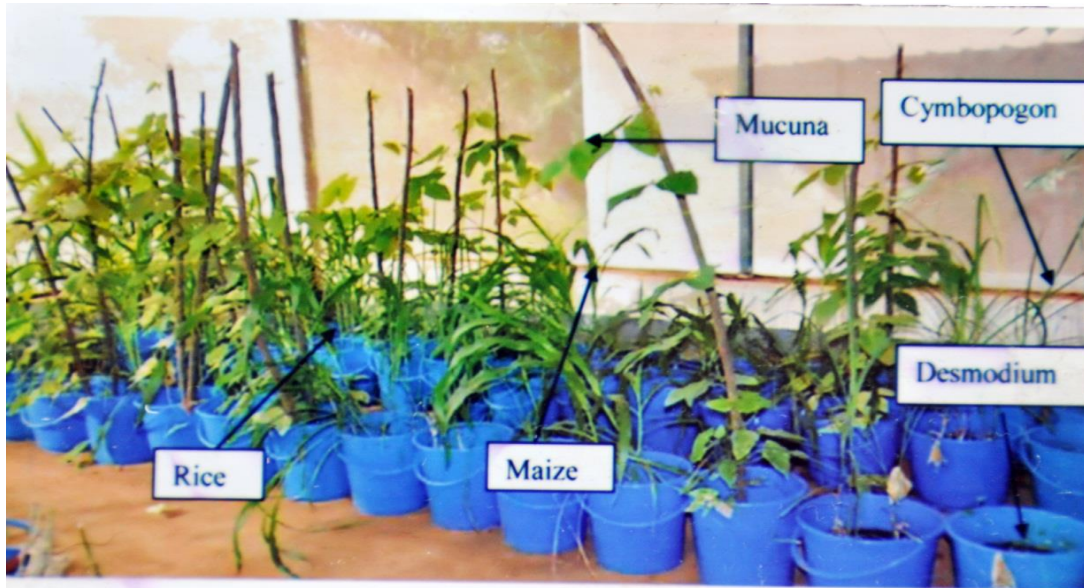
Rice and some of the common intercrops have been reported to exhibit allelopathic properties due to allelochemicals. Allelochemicals influence physiological processes in crops, increase cell membrane permeability, thereby, affecting crop growth rates and may cause death of plant tissue. In addition, allelochemicals can inhibit nutrient absorption and affect normal growth of plants. Tomita *et al.* (2003), reported that aqueous methanol extracts of rice plants inhibited the germination and growth of lettuce seedlings, *Phalaris minor* and *Echinochloa Crus-galli* weeds. Geng *et al.* (2009), reported that a low concentration of dibutyl phthalate increased the absorption of N but decreased P and K uptake. Cheng and Cheng (2015), reported that allelochemicals can inhibit the activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase involved in the absorption and transport of ions at the cell plasma membrane, which suppresses the cellular absorption of K<sup>+</sup>, Na<sup>+</sup>, or other ions. Research on the effects of allelopathy on crop growth, nutrient uptakes and production potential of emerging rice based intercropping systems is limited. The objective of the study was therefore to determine the production potential of rice based intercropping systems with reference to *Striga hermonthica*, nutrient uptake and yield.

## **5.3 Materials and methods**

### **5.3.1 Screen house experiment**

#### **5.3.1.1 Experimental design**

A screen house experiment was conducted at the Uganda National Crops Resources Research Institute, Namulonge, in 2014A. Two different crop species of *Zea mays* (LONGE 6H), *Mucuna pruriens*, *Oryza sativa* (NERICA 1), *Cymbopogon nardus* and *Desmodium uncinatum* were planted in the same pot and as sole crops in a randomised complete block design with five replications. Two of the replicates were used for data collection on destructive samples. Seventy five pots, each measuring 40 cm x 30 cm x 20 cm with a hole at the bottom, were filled with 6 kg of air-dried loam-clay forest soil excavated from 10 cm top depth of regenerated vegetation. Crops were planted in the pots as rice/ maize, maize/ desmodium, cymbopogon/ desmodium, rice/ desmodium, cymbopogon/ mucuna, maize/ mucuna, rice/ mucuna, desmodium/ mucuna, maize/ cymbopogon, rice/ cymbopogon and as sole crops of rice, maize, mucuna, cymbopogon and desmodium. Seven rice, desmodium, mucuna and maize seeds were sown per pot and later thinned at seven days after emergence (DAE) to four plants (rice), desmodium (four plants), mucuna (one plant) and maize (two plants). Two homogenous suckers of cymbopogon trimmed to 10 cm height were also established in similar pots (Plate 5.1). The crops were not supplied with fertilizer but watered with 150 ml of tap water every two days. Hand picking of weeds was done once at 21 days after emergence during the eight week long study and no pests or disease incidences were observed during the experimental period.



**Plate 5.1 Cymbopogon, desmodium, rice, mucuna and maize at 35 days after emergence planted as sole and two companion crops in one pot.**

### **5.3.1.2 Data collection**

Ten days after emergence of rice, one representative mucuna plant and two of each of the crops namely maize, desmodium and cymbopogon were selected per pot and tagged for biometric observations. The plant heights were measured from the top soil level in the pot to the base of the last fully opened leaf. The longest and widest parts of leaves for the tagged plants were measured using a measuring tape and the leaves with more than 75 percent green areas were counted every fortnight up to 56 DAE. During the same period, the root length was measured on harvested samples and dry matter determined on an electronic balance after oven drying the samples at 80° C for 24 hours till constant weight. The relative growth rates (RGR) for the crops were calculated using the formula:  $RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1) =$  Where,  $\ln W_1$  and  $\ln W_2$  are the natural logarithm for transformed plant weight  $W_1$  and  $W_2$  at time  $T_1$  and  $T_2$  respectively. Air-dried soil samples collected at planting and harvesting besides the crop stover and roots at harvest were ground and passed through a 2 mm sieve.

The samples were then subjected to chemical analysis for N, P and K nutrient uptake and nutrient reserves using standard methods described by Okalebo *et al.*, (2002).

### **5.3.1.3 Data analysis**

All data collected were subjected to analysis of variance using 13<sup>th</sup> edition of Genstat software. Fischer's lowest significant difference (LSD) test at  $P \leq 0.05$  was used to separate treatments.

## **5.3.2 Field experiment**

### **5.3.2.1 Site description**

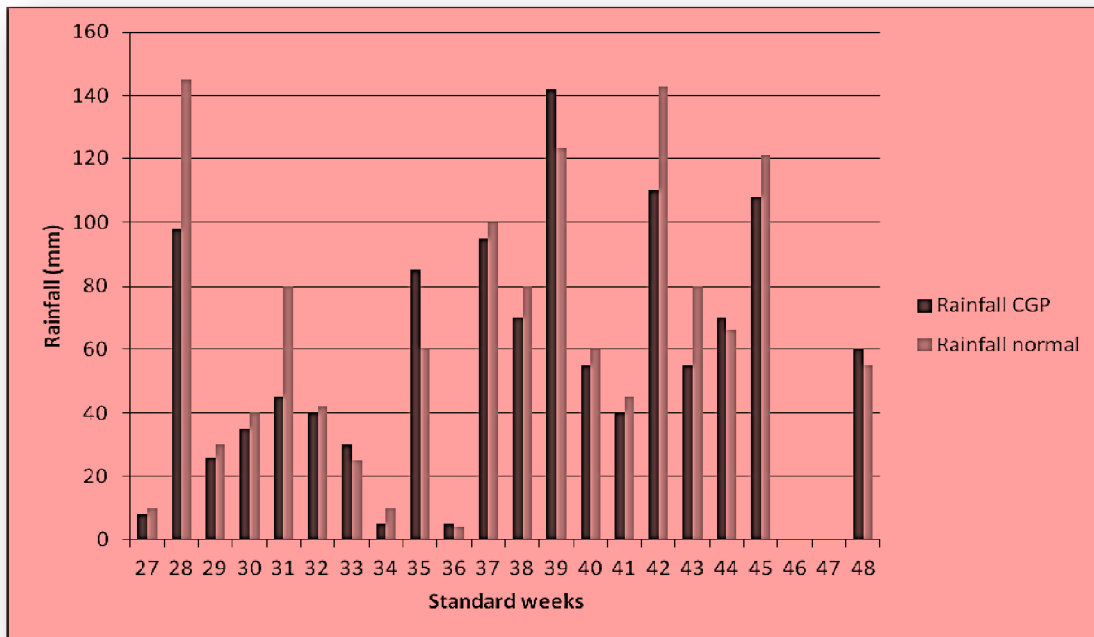
The field study was conducted at Ikulwe research station and on-farm at Pallisa during 2014B (Figure 5.1). Ikulwe is located at  $00^{\circ} 26' 23.2''$ N  $033^{\circ} 28' 40.9''$ E, at 1209 meters above sea level. The area received a total of 543 mm of the 1230 mm annual rainfall during the cropping season (Figure 5.2) with minimum and maximum temperatures of 18 and 30 °C respectively. Pallisa is located at  $1^{\circ} 13' 33.2''$ N and  $33^{\circ} 46' 47.2''$ E. The total precipitation received was 450 mm against the annual rainfall of 990 mm and the temperatures recorded as minimum and maximum were 19 °C and 31°C, respectively. Both experimental sites had sandy loam soils.



**Figure 5.1 Map of Uganda showing the field study sites of Ikulwe station and Pallisa farm.**

### 5.3.2.2 Experimental design and treatments

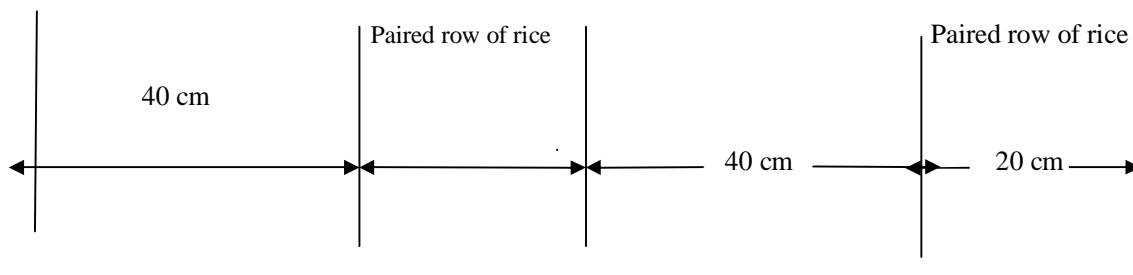
Maize, mucuna, desmodium and cymbopogon were tested as sole and intercrops in rice under a randomized complete block design with three replicates. The experimental units measured 5m x 8 m with 2 m between the plots.



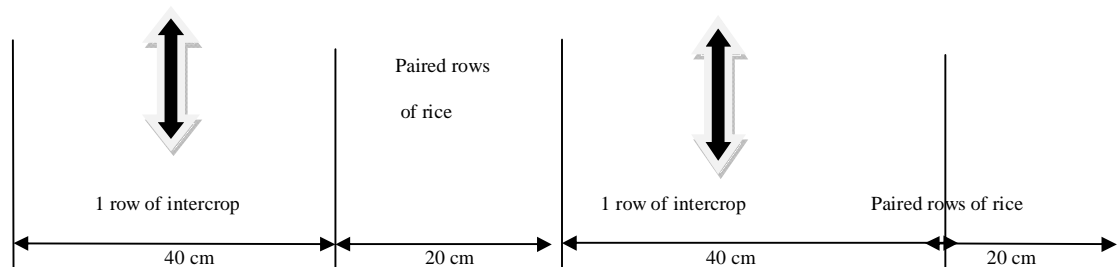
**Figure 5. 2 Rainfall recorded on station at Ikulwe during the crop growing period (CGP) and normal trend during the calendar year (2014B) weeks**

The crops were planted as sole upland rice in paired rows (Figure 5.3) at 40/20 cm x 12.5 cm (one plant/hill) and as rice intercropped with one row (Figure 5.4) of each crop (maize, mucuna or desmodium) thinned to one plant/hill (Plates 5.2-5.4). The within row spacing of the intercrops; desmodium, mucuna and maize were 10 cm, 120 cm and 30 cm respectively. Four uniformly cut 10 cm long cymbopogon suckers were planted per hill as intercrops at a distance of 45 cm within the row. Sole crops of cymbopogon (60 cm x 45 cm), desmodium (45 cm x 10 cm), mucuna (60m c x 120 cm), rice (40/20 cm x 12.5 cm) and maize (75 cm x 30 cm) were sown as in intercropping systems on the same date.

Nitrogen, phosphorus and potassium (60:30:30 kg ha<sup>-1</sup>) fertilisers were applied to rice and none to the associated intercrops. All P and K fertilisers were applied by band placement at planting and N was applied in two equal splits at planting (30 kg ha<sup>-1</sup>) and panicle initiation (30 kg ha<sup>-1</sup>) stages. The sources of N, P and K were urea (46% N), single super phosphate (16% P<sub>2</sub>O<sub>5</sub>) and murriate of potash (60% K<sub>2</sub>O), respectively. Hand hoeing was done uniformly at 21 and 42 days after emergence in all the plots. There were no pests and disease incidences, thus, no control measures were administered in all treatments.

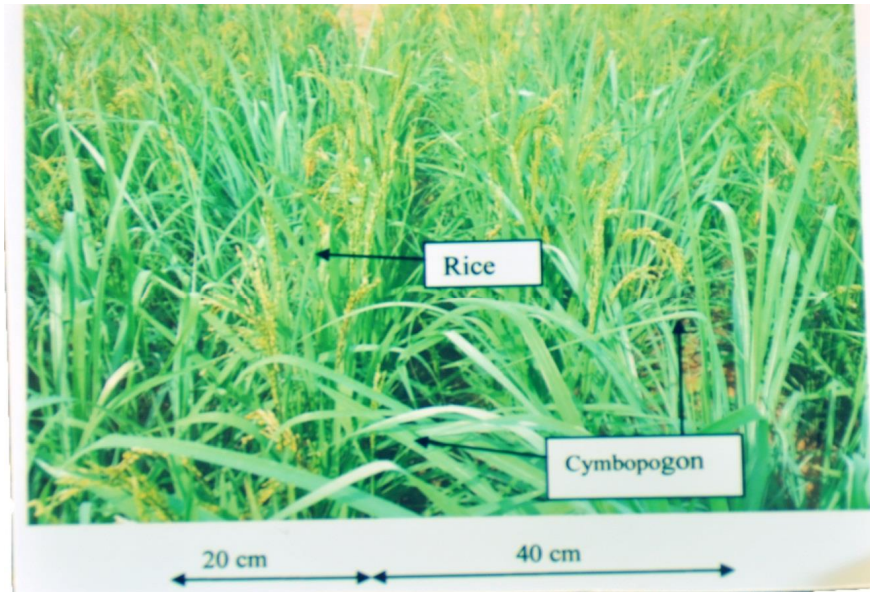


**Figure 5.3 Sole rice in paired rows (40 / 20 cm)**

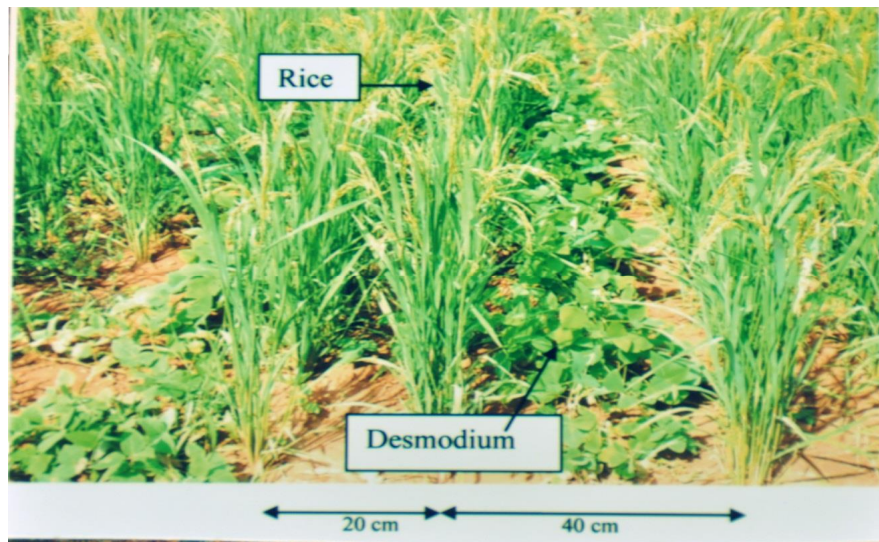


**Figure 5.4 Paired rows of rice + one row of cymbopogon, desmodium, mucuna or maize as intercrop.**

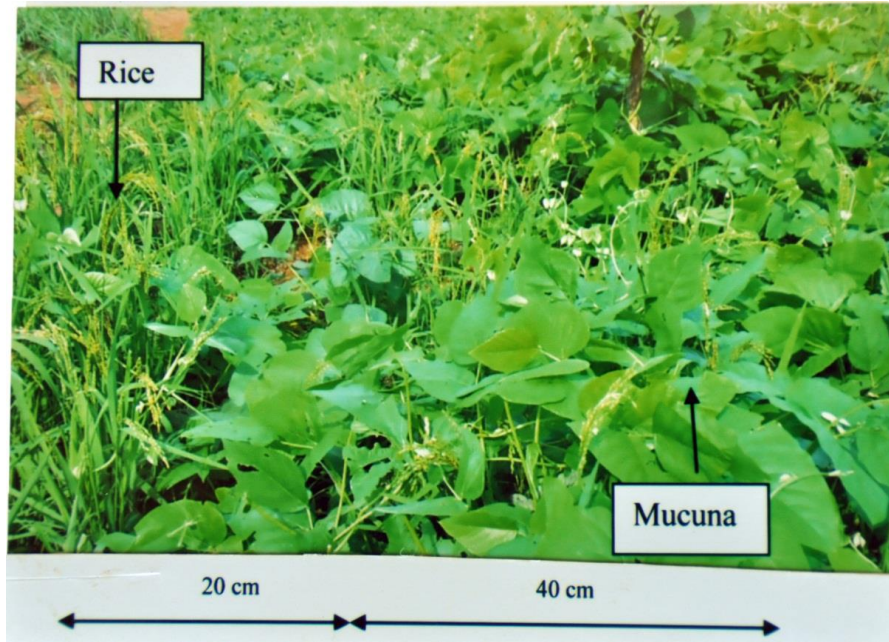




**Plates 5.2 Rice at 70 days after emergence in paired rows (40/20 cm x 12.5 cm) intercropped with one row of cymbopogon**



**Plate 5.3 Rice at 70 days after emergence in paired rows (40/20 cm x 12.5 cm) intercropped with one row of desmodium**



**Plate 5.4 Rice at 60 days after emergence in paired rows (40/20 cm x 12.5 cm) intercropped with one row of mucuna**

### 5.3.3 Competitive indices

The yield advantage for the field experiment was assessed quantitatively by land equivalent ratio (LER) as described by Mead and Willey (1980) and by competitive ratios (CR) by Willey and Rao (1980). Land equivalent ratio (LER) was calculated for each of the components (partial LER) and their combination (combined LER) as follows:  $LER = (Y_{ij} / Y_{ii}) + (Y_{ji} / Y_{jj})$  Where; LER = Combined land equivalent ratio, Y = Yield per hectare, ii and jj = Pure stands of species i and j and ji = Intercrops. The CR index was calculated using the following formula:  $CRA = (LERA / LERB) \times (ZIB / ZIA)$ . Where: CRA = Competitive ratio of A, LERA = Land equivalent ratio of A, ZIA = sown land proportion of crop A (In crop A intercropping with B), ZIC = sown land proportion of crop B (In crop B intercropping with A).

#### **5.3.4. Data collection**

Five plants from individual net plots of each crop were randomly selected and tagged at 14 DAE for biometric observations. The plant heights were measured from the ground to the base of the last fully opened leaf in rice, maize and cymbopogon, and from the ground to the tip of the youngest leaf for desmodium and mucuna. The length and width of the plant leaves were taken by measuring the longest leaf and widest leaf parts, respectively. The green leaves were counted weekly on each tagged plant in all the treatments up to flowering stages. Data was collected on the number of tillers, number of striga per 100 rice plants and the leaf number per plant. Biometric data collection stopped at the panicle initiation stage and tasselling of rice and maize respectively. The two boarder rows per plot together with two plants at both ends of rows in all sole crops were harvested as guard rows. Under intercropping treatments, one row of the base crop and another of the intercrop on each side of the plot were harvested as guard rows. Net plot crops were harvested for data collection. The number of panicles per plant, filled panicles per plant, total grains per plant and filled grains per panicle were determined on 10 earmarked plants. The yield per hectare was determined using harvested grain rice in 30 net plots each measuring 30 m<sup>2</sup> at 90 DAE. Desmodium, cymbopogon and mucuna vegetative materials were harvested from the net plots at 120 DAE. The fresh biomass per net plot and economic yield for mucuna were also determined.

#### **5.3.5 Data analysis**

All data collected were subjected to analysis of variance using 13<sup>th</sup> edition of Genstat software. Fischer's lowest significant difference (LSD) test at  $P \leq 0.05$  was used to separate treatments.

## 5.4 Results

### 5.4.1 Growth parameters of rice potted with cymbopogon, mucuna, desmodium and maize

The results on effects of potting *Cymbopogon nardus*, *Mucuna pruriens*, *Desmodium uncinatum* or *Zea mays* (LONGE 6H) on the growth of *Oryza sativa* (NERICA 1) are indicated in Table 5.1. Rice plant height significantly ( $P \leq 0.05$ ) reduced (12 cm) when potted with maize relative to rice potted with cymbopogon and mucuna (22 cm). No differences were noted in plant height among sole rice plants and plants potted with cymbopogon, desmodium and mucuna. Potting rice with any of the other crops significantly reduced root length. Shoot dry matter, leaf number, leaf length and leaf width of rice plants were not significantly affected by potting rice with other plant species.

**Table 5.1 Growth parameters per rice plant potted with cymbopogon, mucuna, desmodium and maize (2014A)**

Potted crops	PH (cm)	RL (cm)	DM (g)	LN (leaves)	LL (cm)	LW (cm)
Sole rice	17.70a	9.00a	0.20a	5.70a	27.00a	0.60a
Rice/ Cymbopogon	22.00a	5.00b	0.40a	6.00a	36.70a	0.60a
Rice/ Desmodium	17.00a	4.80b	0.20a	4.30a	28.00a	0.50a
Rice/ Mucuna	22.00a	6.30b	0.30a	4.30a	38.30a	0.60a
Rice/ Maize	12.30b	5.00b	0.10a	5.50a	31.50a	0.50a
P-value	0.032	0.005	0.190	0.193	0.204	0.452
LSD ( $P \leq 0.05$ )	6.50	2.00	NS	NS	NS	NS
CV (%)	19.7	18.8	18.3	18.9	19.9	19.8

Values with different letters in a column are significantly different at  $P \leq 0.05$ . NS = Not significant, PH = Plant height, RL = Root length, DM = Dry matter, LN = Leaf number, LL = Leaf length, LW = Leaf width.

#### 5.4.2 Growth parameters per maize plant potted with rice, desmodium, mucuna, cymbopogon and rice

Results on growth parameters as affected by potting *Zea mays* (LONGE 6H) with *Oryza sativa* (NERICA 1), *Cymbopogon nardus*, *Mucuna pruriens* or *Desmodium uncinatum* are indicated in Table 5.2. Potting maize with mucuna and desmodium increased the length of maize leaves by 31 and 15 percent respectively. Plant height, root length, dry matter, leaf number and width of leaves of maize were not significantly affected by potting with other plant species.

**Table 5.2 Growth parameters per maize plant potted with desmodium, mucuna, cymbopogon and rice (2014A)**

Potted crops	PH (cm)	RL (cm)	DM (g)	LN (leaves)	LL (cm)	LW (cm)
Sole maize	40.70a	13.00a	4.10a	8.70a	64.30b	3.40a
Maize/ Rice	6.00a	14.70a	5.00a	8.00a	71.00b	3.30a
Maize/ Cymbopogon	45.70a	18.30a	4.20a	8.70a	72.40b	3.50a
Maize/ Mucuna	46.00a	14.30a	5.00a	8.70a	84.30a	4.00a
Maize/ Desmodium	46.30a	14.30a	6.00a	8.70a	75.70a	3.60a
P-value	0.73	0.44	0.53	0.80	0.04	0.49
LSD (P $\leq$ 0.05)	NS	NS	NS	NS	8.9	NS
CV (%)	24.1	22.9	12.0	14.0	17.2	18.0

Values with different letters in a column are significantly different at  $P \leq 0.05$ . NS = Not significant. PH = Plant height, RL = Root length, LN = Leaf number, LL = Leaf length, LW = Leaf width.

### **5.4.3 Growth parameters of rice and striga as affected by intercropping under field conditions**

Intercropping rice with cymbopogon, desmodium, mucuna and maize had no significant influence on the plant height, number length and width of rice leaves at both stations (Table 5.3). Intercropping rice with mucuna, desmodium and cymbopogon significantly reduced the striga count per 100 rice plants (SHP) in Pallisa by 65, 56 and 36 percent respectively relative to the count under sole rice. Rice-maize intercropping significantly increased the number of striga plants (80%) relative to sole rice with a higher (63%) count of the noxious weed on maize (Plates 5.5-5.6) and only 37 percent of the striga near the rice plants. No striga infection was observed in Ikulwe.

### **5.4.4 Growth of rice tillers**

Intercropping rice with cymbopogon and desmodium significantly increased ( $P \leq 0.05$ ) rice tillers per rice plant between 21 days after emergence (DAE) and 55 DAE (panicle initiation stage) At panicle initiation stage, the tillers for rice intercropped with cymbopogon and desmodium increased by 45 and 11 percent on station and by 21 and 25 percent on-farm respectively (Table 5.4). Intercropping mucuna and maize in rice reduced the number of rice tillers at all crop stages. At panicle initiation stage the rice tillers reduced by 26 percent in rice intercropped with mucuna and maize at Ikulwe and by 18 and 64 percent relative to the control when intercropped with mucuna and maize respectively in Pallisa.

**Table 5.2 Growth parameters per rice plant and striga count per one hundred rice plants under rice intercropping systems (2014B)**

<b>Ikulwe (On station)</b>					
<b>Treatments</b>	<b>Plant height (cm)</b>	<b>LN (leaves)</b>	<b>Leaf length (cm)</b>	<b>Leaf width (cm)</b>	
Sole rice	18.20a	14.70a	30.20a	1.60a	
Rice/ Cymbopogon	16.70a	21.50a	38.30a	1.40a	
Rice/ Desmodium	15.30a	15.50a	31.30a	1.30a	
Rice/ Maize	14.00a	12.30a	24.20a	1.20a	
Rice/ Mucuna	12.80a	10.20a	30.20a	1.50a	
P-value	0.44	0.68	0.70	0.44	
LSD (P≤0.05)	NS	NS	NS	NS	
CV (%)	19.2	16.2	19.4	15.4	
<b>Pallisa (On-farm)</b>					
<b>Treatments</b>	<b>PH (cm)</b>	<b>SHP</b>	<b>LN (leaves)</b>	<b>LL (cm)</b>	<b>LW (cm)</b>
Sole rice	15.00a	61.30ab	13.10a	27.50a	0.90a
Rice/ Cymbopogon	15.00a	39.30b	14.00a	31.80a	1.00a
Rice/ Desmodium	13.30a	26.70b	10.50a	26.70a	0.90a
Rice/ Maize	15.80a	110.30a	10.50a	32.50a	0.90a
Rice/ Mucuna	12.30a	21.70b	9.80a	28.00a	0.90a
P-value	0.09	0.03	0.48	0.07	0.54
LSD (P<0.05)	NS	54.86	NS	NS	NS
CV (%)	15.3	6.8	16.4	18.6	19.4

Values with different letters in a column are significantly different at P≤0.05. PH = Plant Height, SHP = Striga per 100 rice plants, LN = Leaf number, LL = Leaf length, LW = Leaf width, NS = Not significant.

**Table 5.3 Rice plant tillers under different rice cropping systems at Ikulwe and Pallisa (2014B)**

Number of rice tillers per rice plant						
Cropping system	Ikulwe (Station)			Pallisa (On-farm)		
	21 DAE	36DAE	55DAE	21 DAE	36DAE	55DAE
Sole rice	3.20c	6.00b	3.80c	2.80b	4.80b	2.80b
Rice + Maize	2.70d	3.20e	2.70d	1.00e	3.20d	1.00d
Rice + Mucuna	2.80d	4.30d	2.80d	2.30d	3.80c	2.30c
Rice + Desmodium	3.70b	5.30c	4.20b	2.70c	4.70b	3.50a
Rice + Cymbopogon	4.20a	6.80a	5.50a	3.40a	5.80a	3.40a
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD ( $P \leq 0.05$ )	0.18	0.18	0.20	0.17	0.18	0.17
CV (%)	5.0	6.2	3.0	6.4	5.2	3.2

Values with different letters in a column are significantly different at  $P \leq 0.05$ . DAE = Days after emergence of rice.

#### **5.4.5. Growth parameters of maize and cymbopogon intercropped in rice under field conditions**

Intercropping maize with rice significantly reduced (23%) the maize plant height (52 cm) in Pallisa (Plate 5.5) compared to sole maize (67 cm). The number, length and width of maize leaves were not influenced by intercropping systems at both sites (Table 5.5). The height of maize was not influenced by intercropping at Ikulwe and the leaf number, leaf length and leaf width were not significantly affected by intercropping at both sites. Intercropping cymbopogon in rice did not influence cymbopogon growth parameters at both sites.



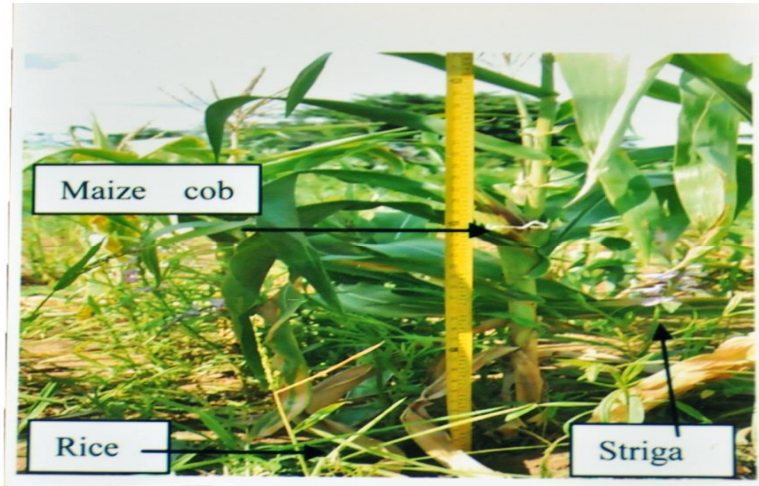


Plate 5.5 Serious *Striga hermonthica* weed attack on rice intercropped with maize

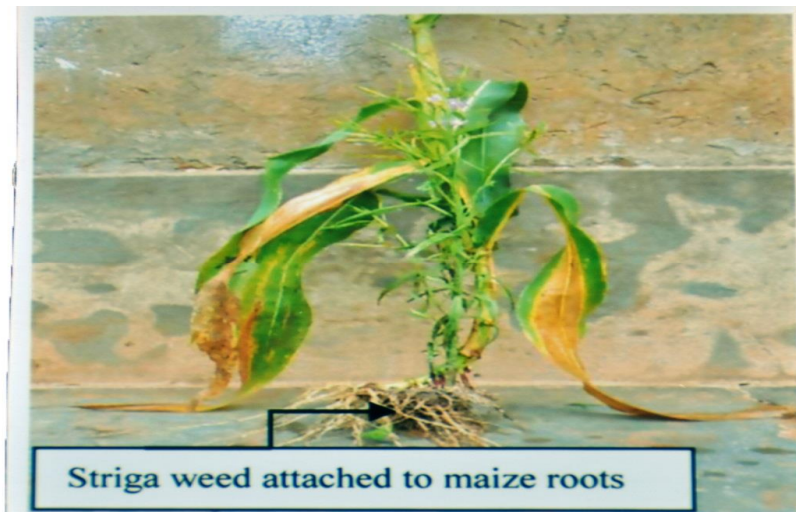


Plate 5.6 Parasitic attack by *Striga hermonthica* on maize plant roots.

**Table 5.4 Growth parameters per maize and cymbopogon plant intercropped with rice (2014B)**

Treatment	Ikulwe				Pallisa			
	PH (cm)	LN (leaves)	LL (cm)	LW (cm)	PH (cm)	LN (leaves)	LL (cm)	LW (cm)
Sole maize	94.30a	11.30a	100.00a	9.20a	67.20a	12.30a	73.70a	8.50a
Rice + Maize	114.50a	13.50a	109.20a	11.00a	52.10b	12.12a	74.40a	8.00a
P-value	0.15	0.08	0.35	0.06	0.04	0.74	0.35	0.23
LSD ( $P \leq 0.005$ )	NS	NS	NS	NS	10.52	NS	NS	NS
CV (%)	21.0	3.0	18.2	4.2	4.0	12.4	14.2	3.2

Treatment	PH (cm)	TN (tillers)	LN (leaves)	LL (cm)	LW (cm)	PH (cm)	TN (tillers)	LN (leaves)	LL (cm)	LW (cm)
	Sole cymbopogon	12.00a	6.50a	29.20a	45.80a	1.42a	11.50a	5.17a	21.20a	35.80a
Rice + Cymbopogon	13.70a	6.00a	28.30a	45.90a	1.42a	13.45a	4.30a	22.30a	35.90a	1.22a
P-value	0.63	0.78	0.93	17.66	1.00	3.22	1.11	7.15	6.36	0.71
LSD ( $P \leq 0.005$ )	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	18.2	4.0	2.8	16.2	18.6	14.5	4.0	12.3	14.0	5.0

Values with different letters in a column are significantly different at  $P \leq 0.05$ . NS = Not significant. PH = Plant height, TN = Tiller number, LN = Leaf number, LL = Leaf length, LW = Leaf width.

#### **5.4.6 Effect of potting rice with cymbopogon, mucuna, desmodium and maize on soil nutrient reserves at harvest of rice**

Potting rice with cymbopogon produced the highest N (0.20 g/100 g) in the soil at harvest relative to the control but gave lower P than sole rice by 12.5% (Table 5.6). Potting rice with desmodium or maize significantly ( $P \leq 0.05$ ) decreased the soil N at harvest by 11 and 16 percent, respectively, but increased the amounts of P and K in the soil at harvest by 7.7 and

5.4 percent under potting with desmodium and mucuna, respectively, relative to sole rice. Potting rice with maize similarly reduced both the P and K soil nutrient uptake levels by 38% compared to sole rice.

**Table 5.5 Soil nutrients at harvest for rice potted with cymbopogon, desmodium, maize and mucuna**

Soil nutrients at harvest			
	Nitrogen	Phosphorus	Potassium
Potted Plants	N (g/100 g)	P (mg/kg)	K (mg/kg)
Sole rice	0.19b	8.95b	144.34b
Rice + Cymbopogon	0.20a	7.83c	144.33b
Rice + Mucuna	0.19b	6.36d	152.13a
Rice+ desmodium	0.17c	9.64a	144.32b
Rice + Maize	0.16d	5.56f	89.72d
P-value	<0.001	<0.001	<0.001
LSD (P $\leq$ 0.05)	0.01	0.01	0.08
CV (%)	2.3	3.0	3.2

Values with different letters in a column are significantly different at P $\leq$ 0.05.

#### **5.4.7. Effects of potting rice with cymbopogon, mucuna, desmodium and maize on nutrient uptake by rice**

Potting rice with cymbopogon increased the plant N, P and K uptake (96, 40 and 73%) in rice (Table 5.7. The N and K uptake by rice significantly (P $\leq$ 0.05) increased when potted with either mucuna (70 and 871%) or desmodium (66 and 75%). Potting rice with mucuna, desmodium or maize did not influence the P uptake. The highest (70.90 mg per plant) K

uptake by rice was under rice potted with mucuna. Maize significantly reduced K uptake by rice (23%) relative to sole rice.

**Table 5.6 Nutrient uptake by rice potted with cymbopogon, desmodium, maize and mucuna**

Nutrient uptake (mg/plant)			
Potted Plants	Nitrogen (N)	Phosphorus (P)	Potassium (K)
Sole rice	2.70c	0.50b	7.30c
Rice + Maize	2.70c	0.40b	5.60d
Rice + Desmodium	4.50b	0.40b	12.80b
Rice + Mucuna	4.60b	0.50b	70.90a
Rice + Cymbopogon	5.30a	0.70a	12.60b
P-value	<0.001	0.05	<0.001
LSD (P≤0.05)	0.30	0.19	1.12
CV (%)	3.0	2.0	4.2

Values with different letters in a column are significantly different at  $P \leq 0.05$ .

#### **5.4.8 Effect of potting maize with cymbopogon mucuna, desmodium and maize on nutrient reserves at harvest of maize**

The results on soil nutrient reserves at harvest due to potting maize with cymbopogon, mucuna, desmodium and maize are indicated in Table 5.8. Potting maize with mucuna produced the highest (0.33 g/100g) N nutrient reserves in the soil at harvest which was 74% higher than under sole maize crop. Potting maize with either cymbopogon or rice significantly ( $P \leq 0.05$ ) reduced (16%) the amounts of N in the soil at harvest relative to the sole maize crop. Potting maize with desmodium, cymbopogon, mucuna and rice significantly ( $P \leq 0.05$ ) reduced (7, 11, 42 & 45) the reserves of P in the soil at harvesting compared with sole maize. Maize potted with mucuna produced lower (42%) P reserves at harvest relative to P under sole maize. Maize potted with cymbopogon increased (13.5%) the amount of K in

the soil relative to sole maize. The amounts of K in the soil at harvest reduced (16%) due to potting with desmodium or mucuna.

**Table 5.7 Soil nutrients reserves at harvest of maize potted with cymbopogon, desmodium, maize and mucuna**

Potted crops	Soil nutrients at harvest		
	N (g/100 g)	P (mg/kg)	K (mg/kg)
Sole maize	0.19c	9.53a	144.32b
Maize + Desmodium	0.19c	8.83b	120.91c
Maize + Cymbopogon	0.16d	8.52c	163.82a
Maize + Rice	0.16d	5.24e	89.72d
Maize + Mucuna	0.33a	5.56d	120.91c
P-value	< 0.001	< 0.001	< 0.001
LSD (P≤0.05)	0.008	0.009	14.6
CV (%)	5.6	4.3	12.5

Values with different letters in a column are significantly different at  $P \leq 0.05$ . N = Nitrogen, P = Phosphorus, K = Potassium

#### **5.4.9 Effect of potting maize with cymbopogon, mucuna, desmodium or rice on nutrient uptake by maize**

The results on nutrient uptake by maize potted with cymbopogon, mucuna, desmodium or rice are indicated in Table 5.9. The N and P uptake by maize increased when potted with desmodium (73% & 42%) and cymbopogon (29% & 9%) relative to sole maize.

**Table 5.8 Nutrient uptake levels for maize potted with cymbopogon, mucuna, desmodium and maize (2014A)**

<b>Nutrient uptake (mg/plant)</b>			
<b>Potted crops</b>	<b>Nitrogen (N)</b>	<b>Phosphorus (P)</b>	<b>Potassium (K)</b>
Sole maize	65.00c	7.50c	167.0a
Maize + Rice	67.50c	5.50e	225.0a
Maize + Mucuna	62.50c	6.40d	168.1a
Maize + Cymbopogon	92.50b	8.20b	168.9a
Maize + Desmodium	112.50a	9.70a	190.5a
P-value	<0.001	<0.001	0.06
LSD (P $\leq$ 0.05)	9.20	0.35	NS
CV (%)	18.5	6.4	24.8

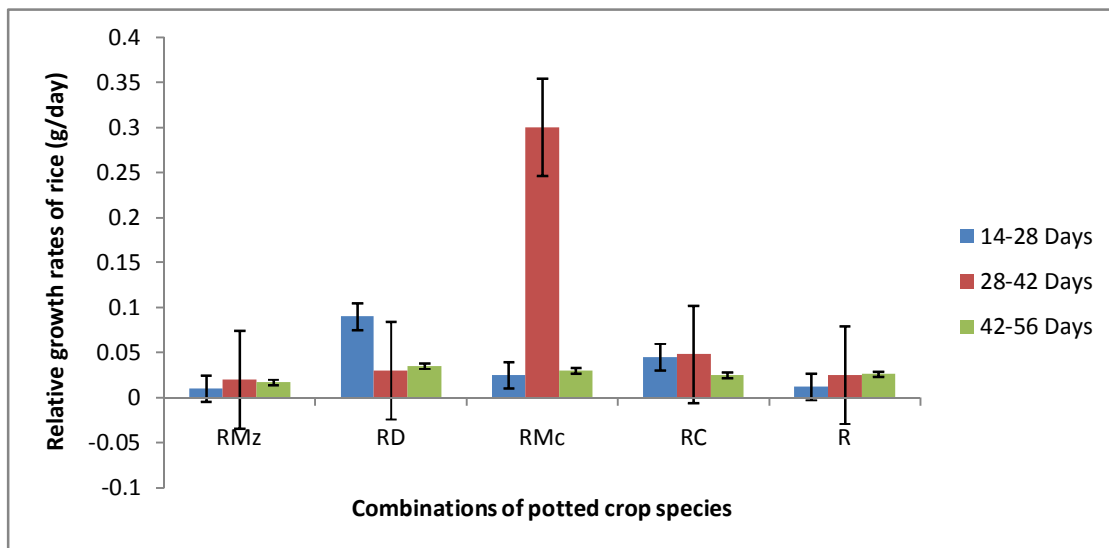
Values with different letters in a column are significantly different at  $p \leq 0.05$ .

Potting maize with desmodium, similarly, increased (73 and 29%) the N and P uptake. Corresponding significant increases (42 and 9%) in N and P nutrient uptake were recorded by maize potted with component rice or cymbopogon. Potting maize with mucuna and rice reduced (15 and 27%) the P uptake by maize but mucuna and rice did not influence N uptake by maize and the K uptake by maize was not significant. The N and P uptake by maize potted with desmodium and cymbopogon increased (73 and 29% and 42 and 9%) relative to sole maize.

## 5.4.10 Relative growth rates

### 5.4.10.1 Effects of potting rice with cymbopogon, mucuna, desmodium or maize on the relative growth rates of rice

The results on effects of potting rice with cymbopogon, mucuna, desmodium or maize on the relative growth rates of rice are indicated in Figure 5.5. The RGR for rice potted with desmodium (RD) was significantly higher ( $P \geq 0.05$ ) between 14-28 days relative to other treatments. The RGR for rice potted with mucuna (RMc) significantly increased between 28-42 days relative to other treatments for the same period and the rice potted with maize (RMz) gave significantly lower RGR between 42 and 56 days relative to other potted plant combinations and sole rice.

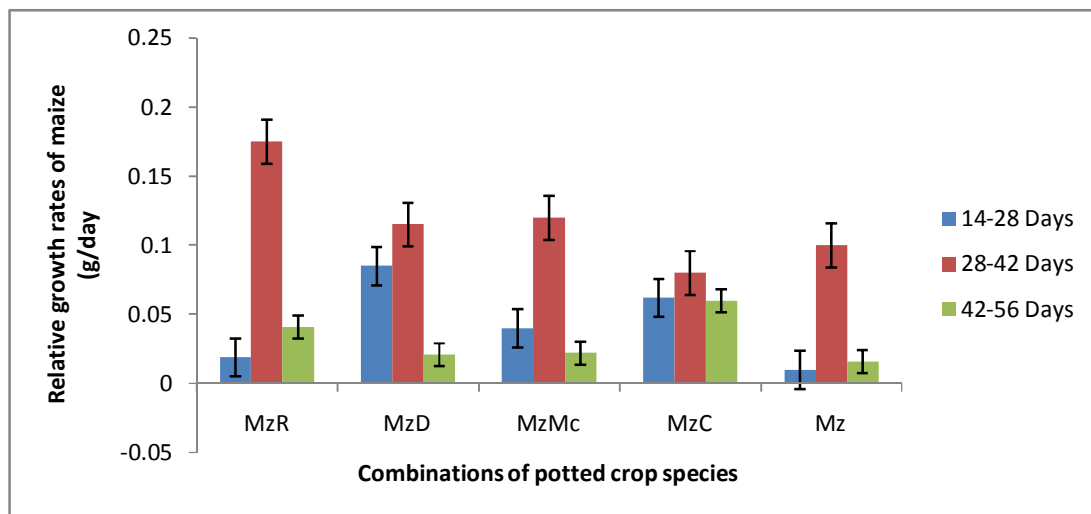


**Figure 5. 5 Relative growth rates of rice (R) potted with maize (RMz), desmodium (RD), mucuna (RMc) and cymbopogon (RC).**

### 5.4.10.2 Effect of potting maize with cymbopogon, mucuna, desmodium and rice on the relative growth rates of maize

The results on effect of potting maize with cymbopogon, mucuna, desmodium or rice on the relative growth of maize are indicated in Figure 5.6. During the period 28-42 days the RGR

for maize potted with rice was significantly higher than under other treatments. Sole maize and maize potted with desmodium (MzD) or mucuna (MzM<sub>c</sub>) produced lower but similar RGR. Maize potted with mucuna gave higher RGR relative to maize potted cymbopogon. Higher RGR between 42-56 days were recorded under maize potted with cymbopogon followed by maize with rice. However, lower RGR were under sole maize and maize potted with desmodium or mucuna.



**Figure 5.6** Relative growth rates of maize (Mz) potted with rice (RMz), desmodium (MzD), mucuna (MzM<sub>c</sub>) and cymbopogon (MzC)

#### 5.4.10.3 Yield attributes and yield of rice under field conditions

The results on yield attributes and yield of sole and rice intercropped with cymbopogon, desmodium, maize or mucuna are indicated in Table 5.10. Intercropping of desmodium, maize and mucuna with rice significantly ( $P \leq 0.05$ ) reduced the number of rice panicles per plant at harvest in Ikulwe. The number of rice panicles per plant significantly reduced when rice was intercropped with mucuna at Pallisa. The percent filled rice panicles and percent filled grains per panicle were not influenced by intercropping treatments at both sites. Sole



rice had significantly higher grain yield than intercropped rice at Pallisa. Similar observations were made at Ikulwe except that the yield of rice intercropped with cymbopogon (1,103 kg ha<sup>-1</sup>) was similar to the sole rice grain yield. Rice intercropped with cymbopogon and desmodium is in plates 5.7 and 5.8 at time of harvesting.

**Table 5.9 Yield attributes and yield for rice intercropped with cymbopogon, desmodium, maize and mucuna plants in the field (2014A).**

Cropping system	Ikulwe				Pallisa			
	PP	PFP (%)	PFG (%)	Y (Kg ha <sup>-1</sup> )	PP	PFP (%)	PFG (%)	Y (kg ha <sup>-1</sup> )
Sole rice	7.80a	46.00a	83.60a	1,111.00a	4.30a	62.70a	84.80a	631.0a
Rice + Cymbopogon	7.60a	47.50a	75.10a	1,103.00a	4.10a	64.80a	76.80a	510.0b
Rice + Desmodium	5.10b	56.00a	74.50a	926.00b	4.30a	59.50a	77.50a	492.0c
Rice + maize	4.60b	67.60a	75.60a	750.00c	3.60a	67.60a	83.20a	394.0d
Rice + Mucuna	3.70b	57.30a	72.80a	728.00c	2.50b	70.30a	81.40a	359.0d
P-value	0.002	0.76	0.36	0.004	0.003	0.43	0.63	0.003
LSD (P <sub>≤</sub> 0.05)	2.40	NS	NS	73.10	1.64	NS	NS	111.30
CV (%)	3.2	15.4	18.4	12.4	4.5	12.6	16.5	5.8

Values with different letters in a column are significantly different at P<sub>≤</sub>0.05, NS = Not significant, PP = Panicles per plant, PFP = Percent filled panicles, PFG = Percent filled grains, Y = Yield

At Pallisa, desmodium intercrop produced lower rice grain yield than rice + cymbopogon. Rice + maize and rice + mucuna intercrops had significantly lower grain yield than all the other cropping systems.

#### 5.4.10.4 Competitive indices of rice at Ikulwe

The results on competitive indices at Ikulwe are indicated in Table 5.11. Intercropping cymbopogon, desmodium, maize and mucuna in rice reduced all the partial land equivalent ratios (LER) for rice to less than 1.00. Cymbopogon gave the highest (1.00) partial LER followed by desmodium (0.8). Rice intercropped with maize or mucuna recorded the lowest partial LER (0.7) for rice.



**Plate 5.7 High rice tillering and rice grains at harvest (90 days after emergence) under intercropping with *Cymbopogon nardus*.**



**Plate 5.8** High rice tillering and rice grains at harvest (90 days after emergence) under intercropping with *Desmodium uncinatum*.

**Table 5.10** Land equivalent ratio for rice and intercrops and competitive ratios for the intercrops at Ikulwe

Rice/intercrop	PLER (Rice)	PLER (Intercrops)	CLER	CR
Sole rice	-	-	1.00b	1.00c
Rice + Cymbopogon	1.00a	0.60b	1.60a	1.60b
Rice + Desmodium	0.80b	0.90a	1.70a	0.70d
Rice + Maize	0.70c	0.30c	1.00b	1.50b
Rice + Mucuna	0.70c	0.30c	1.00b	1.80a
P-value	<0.001	<0.001	<0.001	<0.001
LSD ( $P \leq 0.05$ )	0.05	0.05	0.54	0.17
CV (%)	3.0	4.5	4.0	3.4

Values with different letters in a column are significantly different at  $P \leq 0.05$ . PLER = Partial Land Equivalent Ratio, CLER = Combined land equivalent ratio, CR = Competitive ratio.

The partial land equivalent ratio for intercrops were highest under desmodium (0.9) followed by cymbopogon (0.6) and lowest under maize and mucuna (0.3). The combined land equivalent ratios for rice-cymbopogon and rice-desmodium intercrops were 1.6 and 1.7 respectively. Rice-maize and rice-mucuna intercrops had each LER of 1.0. Mucuna was the most competitive intercrop with rice with a competitive ratio (CR) of 1.8 followed by cymbopogon with a CR of 1.6 and maize with a CR of 1.50. Desmodium had a significantly lower competitive ratio (0.7) than all the other crops.

## **5 Discussions**

### **5.5.1 Effects of potted plants on rice growth, nutrient uptake and soil nutrient reserves**

Rice root length decreased when rice was intercropped with cymbopogon, mucuna, desmodium and maize crops. This may be attributed to allelochemicals produced by the roots of the latter four plants species. Allelochemicals have been reported to influence the physiological and biological processes of plants especially cell division and roots have been reported as being more susceptible than other plant parts. Tomita *et al.*, (2003) and Zhao-Hui *et al.*, (2010) reported inhibited root growth, damages to the seed cell membrane systems for mitochondria and chloroplasts, increases in cell permeability and consequently reduced lettuce (*Lactuca sativa* L.) crop growth rates by allelochemicals from seedlings of *M. pruriens* and other crop species. Prapaipit *et al.*, (2013) and Ali *et al.*, (2015) reported higher sensitivity of the roots of grass weeds and test crops to allelochemicals than the shoots. Some of the compounds identified in the crop leachates (chapter 3 of this thesis) could have inhibited root growth of rice. Height of rice plants potted with maize significantly reduced relative to that of rice potted with cymbopogon or mucuna crops. The lower height of rice

potted with maize may be attributed to competition for nutrients in the rhizosphere by the cereal crops.

### **5.5.2 Effects of potted plants on maize growth, nutrient uptake and soil nutrient reserves**

Maize leaf length and relative growth rates significantly increased when potted with desmodium and mucuna. This may be attributed to the higher N and P uptake with lower N reserves at harvest under maize potted with desmodium observed in chapter 5 of this thesis. Maize potted with mucuna recorded lower P uptake than sole maize and mucuna did not affect maize K uptake. Thus, the increased maize leaf length may partly be associated with possible etiolation under competition for solar radiation between maize and the highly competitive mucuna crop. Mucuna recorded the highest competitive index in chapter 5 of this thesis. The current study indicates high potential in rice intercropping with cymbopogon and in the maize intercropping with desmodium. The maize + rice ecosystem exhibited low nutrient uptake with poor crops growth possible due to competition by both cereal crops and given its low productivity potential, the cropping system may not be sustainable.

### **5.5.3 Striga count and rice tillers in rice based intercropping systems**

Intercropping rice with desmodium, mucuna and cymbopogon reduced the striga weed counts relative to sole rice. Rice-maize intercropping, however, increased the number of striga plants relative to sole rice with a higher (63%) count of the noxious weed on maize. Generally, there is little work on striga control using mucuna and cymbopogon but various studies have shown that intercropping cereals, mainly with legumes such as cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*) and green gram (*Vigna radiate*) can reduce the number of *Striga* plants (Carsky *et al.*, 2000). Potentially, they might be acting as traps crops, stimulating

suicidal *Striga* germination or the microclimate under the crop canopy may be altered and interfere with *Striga* germination and development (Parker and Riches 1993; Khan *et al.*, 2007). This observation may also be attributed to the effects of bio-active compounds exudated by the crops into the rhizosphere that could have interfered with the processes of striga germination and development. As indicated in chapter three of this thesis, mucuna crop roots released five terpenoids, one phenol and an alkane while desmodium plants released three terpenoids, one alkane and a furan and cymbopogon plant released five terpenoids, a phenol and an alkane. Some of these compounds may be associated with inhibited weed and crop growth and could have interfered with the chemical process of striga weed attachment and growth of the rice crops. Pickett (2010) indicated a putative allelopathic mechanism by *D. uncinatum* to be evident in the control of *S. hermonthica*. The allelopathic control of other weeds by the test plants have been observed by Prapaipit *et al.*, (2013) who reported allelopathic inhibitory effects by *C. nardus* (L) on the shoot and root growth of cress, lettuce, rapeseed and Italian rye grass at concentrations  $\times 0.03$  g dry weight equivalent extract / ml. Soares *et al.*, (2014) reported that L-DOPA from mucuna root exudates reduced the growth of neighbouring plants. It is also hypothesized that nitrogen fixed by the legumes might interact with *Striga* growth, as increasing the amount of available nitrogen can reduce *Striga* densities (Pieterse *et al.*, 1991).

Compared to sole crops, cymbopogon and desmodium intercrops increased the tillers per rice plant (11-45%) at panicle initiation stage while mucuna and maize intercrops reduced this parameter in both on station and on-farm. The higher number of rice tillers on rice intercropped with cymbopogon and desmodium may be attributed to the significantly higher N and K nutrient uptake levels by 67 and 75% respectively for rice intercropped with desmodium and significantly higher N, P and K by 96, 80 and 73% respectively under rice

intercropped with cymbopogon than sole rice crop. The reduced number of rice tillers under intercropping with maize may be associated with the higher competitive ability of mucuna and maize with rice for resources. Since rice potted with maize recorded the lowest N and K nutrient uptake in chapter 5 of this thesis, this could have led to the reduced tillering. Intercropped rice with maize reduced maize leaf length and tiller numbers in rice. This could be attributed to competition for nutrients in the rhizosphere and to the high incidence of *Striga hermonthica* parasitic weed. The results further showed that NERICA 1 rice was less susceptible to striga than LONGE 6H maize and this may be attributed to its higher genetic resistance to striga weeds as reported by Jamil *et al.*, (2011) and Cissoko *et al.*, (2011).

#### **5.5.4 Yield and competitive indices of the rice based intercropping systems.**

Sole rice gave higher grain yield than intercropped rice. Intercropping rice with cymbopogon produced higher rice grain yield than other intercropping systems. Rice + Cymbopogon and Rice + Desmodium intercropping systems recorded higher land equivalent ratios than rice-mucuna and rice-maize intercropping systems. The current study indicates that a farmer would need 1.56 acres of land area planted with sole rice to achieve equal productivity from 1.0 acre of rice intercropped with cymbopogon. The good performance of rice when intercropped with cymbopogon could be attributed to the improved N, P and K nutrient uptake by rice with cymbopogon and increased tiller numbers as observed in this study (chapter 5 of the thesis) and could have been influenced by allelopathy.

Other studies have indicated the benefits from rice intercropping with other crops. (Jabbar *et al.*, 2009; Jabbar *et al.*, 2010; Rana *et al.*, 2013). Ram *et al.*, (1998) reported higher land equivalent ratio (1.88) for *Cymbopogon winterianus* intercropped with legumes. Rice intercropping with cymbopogon maximized the productivity with high competitive ability

and clearly demonstrated the benefits from intercropping. The cropping system, however, needs to be further exploited by the farmers for higher productivity and economic returns.

Rice root growth significantly reduced when rice was potted with desmodium, maize, mucuna or cymbopogon. Rice plant height significantly increased when potted with cymbopogon and mucuna but reduced when potted with maize. Maize leaf length, significantly increased when potted with desmodium, cymbopogon and mucuna. Intercropping rice and mucuna significantly reduced the striga weed count relative to sole rice. Intercropping rice with cymbopogon gave higher rice grain yield and land equivalent ratio than rice-mucuna, rice-maize and rice-desmodium intercropping systems. Rice intercropping with cymbopogon is productive under paired row ecosystem.



## CHAPTER 6

### EFFECT OF CROP MULCHES ON WEED INFESTATION AND RICE PRODUCTIVITY

#### 6.1 Abstract

Allelopathic mulches have a high potential as an environmentally friendly weed management technology to improve crop productivity. A study was conducted on station at Ikulwe and on-farm at Pallisa (2015A) in a randomised block design with three replicates to determine the influence of mulches of *Oryza sativa* (NERICA 1), *Zea mays* (LONGE 6H), *Mucuna pruriens* and *Cymbopogon nardus* on the weed growth and rice yield. The 12 mulch treatments were; maize/ rice/ cymbopogon, rice/ mucuna/ cymbopogon, maize/ mucuna/ cymbopogon, maize/ rice/ mucuna, maize/ rice/ cymbopogon + hand roguing once (42DAE) of rice, rice/ mucuna/ cymbopogon + hand roguing once, maize/ mucuna/ cymbopogon + hand roguing once, maize/ rice/ mucuna + hand roguing once, maize/ rice/ cymbopogon + hand hoeing once, rice/ mucuna/ cymbopogon + hand hoeing once, maize/ mucuna/ cymbopogon + hand hoeing once and maize/ rice/ mucuna + hand hoeing once. Four other treatments included hand hoeing twice or thrice (14, 28 and 42 DAE), Butanil (PRE at 2 L ha<sup>-1</sup>) + hand hoeing once and a weedy check. Maize/mucuna mulches increased rice growth much more than rice/ cymbopogon mulches with or without a subsequent weed control. Maize/ rice / cymbopogon and rice/ mucuna/ cymbopogon mulches reduced weed density and biomass the most followed by maize/ mucuna/ cymbopogon and maize/ rice/ mucuna mulches. Hand hoeing twice produced similar rice grain yield to maize/ rice/ mucuna mulch + hand roguing of weeds once. Hand hoeing thrice increased rice growth and yield relative to other treatments. Rice/ mucuna/ cymbopogon + hand hoeing once and Butanil + hand hoeing once produced similar lower rice grain yield. The weedy check reduced rice growth

and tiller development giving zero yields. The highest striga per 100 rice plants (SHP) was recorded at 63 DAE of rice under Butanil + hand hoeing once (8 striga), followed by counts of 3 and 2 striga plants for hand hoeing three and two times, respectively. Striga plants were not observed under cymbopogon mulches and weedy check. The highest returns on investment (ROI) were under hand hoeing twice (0.52 and 0.43) at both sites. Maize/rice/mucuna + hand roguing weeds once recorded high ROI of 0.47 on station, similar to hand hoeing thrice on-farm. Rice/ cymbopogon based mixed mulches most effectively controlled the weeds. Maize/ mucuna based mulches were associated with higher rice growth and yield. Maize, rice, cymbopogon and mucuna have the potential to produce bio-herbicides for weed control and improved rice crop yields.

**Keywords:** bio-compounds, days after emergence, returns on investment, striga, hand hoeing

## 6.2 Introduction

Rice (*Oryza sativa* L.) is a staple food in many regions of the world and the increasing population pressure, demands that more attention be directed towards improving productivity. Phuong *et al.* (2005) reported weeds as the most threatening biological constraint to direct seeded rice cultures with high yield reductions (60%), with even a complete crop failure under heavy weed infestations. Cultural and/or chemical methods are generally employed to control weeds. Hand hoeing, though effective, is getting increasingly unattractive due to labour scarcity, rising wages and its dependence on weather conditions. Rehman *et al.* (2010) reported development of resistance to herbicides in some previously susceptible weed species and serious environmental concerns due to high residual effects of herbicides in soil as major drawbacks associated with herbicide usage. Moreover, allowing weeds to reach sufficient size to be rogued, especially perennial species that fragment on pulling is a serious concern (Rao *et al.* 2007).

The use of allelopathic crop residues is a promising strategy of achieving cost effective, safe and environmentally friendly weed suppression in arable fields (Jabran *et al.*, 2015). Allelopathy is described as the ability of plants to inhibit or stimulate growth of other plants in the environment by effects of biochemicals. Weed suppression using allelopathic mulches can be achieved through crop rotation or intercropping. Residues of allelopathic plants may be left on the soil as mulch after harvesting crops in reduced tillage systems (Ashraf *et al.*, 2017) or may be incorporated into the soil in conventional tillage systems where they release putative allelochemicals during decomposition (Abbas *et al.*, (2017). Ashraf *et al.*, (2017) noted that mulching is only effective against weeds before or during germination and does not provide effective weed control if done after weed emergence. Nevertheless, the use of allelopathic plant residues has been reported to be an effective way of suppressing weeds

because the allelochemicals are released in the soil environment in close proximity to weed seeds or the roots of weed seedlings and can therefore be readily absorbed by the receiver plant (Zohaib *et al.* 2016).

There is little literature on use of mulches and cover crops of mucuna, cymbopogon, desmodium, rice and maize for weed control (Xuan *et al.*, 2005; Pickett *et al.*, 2010; Kato-Noguchi 2012; Jabran *et al.*, 2015). Related studies have indicated that incorporation (*in situ*) of whole sorghum plants or their various parts alone or mixed with each other was found to suppress weed growth in wheat (Cheema and Khaliq, 2000). Cheema *et al.* (2004) reported that sorghum mulch (10-15 Mt ha<sup>-1</sup>) decreased (38-41%) the dry weight of purple nut sedge relative to the control. Cheng and Xu, (2013), however, cautioned about the importance of considering the allelopathic nature of crops before being used as mulches. Mtambanengwe *et al.* (2015) reported that surface mulching with retained cowpea (*Vigna unguiculata* L.) residues suppressed weed emergence between 40% and 60% under conservation agriculture in Zimbabwe.

Allelochemicals released by crop mulches may also influence plant growth indirectly by altering soil characteristics and inhibiting soil micro fauna (Kobayashi., 2003; Zohaib *et al.*, 2016). Consequently, accumulation of allelochemicals in the soil results in suppression of seed germination and plant growth, decrease in the volume of primary roots and increased secondary roots, reduced uptake of water and nutrients and subsequently chlorosis ultimately resulting in the death of the plant (Narwal *et al.*, 2005). Abbas *et al.* (2017) reported that combining sorghum, rice and maize mulches with reduced herbicide mixtures increased little seed canary grass mortality up to 98%, significantly reduced dry weed biomass and provided up to 92% weed control efficiency. Increased weed emergence and seedling growth was

reported from fields treated with allelopathic mulches which could be partially attributed to the hormetic effects of the allelochemicals at low concentrations (Belz *et al.*,2005). The objective of the study was, therefore, to determine the effect of *Mucuna pruriens*, *Cymbopogon nardus*, *Zea mays* (LONGE 6H) and *Oryza sativa* (NERICA 1) mixed mulches on weed growth and rice productivity.

## **6.3 Materials and methods**

### **6.3.1 Field experiment using crop mulches**

#### **6.3.1.1 Site description**

The experiment was conducted at Ikulwe research station and on-farm at Pallisa. Ikulwe is situated at 00° 26' 23.20"N and 033° 28' 40.90"E and lies 1209 m above sea level. The area received a total of 543 of the 1230 mm mean annual rainfall during the cropping season with mean minimum and maximum temperatures of 18.5 and 30 °C against the annual temperature of 18.30 and 32 °C, respectively. Pallisa is located at 1° 13' 33.20"N and 33° 46' 47.20"E. The total precipitation received was 450 mm and the minimum and maximum temperatures recorded were 19 and 31 °C, respectively, against the annual rainfall of 990 mm and mean minimum and maximum temperatures of 23 and 33 °C respectively. Both experimental sites have sandy-loam soils.

#### **6.3.1.2 Experimental design and treatments**

A study was conducted during the rainy seasons of 2015A at Ikulwe research station and on-farm in Pallisa district. *Cymbopogon*, *mucuna*, rice and maize species were each planted in 24 different plots each measuring 5 x 8 m under a randomised complete block design with three replicates, to provide mulch material and give a site with potential allelopathic effects in

the soil for the subsequent study. The crops were uprooted from all the plots at 55 days after emergence (DAE) and combined in sets of three equal proportions of individual stover to give four types of mulches namely maize/ rice / cymbopogon, rice/ mucuna/ cymbopogon, maize/ mucuna/ cymbopogon and maize/ rice/ mucuna. The experimental site was cleared of weeds by slashing and ploughed twice with a tractor. The field was divided into 48 plots (4 x 5 m) arranged in a randomised complete block design with three replicates. At the time of planting rice, mixed stovers were applied as mulches of 3-4 inches thick (10-12.Mt ha<sup>-1</sup>) under 12 treatments namely maize/ rice/ cymbopogon, rice/ mucuna/ cymbopogon, maize/ mucuna/ cymbopogon, maize/ rice/ mucuna, maize/ rice/ cymbopogon + 1 hand roguing (42DAE) of rice, rice/ mucuna/ cymbopogon + 1 hand roguing, maize/ mucuna/ cymbopogon +1 hand roguing, maize/ rice/ mucuna + 1 hand roguing, maize/ rice/ cymbopogon + 1hand hoeing, rice/ mucuna/ cymbopogon + 1 hand hoeing, maize/ mucuna/ cymbopogon +1 hand hoeing and maize/ rice/ mucuna + 1 hand hoeing. Four treatments under twelve plots, which included 2 hand hoeing and 3 hand hoeing (14, 28 and 42 DAE), Butanil (PRE at 2 L ha<sup>-1</sup>) + 1 hand hoeing and a weedy check, were located 2 metres outside the preliminary experimental site to avoid any previous allelopathic effects. The treatments were designated as checks.

For comparison the study was conducted between February and June 2015 in plots (4 m x 5 m) not previously planted with the allelopathic crops on a selected farm at Pallisa under a randomised complete block design, replicated thrice. The eight treatments included application of rice/ mucuna/ cymbopogon + 1 hand hoeing, maize/ mucuna/ cymbopogon + 1 hand hoeing, maize/ rice/ mucuna + 1 hand hoeing, 2 hand hoeing, 3 hand hoeing, Butanil (PRE) + 1 hand hoeing and a weedy check. Application of mulches alone and mulches + 1 hand roguing were not administered because of the observed high weed pressure at the site

prior to the study. Rice was sown at a spacing of 30 cm x 12.5 cm (1 plant) within the row in all the plots at both sites. The full N, P and K fertiliser rates used in the experiment were 60 kg N ha<sup>-1</sup>, 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 30 kg K<sub>2</sub>O ha<sup>-1</sup>. The N fertiliser was applied in splits at 30 and 55 DAE, respectively, and P and K fertilisers were applied at time of planting. The sources of N, P and K were urea (46% N), single super phosphate (16% P<sub>2</sub>O<sub>5</sub>) and muriate of potash (60% K<sub>2</sub>O), respectively.

### **6.3.1.3 Data collection**

Plant height, number of fully opened green leaves, number of tillers per plant, length and width of longest rice leaves were determined on 20 selected rice plants at 21, 42 and 55 days after emergence (panicle initiation stage) in all the treatments. The species of weeds were established, counted and recorded in 1 m x 1 m quadrant for each of the treatments at 21, 42, 55, 63 and 90 days after emergence (Rice harvest). The weeds were oven dried at 80 °C for 12 hours until constant weight and biomass determined. The number of striga plants within 15 cm radius from the base of rice plants and within five inner rice rows was determined during the same sampling period. The count was expressed as the number of striga per 100 rice plants. At harvest, the total number of panicles, filled panicles per plant, total number of grains per panicle and number of filled grains per panicle were determined on a sample of 20 plants. Rice grain yield was established for the net plots (38 m<sup>2</sup>). Data were collected on production costs, gross and net monetary returns per treatment. The economic benefit from weed control per Uganda shilling investment was determined in the ratio of the net returns to the cost of production in each of the treatments (Kaiira *et al.*, 2014).

#### **6.3.1.4 Data analysis**

All data collected were subjected to analysis of variance using 13<sup>th</sup> edition, 2013 of Genstat software. Fischer's least significant difference (LSD) test at  $P \leq 0.05$  was used to separate treatment means.

### **6.4 Results**

#### **6.4.1 Rice growth parameters at Ikulwe station**

Application of maize/ mucuna based mulches with or without a post mulch weed control technology produced taller rice plants (47-60 cm) with higher leaf number (29-37 leaves) and width (1.8-2.0 cm) than rice/ cymbopogon based treatments (Table 6.1). Hand hoeing after mulching with maize/ rice/ mucuna mulch significantly ( $P \leq 0.05$ ) reduced rice plant height and increased leaf number and leaf width relative to the maize / rice / cymbopogon and rice/mucuna/cymbopogon mulches alone. Maize/ rice/ cymbopogon mulches alone recorded lower (4.30 tillers) number of tillers per rice plant than other treatments. Leaf length was not significantly affected by treatments. Field observations at Ikulwe station indicated that more weeds germinated in the seeded, un-mulched rice rows, than under the mulched inter-rows. The weedy check recorded the lowest observations.

#### **6.4.2 Rice and striga growth under different weed control treatments in Pallisa**

Butanil (PRE) application, hand hoeing twice, hand hoeing thrice and maize/mucuna mulches each followed by hand hoeing once, significantly ( $P \leq 0.05$ ) increased the plant height and leaf width relative to rice/cymbopogon mulches (Table 6.2).



**Table 6.1 Rice growth parameters under different weed control treatments at Ikuwe station during 2014A**

Treatments	PH (cm)	Tillers	LN/ plant	LL (cm)	LW (cm)
Butanil (PRE) + 1 hh	59.30a	8.70a	42.00a	37.30a	2.00a
Hand hoeing twice	53.70a	6.70a	31.00a	37.70a	2.00a
Hand hoeing thrice	52.30a	9.00a	39.70b	37.70a	2.00a
Maize/ mucuna/ cymbopogon + 1 hr	47.00ab	10.00a	37.00a	39.00a	1.80a
Maize/ rice/ mucuna + 1 hr	47.10ab	7.00a	36.00a	38.00a	2.00a
Rice/ mucuna/ cymbopogon +1 hr	47.10ab	9.00a	28.00b	34.00a	1.50b
Maize/ rice/ cymbopogon +1 hr	47.30ab	8.00a	24.30b	42.00a	1.50b
Maize/ mucuna/ cymbopogon +1 hh	52.00a	5.00a	31.00a	39.70a	1.80a
Maize/ rice/ mucuna +1 hh	38.00b	5.67a	30.00a	33.70a	1.80a
Rice/ mucuna/ cymbopogon +1 hh	44.00b	6.00a	24.30b	37.30a	1.50b
Maize/ rice/ cymbopogon + 1 hh	44.70b	4.67a	19.00b	34.70a	1.50b
Maize/ mucuna/ cymbopogon	60.30a	5.00a	29.30a	37.30a	2.00a
Maize/ rice /mucuna	60.70a	6.00a	28.20a	39.70a	2.00a
Rice/ mucuna/ cymbopogon	45.30b	6.00a	26.30b	33.70a	1.50b
Maize/ rice/ cymbopogon	45.70b	4.33b	17.00b	32.30a	1.20c
Weedy check	20.00c	0.00b	5.70c	30.30a	1.20c
P-value	<0.001	0.049	<0.001	0.760	0.002
LSD (P Ö0.05)	13.81	5.37	13.8	NS	0.46
CV (%)	17.20	49.7	29.0	21.2	16.2

Values with different letters in a column are significantly different at  $P \leq 0.05$ , NS = Not significant, hr = hand roguing, hh = hand hoeing. PH = plant height, LN = leaf number, LL = leaf length, LW = leaf width.

**Table 6.2 Rice growth parameters and striga per hundred rice plants in Pallisa (2015A)**

<b>Treatments</b>	<b>PH (cm)</b>	<b>Tillers</b>	<b>SHP</b>	<b>LN/ plant</b>	<b>LL (cm)</b>	<b>LW (cm)</b>
Butanil (PRE) + hand hoeing once	33.00a	4.33a	8.00a	20.67a	33.67a	1.00a
Hand hoeing twice	30.67a	5.00a	2.00b	20.67a	33.00a	1.00a
Hand hoeing thrice	30.33a	4.67a	3.00b	20.00a	34.33a	1.03a
Maize/mucuna/cymbopogon + 1hh	31.00a	3.33b	0.00c	26.33a	29.67a	1.13a
Maize/rice/mucuna +1 hh	32.00a	3.33b	3.00b	11.67b	25.00b	1.00a
Rice/mucuna/cymbopogon +1hh	26.33b	2.00b	0.00c	7.33b	19.67c	0.83b
Maize/rice/cymbopogon +1hh	29.00b	2.67b	0.00c	11.67b	24.33b	0.80b
Weedy check	11.00c	0.00c	0.00c	3.00c	18.33c	0.50c
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
LSD (P Ö0.05)	3.52	1.58	1.66	6.51	4.78	0.26
CV (%)	7.5	32.7	35.4	27.8	10.1	16.9

Values with different letters in a column are different at (P Ö0.05), SHP = Striga per hundred rice plants, 1hh = hand hoeing once, PH = Plant height, LN = Leaf number, LL = Leaf length, LW = Leaf width

Butanil application + hand hoeing once, hand hoeing twice and hand hoeing thrice produced significantly more tillers per rice plant than mulched treatments. Like the weedy check, mulching rice with cymbopogon stover recorded no striga attack. Application of maize/rice/mucuna mulch recorded higher striga weed count (3 weeds) than the cymbopogon mulches. Application of Butanil produced the highest number of striga (8 striga plants) at the peak count date (63 DAE). This was followed by counts of 3 and 2 striga for hand hoeing thrice and hand hoeing twice respectively. Butanil application, hand hoeing twice, hand

hoeing thrice and maize/mucuna/cymbopogon mulch produced significantly more and longer rice leaves than other treatments.



Plate 6.1 Rice (55 days after emergence of weeds) with maize/rice/mucuna (MzRMc) mulch followed by 1 hand roguing of weeds (42 DAE) showing good weed suppression ability and good rice growth.

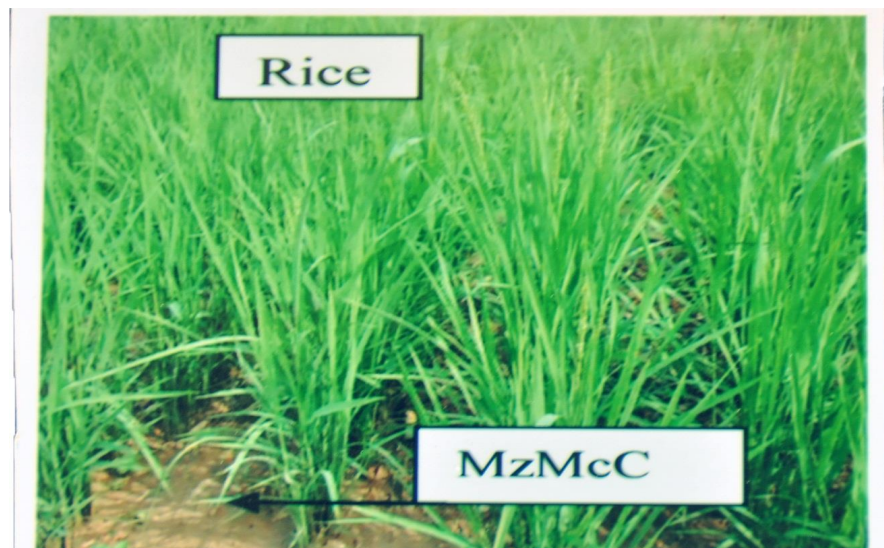


Plate 6.2 Rice (55 days after emergence of weeds) with maize/mucuna/cymbopogon (MzMcC) mulch followed by 1 hand roguing (42 days after emergence) of weed showing good weed suppression ability and good rice growth.

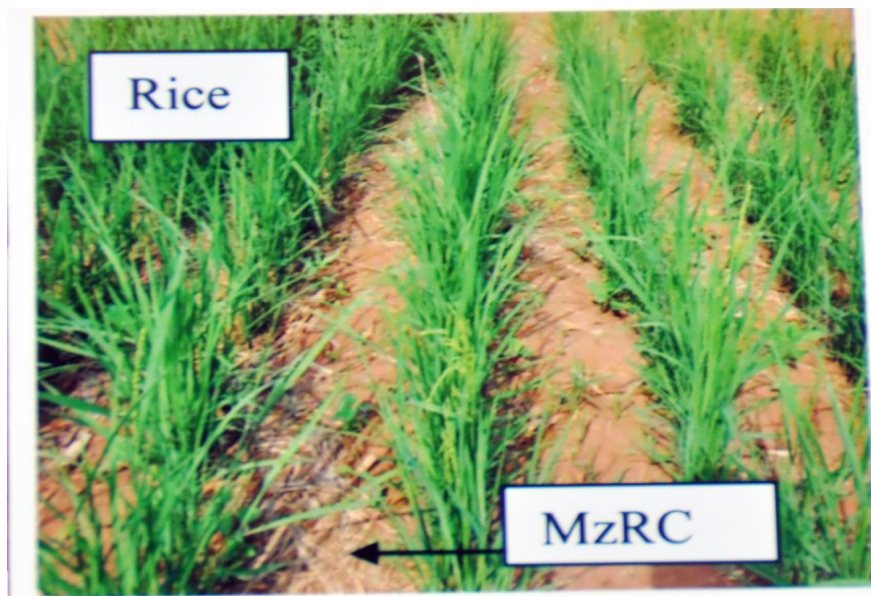


Plate 6.3 Rice with maize/rice/cymbopogon (MzRC) mulch at 55 days after emergence (DAE) of weeds followed by 1 hand rogueing of weeds (42 DAE) showing good weed suppression ability and poor rice growth.

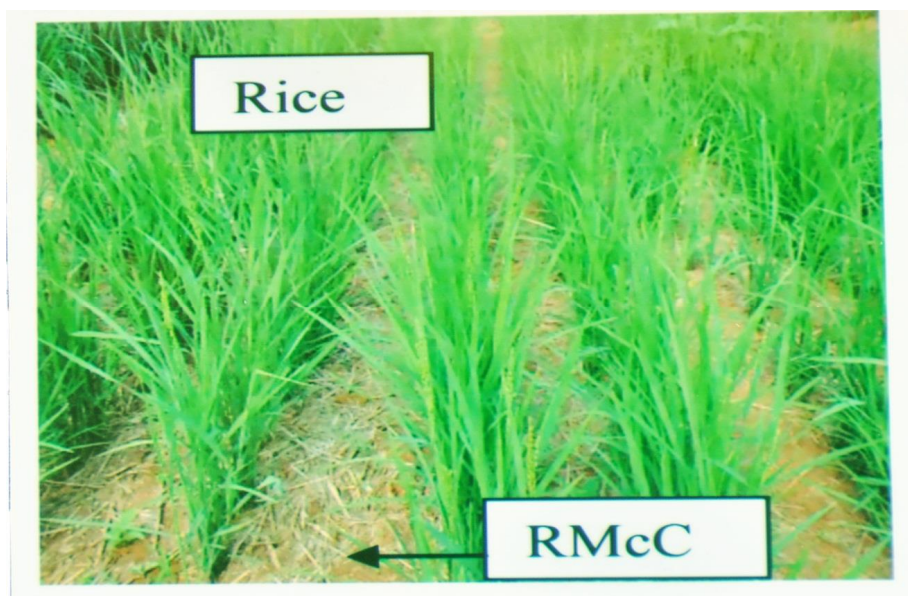


Plate 6.4 Rice with rice/mucuna/cymbopogon (RMcC) mulch at 55 days after emergence (DAE) followed by 1 hand rogueing of weeds (42 DAE) showing good weed suppression ability and poor rice growth.

Application of rice + mucuna + cymbopogon mulches produced rice with the shortest leaves. The weedy check gave the lowest rice plant height, number of tillers, leaf number, leaf length and leaf width.

### **6.3.3 Weed type, species, density and biomass under different mulches at Ikulwe site.**

At 42 days after application of the treatments (DAE), maize/ rice/ mucuna, rice/ mucuna/ cymbopogon, maize/ rice/ cymbopogon and maize/ mucuna/ cymbopogon mulches inhibited the germination of grasses more than broad leaved weeds but the weedy check produced the same density (60 weeds m<sup>-2</sup>) of both broad leaved weeds and grasses (Table 6.3). Application of maize/ rice/ mucuna mixed mulches significantly ( $P \leq 0.05$ ) reduced both the broad leaved and grass weeds. Maize/ rice/ mucuna mixed mulches produced the lowest weed density (46 weeds, 75 weeds and 35 weeds m<sup>-2</sup>) and biomass (120 g, 148g and 109 g m<sup>-2</sup>) per unit area at 42, 65 and 90 DAE of rice. Application of mixed mulches of rice/ mucuna/ cymbopogon, maize/ rice/ cymbopogon and maize/ mucuna/ cymbopogon produced higher weed density (50-99 weeds m<sup>-2</sup>) and biomass (136-213 weeds m<sup>-2</sup>) than the maize/ rice/ mucuna mulches between 42 and 90 DAE.

The weedy check recorded the highest weed density (120-150 weeds m<sup>-2</sup>) and biomass (283-346g m<sup>-2</sup>) per unit area between 42 and 90 DAE. The number of weed species namely *Digitaria scalarum*, *Ageratum conyzoides*, *Gallinsoga parviflora*, *Commellina bengalensis*, *Amaranthus retroflexus*, *Eleusine indica*, *Spigelia anthelmisa*, *Euphorbia heterophylla*, *Bracharia scalaris*, *Portulaca olerace*, *Bidens pilosa*, *Eleusine indica* and *Sorghum halepense* were not influenced by the treatments.

**Table 6.3 Weed types, species, density and biomass under combined mulches at Ikulwe during 2014A**

Treatment	Weed Type / Spp.			Days after emergence					
	(42 DAE)			42	65	90	42	65	90
	BW	G	WS.	Weed density (Weeds m <sup>-2</sup> )			Biomass (g m <sup>-2</sup> )		
Maize/rice/mucuna	45.0c	1.0d	12.0a	46.0b	74.5b	34.50b	120.0c	148.0c	109.0d
Rice/mucuna/cymbopogon	81.0a	5.0c	12.0a	86.0a	99.0a	49.5b	136.0c	158.0b	142.50c
Maize/rice/cymbopogon	78.0a	12.0b	13.0a	90.0a	87.0a	51.0b	150.0b	178.0b	171.0b
Maize/mucuna/cymbopogon	80.0a	16.0b	12.0a	96.0a	88.3a	69.0a	180.0b	213.0b	188.0b
Weedy check	60.0b	60.0a	14.0a	120.0a	150.0a	122.0a	315.0a	346.0a	283.0a
P-value	<0.01	< 0.01	0.54	0.04	0.11	0.05	0.02	0.02	<0.001
LSD (P 0.05)	3.6	4.8	NS	38.8	64.3	57.8	38.6	96.7	32.2
CV (%)	2.5	3.0	8.0	12.0	7.0	7.2	18.40	21.00	18.0

Values with different letters in a column are different at (P 0.05), BLW = Broad leaved weeds, NS = Not significant. DAE = Days after emergence of rice, BW = Broad leaved, G = Grasses, Weed species.

#### 6.4.4 Weed density and Biomass in Pallisa

Application of maize/ rice/ mucuna mixed mulches significantly reduced the weed density and biomass per unit area at 42, 65 and 90 DAE amongst all treatments. The weed density ranged from 340-840 weeds m<sup>-2</sup> (Table 6.4). Maize/ rice/ cymbopogon, rice/ mucuna/ cymbopogon and maize/ mucuna/ cymbopogon mixed mulches produced higher weed density and biomass per unit area at 90 DAE than mixed mulches of maize/ rice/ mucuna. The weedy

check recorded the highest weed density (215-931 weeds m<sup>-2</sup>) and biomass (150-272 g m<sup>-2</sup>) at 42, 65 and 90 DAE.

**Table 6.4 Weed density and biomass as influenced by mixed crop mulches in Pallisa (2015A)**

Treatments	Weed Density (Weeds m <sup>-2</sup> )			Weed Biomass (g m <sup>-2</sup> )		
	42 DAE	65 DAE	90 DAE	42 DAE	65 DAE	90 DAE
Maize/Rice/Mucuna	105.00c	302.00b	340.00d	126.00b	138.00c	167.00c
Maize/Rice/Cymbopogon	125.00c	144.00c	345.00d	103.60b	142.00b	190.00b
Rice/Mucuna/Cymbopogon	158.00b	200.00b	525.00c	129.40b	161.80b	194.00b
Maize/Mucuna/Cymbopogon	175.00b	233.00b	573.00b	131.00b	154.90b	201.00b
Weedy check (Control)	215.00a	931.00a	840.00a	150.00a	272.00a	249.00a
P-value	0.002	<0.001	<0.001	0.002	<0.001	<0.001
LSD (P 0.05)	26.96	87.60	26.96	18.18	22.99	18.18
CV (%)	13.0	8.0	8.1	15.3	14.0	12.8

Values with different letters in a column are significantly different at (P 0.05), DAE = Days after emergence of rice

## 6.4.5 Yield parameters and yield at Ikulwe and Pallisa

### 6.4.5.1 Ikulwe station

The highest number of rice panicles per plant was produced when the plots were hand hoed twice or thrice (Table 6.5). Mulches of maize/ rice/ mucuna, rice/ mucuna/ cymbopogon, maize/ mucuna/ cymbopogon and maize/ rice/ cymbopogon each followed by hand-roguing of weeds once produced similar number of rice panicles per plant to hand hoeing twice or thrice treatments. Hand-hoeing alone and mulching followed by post hand-roguing had higher number of panicles than application of Butanil followed by hand hoeing. The latter

treatment gave similar number of panicles per plant to treatments with or without a post mulch hand hoeing. Application of the different weed control treatments did not significantly influence the filling of panicles per plant. Rice/ mucuna/ cymbopogon mixed mulch produced the lowest number of grains per panicle among all the treatments. Hand hoeing twice, hand hoeing thrice, application of Butanil + hand hoeing once, maize/ rice/ mucuna mulch + hand roguing once and rice/ mucuna/ cymbopogon mulch + hand-hoeing once treatments produced the highest filled grains per panicle.

Application of different mixed mulches without a post weed control operation produced the lowest percent filled grains per panicle. The weedy check produced no panicles and zero grain yields. Hand hoeing thrice gave the highest grain yield ( $1125 \text{ kg ha}^{-1}$ ) followed by hand hoeing twice ( $1031 \text{ kg ha}^{-1}$ ) and maize/ rice/ mucuna + hand-roguing once ( $1037.50 \text{ kg ha}^{-1}$ ). The grain yield under Butanil ( $796 \text{ kg ha}^{-1}$ ) and rice/ mucuna/ cymbopogon followed by hand hoeing once ( $876 \text{ kg ha}^{-1}$ ) were not significantly different. Generally, application of a post-mulch hand hoeing gave higher grain yield than treatments given post mulch hand roguing except for maize/ rice/ mucuna followed by one hand-roguing of weeds. The lowest rice grain yield was recorded under application of mulches without a post mulch weed control operation. The maize/ cymbopogon mulch treatments with or without a post mulch weed control option produced lower rice grain yield than rice/ mucuna treatment. The weedy check had zero rice grain yields.



**Table 6.5 Yield parameters and grain yield of rice at Ikulwe station (2014A)**

Treatments	PP	PFPP (%)	GP	PFGP (%)	Yield (kg ha <sup>-1</sup> )
Three hand-hoeing (hh)	4.50a	45.00a	117.70a	79.40a	1125.0a
Two hand-hoeing (hh)	6.50a	55.30a	112.50a	85.40a	1031.0b
Maize/rice/mucuna +1hr	4.80a	65.00a	124.80a	72.90a	1037.5b
Butanil-70 (PRE) + 1hh	3.90b	45.50a	130.70a	85.30a	796.0c
Rice/mucuna/cymbopogon + 1hh	3.10b	41.50a	112.80a	70.30a	785.5c
Maize/rice/mucuna +1hh	3.70b	47.30a	114.10a	61.40b	730.0d
Maize/mucuna/cymbopogon +1 hh	3.70b	46.00a	131.60a	46.10b	637.5e
Maize/rice/cymbopogon + 1hh	2.50b	72.90a	112.80a	62.10b	600.0e
Rice/mucuna/cymbopogon +1hr	5.40a	39.70a	124.70a	64.60b	640.9e
Maize/mucuna/cymbopogon +1hr	5.50a	43.30a	123.60a	65.00b	631.0e
Maize/rice/cymbopogon +1hr	4.50a	72.90a	113.50a	64.90b	400.0f
Maize/ rice/mucuna	3.50b	42.30a	128.40a	38.00c	626.2e
Rice/mucuna/cymbopogon	3.40b	35.30a	85.10b	43.40c	371.2f
Maize/mucuna/cymbopogon	3.50b	45.70a	128.10a	59.90b	246.2g
Maize/rice/cymbopogon	3.00b	69.70a	113.20a	45.10c	262.5g
Weedy Check	0.00c	0.00b	0.00c	0.00d	0.00i
P-value	0.001	0.024	<0.001	<0.001	<0.001
LSD (P<0.05)	2.28	37.69	28.58	19.14	45.05
CV (%)	2.0	4.0	4.0	3.0	12.0

Values with different letters in a column are significantly different at (P<0.05), PP = Panicles per plant, PFPP = Percent filled panicles per plant, PFGP = Percent filled grains per panicle, GP = Grains per panicle, hh = hand hoeing, hr = hand roguing. % = Percent, ha = hectare, Kg = Kilograms.

#### 6.4.5.2 Pallisa site

Hand hoeing twice and hand hoeing thrice produced a higher number of grains per panicle in Pallisa site than other treatments (Table 6.6). Maize/ mucuna/ cymbopogon mulch followed by hand hoeing once also had higher number of grains per panicle than most treatments. Hand hoeing thrice and hand hoeing twice gave higher rice grain yield than application of butanil and rice/ mucuna/ cymbopogon, maize/ mucuna/ cymbopogon, maize/ rice/ mucuna and maize/ rice/ cymbopogon mixed mulches which gave significantly lower grains per panicle and yield than hand hoeing alone. Maize/ rice/ cymbopogon mulches produced the lowest rice grain yield and the weedy check yielded no grains.

**Table 6.6 Yield parameters and grain yield of rice on-farm at Pallisa**

Treatments	GP (Grains)	PFGP (%)	Yield (kg ha <sup>-1</sup> )
Hand-hoeing thrice	123.50a	81.70a	1096.00a
Hand-hoeing twice	107.00a	79.60a	969.40b
Butanil (PRE) + 1hh	81.00c	77.30a	485.50c
Rice/mucuna/cymbopogon + 1hh	77.00c	87.50a	539.40c
Maize/mucuna/cymbopogon +1hh	88.50b	76.10a	414.00d
Maize/rice/mucuna +1hh	76.00c	85.20a	461.00d
Maize/rice /cymbopogon + 1hh	66.00c	89.40a	105.60e
Weedy Check	0.00d	0.00b	0.00f
P-value	0.001	0.65	<0.001
LSD (P<0.05)	25.35	NS	69.23
CV (%)	15.0	18.0	14.5

Values with different letters in a column are significantly different at (P<0.05), GP = Grains per panicle, PFGP = percent filled grains per panicle, % = percent, ha = hectare, kg = kilograms

## 6.4.6 Economics of weed control methods for rice at Ikulwe and Pallisa

### 6.4.6.1 Ikulwe site

Hand hoeing twice, maize/ rice/ mucuna + hand-rogueing once, hand-hoeing thrice, Butanil + hand-hoeing once, rice/ mucuna/ cymbopogon + hand-hoeing once gave higher gross and net monetary returns than other treatments (Table 6.7).

**Table 6.7 Economics of weed control methods for rice at Ikulwe site**

Treatments	Production costs (Ush)	Gross returns ha <sup>-1</sup> (Ush)	Net returns ha <sup>-1</sup> (Ush)	ROI
Hand-hoeing twice	1,353,000	2,062,000	709,000	0.52
Maize/rice/mucuna +1 hr	1,404,000	2,070,000	666,000	0.47
Three hand-hoeing	1,562,000	2,250,000	688,000	0.44
Butanil + 1hh	1,230,000	1,592,000	362,000	0.29
Rice/ mucuna/ cymbopogon + 1hh	1,151,000	1,571,000	420,000	0.36
Maize/ rice/ mucuna mulch + 1hh	1,524,000	1,252,000	-271,000	-0.17
Maize/ mucuna/ cymbopogon +1hr	1,430,000	1,262,000	-168,000	-0.12
Maize/ mucuna/ cymbopogon + 1hh	1,514,000	1,275,000	-239,000	-0.16
Rice/ mucuna/ cymbopogon +1hr	1,540,000	1,280,000	-260,000	-0.17
Rice/ mucuna/ cymbopogon	1,090,000	742,000	-348,000	-0.32
Maize/ rice/ mucuna +1hh	1,523,000	1,460,000	-63,000	-0.04
Maize/ rice/ cymbopogon +1hr	1,390,000	800,000	-590,000	-0.4
Maize/ rice/ cymbopogon	1,076,000	524,000	-552,000	-0.5
Maize/ mucuna/ cymbopogon	1,075,000	492,000	-583,000	-0.5
Maize/ rice/ cymbopogon + 1hh	1,390,000	1,200,000	-190,000	-0.14
Weedy Check	1,050,000	0.000	-1,050,000	-1.00

ROI = Returns per Uganda shilling investment, hh = hand-hoeing, hr = hand-rogueing, Ush = Uganda shilling

The treatments also produced positive returns per shilling investment. Mulches of maize/rice/cymbopogon and maize/mucuna/cymbopogon mulches without a post mulch operation gave the lowest monetary gross returns, net returns and returns per shilling investment. Generally, application of maize/mucuna mulches recorded higher returns than rice/cymbopogon crop mulches with or without a subsequent weed control operation. The weedy check gave the lowest (-1.00) net returns on investment.

#### **6.4.6.2 Pallisa**

Hand hoeing twice and hand-hoeing thrice gave higher gross returns, net monetary returns and positive returns per shilling invested than application of Butanil + hand-hoeing once and other treatments (Table 6.8). Application of Butanil + hand hoeing once gave negative returns in investment (-0.21). Maize/ rice/ cymbopogon and maize/ mucuna/ cymbopogon mulches each followed by hand hoeing once gave the lowest gross returns and returns per shilling invested. The weedy check gave the lowest (-1.00) returns on investment amongst all treatments

**Table 6.8 Economics of different weed control options tin rice at Pallisa site**

<b>Treatments</b>	<b>Production costs (Ush)</b>	<b>Gross returns ha<sup>-1</sup> (Ush)</b>	<b>Net returns ha<sup>-1</sup> (Ush)</b>	<b>ROI</b>
2 hand hoeing	1,353,000	1,938,800	585,000	0.43
3 hand hoeing	1,562,000	2,192,000	630,000	0.40
Butanil (PRE) + 1hh	1,230,000	971,000	-259,000	-0.21
Rice/ mucuna/ cymbopogon + 1hh	1,151,000	1,078,000	-450,200	-0.39
Maize/ rice/ mucuna +1 hh	1,523,000	922,000	-601,000	-0.39
Maize/ mucuna/ cymbopogon + 1 hh	1,514,000	828,000	-686,800	-0.45
Maize/ rice/ cymbopogon + 1 hh	1,390,000	211,200	-1,317,800	- 0.94
Weedy Check	1,050,000	0.000	-1,050,000	-1.00

PC = Production cost, GR = Gross returns, NR = Net returns, ROI = Returns on Investment,

Ush = Uganda shillings

## **6.5. Discussion**

### **6.5.1 Rice growth under different weed control treatments at Ikulwe station and on-farm in Pallisa**

Mulches of maize/mucuna/cymbopogon with or without a post mulch weed control technology, Butanil + hand hoeing once, hand hoeing twice or thrice increased rice plant height, leaf number and leaf width. The improved rice growth parameters under hand weeding and Butanil herbicide application may be attributed to increased uptake of water, nutrients and absorption of solar radiation under conditions of reduced competition between rice and weeds. Labrada (2003) reported the effects of weeds on rice as reduced yield and quality mostly due to competition for nutrients, water and sunlight. In upland direct seeded rice, yield reductions were reported to range between 35 and 45%. The enhanced rice growth parameters with application of maize/mucuna/cymbopogon mulches may be attributed to additive positive allelopathic effects of the bio-compounds particularly 1,4-Eicosadiene, 2,5-Di-tert-butylphenol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol which were commonly exuded by maize and mucuna (Chapter 3 of this thesis) on physiological processes for rice growth such as nutrient uptake. Belz *et al.* (2005) and Abbas *et al.* (2017) reported increased weed emergence and seedling growth from wheat fields treated with allelopathic mulches which were partially attributed to the hormetic effects of the allelochemicals at low concentrations. Munir, (2011) reported that allelopathic extracts lethal to young weeds but stimulatory to crops may be attributed to enhanced membrane stability and water relations among other mechanisms.

Treatment with maize/ rice/ cymbopogon mulch and the weedy check recorded the lowest number of tillers per rice plant. The low number of tillers per rice plant under the mixed

mulches may be attributed to antagonistic inhibitory effects of some molecules in the compounds identified in the plant stover (Chapter 3 of the thesis) on rice nutrient uptake, growth and tiller development. In chapter 3 of this thesis, 2-Ethylhexanol phenol, Tert-Amylbenzene, pentamethylbenzine, 1,2-Di-tert-butylbenzene, 2,3-Dimethylundecane, naphthalene and 1-Sec-Butyl-4-methylbenzene compounds were identified in stover of each of rice and maize and could have by additive and synergy actions inhibited rice growth and development processes.

The reduced number of rice tillers under the weedy check could have also resulted from allelopathy or from competitions for light, water, nutrients and other resources between weeds and rice under the weedy check. Labrada, (2003) reported some of the effects of weeds on rice as reduced yield and quality, mostly due to competition for nutrients, water and sunlight.. Weeds have been reported by Hajizadeh and Mirshekari, (2011) as a serious biotic stress in cropping systems that cause a reduction in the growth and yield of crops by interfering with different metabolic processes. Allelopathic inhibitory effects of weeds on crops were observed by Zohaib *et al.* (2016) who noted that the released allelochemicals by allelopathic weeds cause substantial reduction in germination, growth and yield of the crop plants by altering various physiological processes such as enzyme activity, protein synthesis, photosynthesis, respiration, cell division and enlargement. . Kobayashi, (2003) and Zohaib *et al.* (2016) reported that allelochemicals from crop mulches influence crop growth indirectly by altering soil properties and inhibiting soil micro fauna. Touré *et al.* (2013) reported the critical period for weed control in rice to be between 14 and 42 DAE of rice.

### 6.5.2 Striga growth

The striga count in Pallisa significantly ( $P < 0.05$ ) reduced under treatments with cymbopogon mulches and increased under maize/rice based mulch. This suggests that the secondary metabolites produced by cymbopogon have inhibitory effects on striga attachment on rice roots. The major secondary metabolites produced by cymbopogon stover in chapter 3 of this thesis included citronellal,  $\alpha$ -Citral, cis-Geraniol, Trans-Carane, eugenol, geraniol acetate,  $\alpha$ -Elemen, caryophyllene,  $\alpha$ -Gurjunene,  $\alpha$ -Cadinene and citronellyl butyrate. The bio-compounds possibly reduced the quality and quantity of strigolactones; the chemical elements, responsible for successful striga attachment and development. The inhibitory effect of *C. nardus* have been observed on the shoot and root growth of cress, lettuce, rapeseed and Italian rye grass at concentrations  $\times 0.03$  g dry weight equivalent extract per milliliter (Prapaipit *et al.*, 2013). Citral of cymbopogon was reported to significantly reduce the chlorophyll and carotenoid contents of barnyard grass by Poonpaiboopipat *et al.* (2013).

The higher striga count due to maize and rice mulch could be attributed to two phenolic compounds namely 2,5-di-tert-butyl- Phenol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and one terpenoid namely 1,4 Eicosadiene that were identified as common compounds in both maize and rice mulch (Chapter 3 of this thesis) which may have stimulated the striga attachment in a similar manner to the strigolactones. Pandey *et al.* (2016) reported strigolactones from root exudates of rice to stimulate the germination of parasitic plant seeds.

### 6.5.3 Weed density and biomass under mulched rice

Mixed maize, rice, mucuna and cymbopogon mulches inhibited the germination of grasses more than the broad leaved weeds. This may be attributed to possible phytotoxic effects of



compounds identified in chapter three of this thesis from mixed mulches of maize, rice, mucuna and cymbopogon on enzymic processes involved in the germination and development of grass seeds. Germination of grass seeds is influenced by a chemical effect on amylase enzyme in seeds which catalyses the hydrolysis of starch, following imbibition of water, into simple sugars using Gibberellic acid (GA). This is coupled with the hydrolysis of stored protein into amino acids (Jacobsen, 1995). The results are supported by Iman *et al.* (2006) who reported that allelopathy influenced seed germination and seedling development by preventing cell division and inhibiting cell elongation. Li *et al.* (2010) similarly reported that the inhibition of germination and seedling growth by allelochemicals is caused by disturbance in hormonal balance, respiration, photosynthesis and interference in cell growth.

Maize, rice and mucuna mixed mulches reduced the density and biomass of weeds more than mulches with cymbopogon at all crop stages under this study. Inhibitory effects on weed growth by the different mixed mulches may be attributed to higher potency of their allelochemicals on target weeds. In chapter 3 of this thesis, five compounds namely 1,4-Eicosadiene; 2,5-di-tert-butyl- Phenol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; (9Z)-9-Icosen-1-ol and Butylated Hydroxytoluene were commonly identified in the stover of maize, rice and mucuna) and the molecules in the compounds possibly additively inhibited processes that promote the growth of weeds.

The cymbopogon based mulch treatments namely maize/ rice/ cymbopogon, rice/ mucuna/ cymbopogon and maize/ mucuna/ cymbopogon, each had four similar compounds namely 1,4-Eicosadiene, 2,5-di-tert-butyl- Phenol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and (9Z)-9-Icosen-1-ol but had no compound in common with cymbopogon crop mulches. This probably explains the lower efficacy by the cymbopogon based treatments on weed density

and biomass. The low efficacy may be attributed to possible antagonistic effects by molecules on processes of weed growth and development. Allelopathic effects of bio-compounds in related crop surface mulches indicate that sorghum surface mulch (10-15Mt ha<sup>-1</sup>) applied at sowing controlled weeds and increased maize yield significantly (Cheema *et al.*, 2004). Cheema *et al.*, (2010a) reported mixture of sorghum + sunflower (18 L ha<sup>-1</sup>) to suppress the density and dry weight of weeds.

#### **6.5.4 Yield parameters**

Hand hoeing twice, hand hoeing thrice, maize/rice/mucuna mulches + hand roguing of weeds once and Butanil herbicide + hand hoeing once produced the highest number of panicles per rice plant, percentage filled grains and grain yield. This may be attributed to lower weed biomass and density that could have led to improved nutrient uptake and reduced competition for crop growth resources. Weeds were reported to reduce yields by 35 to 45 percent by Labrada (2003) due to competition for nutrients, water and sunlight. Devasinghe *et al.*, (2011) found an inverse correlation between the rice grain yields, weed biomass and weed density. Mahajan and Chauhan. (2013) observed reduced total weed biomass (68-75%) with an increased corresponding grain yield (119-149%) for the non-treated control under a single application of herbicides. Zohaib *et al.*, (2016) reported that weeds interfere with crops through competition and allelopathy.

Percent filling of panicles per plant was lowest under mulched treatments without a post mulch weed control operation and the weedy check produced zero grain yields. Reduced productivity under relatively weedy conditions may be associated with increased chemical interference of allelochemicals in the mulch and weeds on rice reproductive functions.

Results may also be attributed to increased competition between rice and weeds for water, nutrient and solar radiation. Abouziena and Haggag (2016) reported crop yield reductions of more than 50 percent due to weeds under water stress conditions.

### **6.5.5 Economics of allelopathic weed control methods in rice**

Treatments of two and three hand hoeing, maize/ rice/ mucuna mulch + hand roguing once, Butanil + hand hoeing once and rice/ mucuna/ cymbopogon mulch + hand hoeing once gave higher gross and net monetary returns than other treatments. The treatments also produced positive returns on investment (ROI) per Uganda shilling invested. The increased gross returns, net monetary returns and high ROI observed may be attributed to higher yields and gross returns under the treatments. Higher ROI were under maize + mucuna than under rice/ cymbopogon mixed mulches. This may be associated with the enhanced rice growth with higher leaf number, leaf width and higher grain yields observed under the maize + mucuna treatments (chapter six of the thesis).

The reduced growth and yield under rice/cymbopogon mulches are associated with inhibitory effects of the phytotoxins in the mulches on physiological processes vital for growth such as nutrient uptake. Several of the associated compounds were identified in chapter three of the thesis. The negative ROI under rice/cymbopogon based mulches and other mulched treatments were due to the low yields associated with the poor rice growth, low yield and yield attributes under the treatments. The weedy check produced no grain yield and gave the lowest returns on investments. Kaiira *et al.* (2014) reported higher returns on investment under pre-Atrazine + hand hoeing once and hand hoeing twice (180%), followed by post Atrazine + hand hoeing once (167%) in maize. The no weeding treatment registered the

lowest value (67%). Punit *et al.*(2018) reported the traditional method of growing rice as more expensive (Rs. 14014.54 per acre) than improved SRI method (Rs.12154.63 per acre). Hand hoeing twice, hand hoeing thrice and application of Butanil followed by hand hoeing once increased rice growth, yield parameters, yield and returns on investment (ROI) relative to other treatments. Rice /cymbopogon based mulches most effectively controlled weeds including the noxious striga in upland rice, but maize /mucuna mulches had higher rice growth, grain yield and ROI than under rice/cymbopogon mulches. Cymbopogon based mulches effectively controlled striga and other weeds and the lowest effects were with maize and rice mulches. All mulches controlled grasses much more than broad leaved weeds. Maize, rice, cymbopogon and mucuna have the potential to produce bio-herbicides for weed control in the drylands.

## CHAPTER 7

### WEED CONTROL USING PLANT POWDERS AND WATER EXTRACTS OF RICE, MAIZE, CYMBOPOGON, MUCUNA AND MAIZE

#### 7.1 Abstract

Sustainable agriculture is being emphasised and increased concerns about the adverse effects of extensive use of farm chemicals have been raised. Allelopathic water bio-extracts have a high potential in weed management. A screen house experiment was conducted during 2013B at Namulonge Research station under a completely randomised design and a field study was carried out during 2015A at Ikulwe station in Uganda in a randomised complete block design to determine the efficacy of leaf, stem and root (LSR) powders and water extracts from *Mucuna pruriens*, *Cymbopogon nardus*, *Desmodium uncinatum*, *Zea mays* and *Oryza sativa* in weed control. The treatments were arranged in a randomised complete block design and replicated thrice. The screen house study included eight mixed plant LSR powders combined as mucuna/ desmodium/ cymbopogon, maize/ mucuna/ cymbopogon, rice/ desmodium/ cymbopogon, mucuna/ desmodium/ cymbopogon, maize/ rice/ mucuna, maize/ desmodium/ mucuna, rice/ mucuna/ cymbopogon and maize/ rice/ cymbopogon. The LSR powders were mixed with soil in the ratios of 15, 30 and 45 % (w/w) with a control without powders. The mixtures were then put in pots measuring 10 x 10 x 10 cm and 20 seeds of *Bidens pilosa* were sown in each pot and watered every two days for four weeks. In the field study, 24 kg of stover each from rice, cymbopogon, mucuna and maize were chopped into 2 cm pieces and soaked in 24 litres of tap water for 48 hours to make 100 percent extract solutions. The solutions were filtered and mixed to make 100 percent combined extracts of maize/ rice/ mucuna (MzRMc), rice/ mucuna/ cymbopogon (RMcC), maize/ rice/ cymbopogon (MzRC), maize/ mucuna/ cymbopogon (MzMCC), maize/ rice/ desmodium (MzRD), maize/

desmodium/ mucuna (MzDMc), rice/ mucuna/ desmodium (RMcD), rice/ cymbopogon / desmodium (RCD), maize/ cymbopogon/ desmodium (MzCD) and maize/ mucuna/ desmodium (MzMcD). The water extracts and Butanil were applied to weed free soil. The treatments were laid out in a randomized complete block design with three replications. A weedy check was also used as a control. The data were collected at 42 and 65 days after application of the extracts (DAA). In the screen house, increasing the concentrations (15-45 percent; w/w) of MzRC, RDC, RMcC, MzDMc, MzRMc, MzMcC, McDC and MzDC powders, significantly ( $P \leq 0.05$ ) reduced the number of weeds and dry biomass at 30 days after application (DAA) of powders. Rice and cymbopogon based powders and water extracts controlled the weeds more than maize and mucuna powders and extracts. Cymbopogon/ desmodium based powders and water extracts had the lowest weed control effect. Rice/ cymbopogon and maize/mucuna based water extracts most effectively reduced weed density and biomass per unit area and the crops have high potential for the development of herbicides.

**Key words:** *Bidens pilosa*, leaf/ stem/ root, water extracts, weed biomass, weed density.

## 7.2 Introduction

Weeds are a major constraint to direct seeded rice (Rao *et al.*, 2007). The development of resistance in some previously susceptible weed species and environmental pollution in soils are some of the major drawbacks associated with chemical herbicide use (Macias *et al.*, 2007). The potential of plant species to control weeds can be exploited in many ways including utilisation of water extracts from plant species. Jamil, (2009) reported that weed management using allelopathy is an environmentally friendly alternative approach in field crops and improves the crop yields without detrimental effects. Allelopathy is described as any direct or indirect harmful or beneficial effects of one organism on another through the production of chemical compounds that it releases into the environment (Rice, 1984).

Allelopathy can be divided into two categories namely 'true allelopathy' and 'functional allelopathy' (Zimdahl, 2013). True allelopathy involves the direct release of allelochemicals into the environment without any chemical or microbial metabolism via leaf leachates, volatiles or root exudates (Baratelli, 2012). Allelochemicals can also be released from seeds and flowers (Farooq *et al.*, 2011). On the other hand, allelochemicals may be released from plant mulches usually applied under field conditions which decompose to produce phyto-active secondary compounds by chemical or microbial metabolism. This phenomenon is called functional allelopathy (Zimdahl, 2007).

Besides their other potential values, the bio-active compounds in mucuna, cymbopogon, desmodium, rice and maize may be utilised for weed control as liquid synthetic derivatives of

naturally occurring compounds. Several researchers, including Iqbar and Cheema, (2010b) and Jabran *et al.* (2015), have demonstrated that exploitation of allelopathic water extracts has great potential for effective and sustainable weed control in agriculture. Cheema *et al.*, (2010a) reported suppressed weed dry weight by 86-100% when reduced rates of atrazine were used in combination with sorghum and sunflower water extracts at 10 L ha<sup>-1</sup>. Sorgaab at 12 L ha<sup>-1</sup> combined with Ethoxy sulfuron at 15 g a.i. ha<sup>-1</sup> and Butachlor at 600 g a.i. ha<sup>-1</sup> reduced weeds by 77 and 68%, respectively, which was to the equivalent to the effects of these herbicides.

Widespread herbicide resistance in weeds has created great interest in the development of herbicides with novel target sites, particularly herbicides developed from natural plant products (Duke *et al.*, 2000). The triketone herbicides, including mesotrione, the few known bio-herbicides were developed through the optimization of leptospermone, a natural plant product produced by the bottlebrush plant (*Callistemon citrinus*) that exhibits herbicidal activity (Mitchell *et al.*, 2001). In Eastern Uganda, the problematic weeds in rice were classified as broad leaved, grasses and sedges. Hand hoeing is the most common weed control method followed by the use of Butanil 70 (Butachlor + Propanil) as both a pre and post emergence herbicide. Hand hoeing labour was however, reported to be expensive and unreliable (Kikafunda, 2000). The 2-4 D amine herbicide is the common post emergence synthetic herbicide for broad leaved weeds. The objective of the study was therefore to determine the allelopathic potential of mixed leaf, stem and root powders and liquid formulations from bio-extracts of rice, cymbopogon, mucuna and maize in controlling weeds.



## **7.3 Materials and methods**

### **7.3.1 Screen house study of powdered formulations**

#### **7.3.1.1 Experimental design and treatments**

A screen house experiment was conducted during 2013B at Namulonge Research Station in Uganda to determine the herbicidal effects of powdered formulations from dry leaves, stems and roots (LSR) of three months old *D. uncinatum*, *M. pruriens*, *O. sativa* and *Z. mays* and one year old *C. nardus* powders on weeds. The LSR powders were combined in equal proportions of three to make eight powdered mixtures namely maize/ desmodium/ cymbopogon, maize/ mucuna/ cymbopogon, rice/ desmodium/ cymbopogon, mucuna/ desmodium/ cymbopogon, maize/ rice/ mucuna, maize/ desmodium / mucuna, rice/ mucuna/ cymbopogon and maize/ rice/ cymbopogon. The powders were mixed with sandy loam soil to make ratios of 15, 30 and 45% w/w (LSR powders to soil mixtures) and applied to seventy two pots filled with air dried soil. The treatments were arranged in a randomised complete block design with three replicates. The control pots were not supplied with LSR powders. Twenty seeds of *Bidens pilosa* were sown in each pot and watered initially with 250 ml and later 100 ml of tap water every two days for 4 weeks and data was collected on number and biomass of weeds. The collected data was used to determine four allelopathic LSR powders with the highest efficacy in weed control. The corresponding test plants were identified for subsequent experiments. The vegetative parts of the test plants were combined as bio-active mulches for Ikulwe station (2014A) and Pallisa on farm (2015A) studies and for the liquid bio-extracts (2015B) study.

### **7.3.1.2 Data collection**

Both the sown and volunteer weeds were counted on a weekly basis and data was collected on the species and total number of weeds. All the weeds in each pot were harvested at 30 days after emergence, counted and oven-dried at 80 °C for 12 hours till constant weight and weed dry biomass was determined.

## **7.3.2 Field experiment using liquid extracts**

### **7.3.2.1 Experimental design and treatments**

A field study was carried out at Ikulwe station during 2015B. Liquid bio-extracts were prepared from mature leaves, stems and roots of 90 days old fresh plant stover of cymbopogon, desmodium, mucuna, rice and maize. Twenty four kilograms of the stover were chopped into 2 cm pieces with a livestock feed chopper and soaked in 24 litres of tap water for 48 hours to make 100% extract solutions. The solutions were filtered and mixed in equal combinations of three extracts from the plants that exhibited the best weed control in both the screen house and mulch experiments (Chapter 6 of the thesis ) to make ten extracts namely maize/ rice/ mucuna, rice/ mucuna/ cymbopogon, maize/ rice/ cymbopogon, maize/ mucuna/ cymbopogon, maize/ rice/ desmodium, maize/ desmodium/ mucuna, rice/ mucuna/ desmodium, rice/ cymbopogon/ desmodium, maize/ cymbopogon/ desmodium and maize/ mucuna/ desmodium. The water extracts were applied to weed free soil. Thirty plots (4 m x 5 m) were laid out in a randomised complete block design with three replications. For comparison, a weedy check control and the recommended rate of Butanil (Butachlor + Propanil) at 1.8 kg a.i. ha<sup>-1</sup> was applied as a standard synthetic pre-emergence herbicide treatment.

### **7.3.2.2 Data collection and analysis**

Data were recorded at 42 and 65 days after application (DAA) of extracts, on the number of weeds and weed dry biomass per square metre using a 1 m x 1 m quadrant. Data collected were subjected to analysis of variance (ANOVA) using Genstat statistical package 13<sup>th</sup> edition, 2013. Fischer's least significant difference (LSD) test at  $P \leq 0.05$  was used to compare the treatment means.

## **7.4 Results**

### **7.4.1 Growth of weeds under leaf stem and root powders in a screen house**

Increasing the LSR powder concentrations from 15 to 45% significantly ( $P \leq 0.05$ ) reduced the number of sown *Bidens pilosa* weeds, volunteer weeds and weed biomass for all the treatments at 30 days after application (DAA) of powders (Table 7.1). Application of the rice/cymbopogon based powders namely rice/mucuna/cymbopogon and maize/ rice/ cymbopogon most effectively reduced the weed number and dry biomass. This was followed by the maize/mucuna based powders namely maize/ rice/ mucuna and maize/desmodium/mucuna. Application of rice/ desmodium and desmodium/ cymbopogon based powders had the lowest inhibitory effects on weed number and biomass. The treatment that was not supplied with any LSR powders produced the highest number of *Biden pilosa* weeds, total number of weeds and biomass.

**Table 7.1 Number of weeds and dry biomass at 30 days after application under leaf, stem and root powdered formulations**

<b>Treatments / LSR concentration (%)</b>	<b><i>Bidens pilosa</i> per pot</b>	<b>Total weeds per pot</b>	<b>Weed biomass per pot (g)</b>
Control (No LSR powder)	16.50a	26.50a	1.22a
Maize/ cymbopogon/ desmodium (15)	15.50a	25.50b	0.84b
Maize/ cymbopogon/ desmodium (30)	8.50c	15.50f	0.45d
Maize/ cymbopogon/ desmodium (45)	9.50b	13.50f	0.51d
Rice/ desmodium/ cymbopogon (15)	7.50c	20.50c	0.78c
Rice/ desmodium/ cymbopogon (30)	8.50c	17.50d	0.54d
Rice/ desmodium/ cymbopogon (45)	3.50e	12.50h	0.25e
Mucuna/ desmodium/ cymbopogon (15)	9.50b	18.50d	0.65c
Mucuna/ desmodium/ cymbopogon (30)	7.50c	16.50e	0.48d
Mucuna/ desmodium/ cymbopogon (45)	5.50d	13.50g	0.36e
Maize/ mucuna/ cymbopogon (15)	10.50b	20.50c	0.59d
Maize/ mucuna/ cymbopogon (30)	8.50c	20.50c	0.53d
Maize/ mucuna/ cymbopogon (45)	5.50d	12.50h	0.30e
Maize/ desmodium/ mucuna (15)	6.50d	13.50g	0.39e
Maize/ desmodium/ mucuna (30)	6.00d	14.50g	0.41e
Maize/ desmodium/ mucuna (45)	4.50e	10.50i	0.24f
Maize/ rice/ mucuna (15)	9.50b	17.50d	0.74c
Maize/ rice/ mucuna (30)	8.50c	16.50e	0.63d
Maize/ rice/ mucuna (45)	3.50e	16.50e	0.40e
Maize/ rice/ cymbopogon (15)	10.50b	16.50e	0.66c
Maize/ rice/ cymbopogon (30)	9.50b	14.50g	0.57d
Maize/ rice/ cymbopogon (45)	6.50d	10.50i	0.28e
Rice/ mucuna/ cymbopogon (15)	10.50b	16.50e	0.74c
Rice/ mucuna/ cymbopogon (30)	6.50d	12.50h	0.48d
Rice/ mucuna/ cymbopogon (45)	5.50d	13.00h	0.38e
P-value	< 0.001	< 0.001	< 0.001
L.S.D (P≤0.05)	1.42	1.43	0.20
CV (%)	3.0	1.5	0.6

Values with different letters in a column are different at p=0.05, LSR = Leaf/stem/root powder

#### 7.4.2: Aqueous formulations for weed control

Mixed aqueous extracts of maize and rice with cymbopogon or mucuna and extracts of rice or maize each mixed with mucuna and cymbopogon aqueous extract significantly ( $P \leq 0.05$ ) reduced the weed density, biomass per unit area and biomass per weed at 42 DAA compared to mixed extracts of rice or maize with cymbopogon, rice extract mixed with maize or mucuna and mixed maize and mucuna bio-extracts each mixed with desmodium (Desmodium based) bio-extracts (Table 7.2). Weed densities and biomass per unit area increased for all the treatments at 65 DAA relative to the observations at 42 DAA of the water extracts. Maize/ rice/ cymbopogon, rice/ mucuna/ cymbopogon, maize/ rice/ mucuna and maize/ mucuna/ cymbopogon extracts reduced the biomass per weed (0.1 g) at 42 DAA but the biomass per weed was not influenced under all desmodium water extracts (0.2g).. Desmodium water extracts namely rice/ cymbopogon/ desmodium, maize/ cymbopogon/ desmodium, rice/ mucuna/ desmodium, maize/ rice/ desmodium and maize/ mucuna/ desmodium increased weed biomass per unit area ( $54.3-74.3 \text{ g m}^{-2}$ ) and per weed (0.3-0.5 g) at 65 DAA. Maize/ rice/ mucuna, maize/ mucuna/ cymbopogon, rice/ mucuna/ cymbopogon and maize/ rice/ cymbopogon water extracts produced lower weed biomass per unit area ( $0.9-3.2 \text{ g m}^{-2}$ ) relative to all extracts with desmodium ( $3.3-38.5 \text{ g m}^{-2}$ ) at 42 DAA Application of Butanil produced the lowest weed density and biomass per unit area.

**Table 7.2 Weed density and biomass as influenced by water extracts in the field at Ikulwe site**

Treatment (Water-extract)	42 DAA			65DAA		
	Density (weeds m <sup>-2</sup> )	B/area (g m <sup>-2</sup> )	B/weed (g)	Density (weeds m <sup>-2</sup> )	B/area (g m <sup>-2</sup> )	B/weed (g)
Control (No bio-extracts)	201.0c	12.8c	0.2a	232.0g	72.7a	0.4b
Rice/Cymbopogon/Desmodium	216.0a	29.7b	0.2a	155.0i	74.3a	0.5a
Maize/Cymbopogon/Desmodium	209.0b	38.5a	0.2a	185.0h	48.5c	0.4b
Rice/Mucuna/Desmodium	79.0d	11.7d	0.2a	241.0g	60.6b	0.3c
Maize/Rice/Desmodium	16.0f	3.2e	0.2a	405.0e	54.3c	0.2d
Maize/Desmodium/Mucuna	28.0e	3.3e	0.2a	291.0f	57.7b	0.1e
Maize/Mucuna/Cymbopogon	29.0e	1.6f	0.1b	478.0d	37.3d	0.1e
Maize/Rice/Mucuna	16.0f	1.5f	0.1b	605.0a	49.3c	0.1e
Rice/Mucuna/Cymbopogon	7.0g	1.3g	0.1b	578.0b	29.3e	0.1e
Maize/Rice/Cymbopogon	17.0f	0.9g	0.1b	521.0c	24.3f	0.1e
Butanil 70	4.0g	0.6h	0.2a	85.0j	13.0g	0.2d
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (P ≤0.005)	5.76	0.55	0.05	15.56	3.46	0.01
CV (%)	4.0	3.4	3.5	12.4	6.4	2.3

Values with different letters in a column are significantly different at P≤0.05, DAA = Days after application of bio-extracts, B = Biomass

## 7.5 Discussion

### 7.5.1 Effect of leaf stem and root mixed powders and liquid extracts on weed growth

The number of volunteer weeds, sown *B. pilosa* and weight per weed significantly reduced with increased leaf, stem and root (LSR) powder and liquid extract concentration. The weed control efficacy by LSR powders declined in the order of rice/ cymbopogon + (maize or mucuna) > maize/ mucuna + (rice or cymbopogon) > maize/ rice or rice/ mucuna + (desmodium) > cymbopogon/ desmodium + (maize or rice). The higher weed control at increased powder concentrations may be attributed to higher concentrations of chemicals in the powders with allelopathic potential. Berendji *et al.* (2008) observed significant correlation between phenolic contents and percent inhibition of root growth in barnyard grass. Cai and Mu. (2012), similarly, observed inhibited growth of soybean at higher concentrations of *D. stramonium* extracts and associated the observations with allelochemical effects. Pereira *et al.* (2015) reported inhibited seedling growth of *Sesamum indicum* L. by different concentrations of ethanolic leaf extracts of *Serjania lethalis* leaf extracts. Roots of the test plants were most susceptible to the extracts than their shoots.

Rice/ cymbopogon/ mucuna and maize/ mucuna/ rice mixed powders and water extracts exhibited higher efficacies in weed control than maize/mucuna based mixtures and the lowest control was by cymbopogon/desmodium based powdered mixtures and liquid extracts. This may be attributed to additive effects of the identified compounds in the stover of the crops. In chapter 3 of this thesis rice and maize and mucuna stover had in common the metabolites namely 2 terpenoids namely Butylated Hydroxytoluene, 3 and 4 - Diethyl -1,1'-biphenyl and 3 phenols called 2, 5-Di-tert-butylphenol, 3, 7, 11, 15- Tetraethyl- 2- hexadecen-1 -ol and

(9Z)- 9- Icosen- 1- ol. Reduced weed control with application of desmodium extracts was probably due to antagonism between 2'-Diethylbiphenyl, 1-Ethyl-2-(1-phenylethyl) benzene metabolites identified only in desmodium stover and the five common compounds identified in rice, maize and mucuna stover.

Blouin (2004) indicated that herbicides applied in mixtures exhibited synergy compared with single herbicides under low infestation by weeds. Related studies on weed control through allelopathy by foliar sprays of allelopathic plant extracts have been conducted by several researchers. Cheema *et al.*, (2004) and Iqbar and Cheema (2008) indicated that Sorghum foliar spray controlled weeds relative to hand weeding, chemical herbicides and sorghum mulch. Hegazy and Farrag (2007) found that water, methanol and oil extracts of *Chenopodium ambrosioides* contained flavonoids, alkaloids, terpenoids and volatile oils that were inhibitory to the germination of weeds. Ali *et al.*, (2015) reported significant reductions in both germination and seedling growth of grass weeds at 2.5-10 percent concentration of aqueous leaf extracts of *Pinus eldarica*. The results in the current study are supported by Namkeleja *et al.*, (2013) who observed reduced seed germination and growth of *B. dictyoneura* and *C. ternatea* weeds by leaf and seed extracts of *Argemone mexicana*. Inhibition of *Brassica tournefortii* weed germination and seedling growth due to water extracts applied at a concentration of 10 g L<sup>-1</sup> has been reported by El-Gawad (2014). It is inferred from the study that the identified compounds in the stover of rice, cymbopogon, maize and mucuna could, at lethal doses, if appropriately combined be utilized to synthesize bio-herbicides for the control of weeds.



The increase in weed densities and biomass per unit area at 65 days after application of water extracts (DAA) compared to 42 DAA may be attributed to reduced efficacy of the extracts due to declines in concentrations of the bio-compounds within two months of being released into the soil. Barto and Cipollini (2009) reported that the half-life of allelochemicals varies from a few hours to a few months. Kong *et al.* (2008) reported that allelochemical degradation varied with allelochemical concentration, soil type, soil enzymes, soil microbial population and community structure.

There was increased weed density and biomass per unit area under rice/ cymbopogon/ desmodium and maize/ cymbopogon/ desmodium treatments, compared to the control that was not given any bio-extracts. The enhanced weed germination and growth could be attributed to growth stimulation at sub-optimal allelochemical doses (hormesis) by metabolites in the stover of rice, maize, desmodium and cymbopogon. The compounds namely 2 terpenoids namely 2, 2'-Diethylbiphenyl and 1-Ethyl-2-(1-phenylethyl) benzene were uniquely profiled in the stover of both rice and desmodium and an ester named Hexadecanoic acid was found in the rice stover alone. Falcarinol phenol and a terpenoid named Ionene were identified in the maize stover alone. The metabolites could have by antagonism interacted with compounds profiled in cymbopogon stover namely Citronellyl butyrate ester and the ten terpenoids called Citronellal, -Citral, cis-Geraniol, trans-Carane, Eugenol, Geraniol acetate, -Elemen, Caryophyllene, -Gurjunene, -Cadinene, causing the observed reduction in weed control. Hormesis is the stimulation of growth at sub-optimal levels of allelochemicals common in auxin herbicides which mimic the growth hormone auxin but which are lethal at higher doses (Allender 1997). Hormesis was also reported by Cedergreen and Olen (2010) when Glyphosate at 10% of the rate for field conditions

promoted crop growth. Investigations by Abbas *et al.* (2015) in a related study observed that Glyphosate hormesis at low doses (18-72 g a.i. ha<sup>-1</sup>) caused maximum growth and yield parameters of chick pea (*Cicer arietinum* L).

The lowest weed density and biomass per unit area under Butanil at 42 DAA may be associated with probable higher efficacy of the synthetic Butanil chemical in the control of germination and growth of weeds. The SLR powders and bio-extracts from rice, cymbopogon, maize and mucuna should be further investigated for potential production of triketone bio-herbicides. The bio-herbicides have high potential to control weeds since there are no reports of naturally occurring herbicide resistance to triketone herbicides (Heap (2007) and yet they are reported to have high efficacy (Danijela 2014). It is inferred from the study that allelopathic compounds at lethal doses from rice, mucuna, desmodium, cymbopogon and maize, if appropriately combined, could be utilized to control weeds and the weed control could be attributed to allelopathic effects of the compounds identified in chapter 3 of this thesis.

## **7.6 Conclusion**

Increasing the leaf, stem and root (LSR) powder concentrations (15-45 % w/w) significantly reduced the number of sown *B. pilosa*, volunteer weeds and weed weight at 30 days after application (DAA) of the powders. Rice/cymbopogon, maize/mucuna based LSR powder and water extract were the most effective in reducing weed density and biomass which was associated with possible influences of allelochemicals. Cymbopogon/desmodium based mixed powders and liquid formulations expressed the lowest inhibitory effects on weed seed germination and growth. Application of cymbopogon/desmodium liquid extracts increased weed density, biomass and weight per weed at 42 DAA but the density and biomass reduced

at 65 DAA, attributed to hormesis. Butanil gave the lowest weed count and weight per unit area at 42 DAA and 65 DAA.

## CHAPTER 8

### GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

#### 8.1. Discussion

In the current study, compounds in the stover and compounds exudated into the soils potted with rice, desmodium, cymbopogon, mucuna and maize were identified. The control soil produced 15 terpenoids, two alcohols and one each of trihalomethanes, ethers, phenols, ketones, furans, alkanes and aldehydes. Cymbopogon exuded five terpenoids, one phenol and an alkane. Desmodium plant roots released three terpenoids, one alkane and a phenol. Rice crop produced eight terpenoids, two alkanes and a furan. Five terpenoids, one phenol and an alkane were released by mucuna crop, while six terpenoids were found in maize root exudates. Ten terpenoids and one ester were identified in cymbopogon stover and desmodium stover released six terpenoids and three phenols. Rice stover produced six terpenoids, three phenols and an ester. *Mucuna pruriens* stover produced two terpenoids and four Phenols and *Zea mays* (LONGE 6H) stover released five terpenoids and four phenols. The identified compounds could have inhibited the growth of weeds in the pot screening, equal compartment agar and donor-receiver plants in one pot studies as well as mulching, leaf/stem/root powder and water extract studies, influencing nutrient uptake and other processes in weeds and crops. Phytotoxins are reported to move from an allelopathic donor to a receiver plants via root exudates and cause direct biological and physiological effects on the receiver plants under intercropping systems and in crops under succession. Root leachates may have inhibited the growth of weeds and crop plant due to action of the allelochemicals on the target sites. The results indicate the possibility of developing selective bio-herbicides from cymbopogon, rice, desmodium, mucuna and maize plants.

Higher concentrations of the mixed crop leachates significantly reduced the seedling emergence and growth in mucuna, desmodium and rice plants due to possible adverse effects of allelochemicals in the test plants on processes such as synthesis of protein and carbohydrates and storage of oil in the seeds to support seedling germination. Seedling emergence, crop growth and biomass of plants increased at higher concentrations of rice + desmodium + maize leachates. This may be attributed to hormesis, a condition of growth stimulation at sub-optimal levels of allelochemicals. The higher relative potency and inhibitory effects by different mixtures of the leachates on seed germination could be attributed to the stronger synergistic effects of molecules of the putative compounds in test plants. This relates to the additive model assumptions that chemicals in a mixture may have similar molecular targets in the receiver plant and can replace each other on the basis of their biological exchange rate or have different molecular targets and exert their effects independent of each other in the receiver plants.

Potting rice with maize significantly reduced the rice plant height while mucuna and cymbopogon component crops reduced the rice root length when potted with rice. The reduced rice growth may be partly attributed to competition for below and above ground resources and to allelopathic phytotoxic effects of the compounds via root exudates from maize, mucuna and cymbopogon to rice plants. Possible shading and competition for some resources could have occurred since mucuna, cymbopogon and maize crops also exhibited higher competitive ratios in chapter 5 of this thesis. Bio-active compounds influence plant nutrient uptake, cell division and development which could have eventually affected the growth of rice plants. Potting of rice with cymbopogon, mucuna or desmodium did not influence rice plant height, relative to sole rice, but the height of rice potted with maize significantly reduced relative to rice potted with cymbopogon and mucuna crops. This relates

to the observed high relative growth rates for rice potted with cymbopogon and mucuna. The low height of rice potted with maize may be attributed to the significantly lower N and K nutrient uptake levels by rice than by sole crops relative to the significantly higher N, P and K uptake levels when potted with cymbopogon. Higher N and K uptake levels by rice potted with mucuna crops relative to the uptake by sole crops were also recorded. Rice and maize being both cereal crops could have expressed higher competition for the same nutrients relative to other component crops. The maize leaf length increased when potted with desmodium, cymbopogon or mucuna, compared to sole maize and this was related to the higher N and P uptake by maize when potted with these companion crops relative to sole maize. Nutrient uptake levels were attributed to possible influences of the compounds identified.

Higher relative growth rates for maize potted with desmodium or cymbopogon crops were observed. This stimulation of plant processes at low concentrations or sub-optimal levels of metabolites with inhibition at higher concentrations (hormesis) is associated with effects of allelochemicals. Intercropping rice with cymbopogon and maize with desmodium are potentially productive intercropping systems, while intercropping rice with maize which was characterised by inhibited nutrient uptake by both crops and poor plant growth was apparently unproductive.

Intercropping rice with mucuna, desmodium and cymbopogon reduced the striga weed count relative to sole rice. This was attributed to chemical interference by compounds in root exudates of mucuna, desmodium and cymbopogon on striga attachment and growth. Intercropping mucuna and maize with rice significantly inhibited rice tiller development. This

could be attributed to the higher competitive ability of mucuna crop as observed and to inhibitory allelopathic effects of maize on rice root growth, crop nutrient uptake and growth.

Intercropping maize in rice produced shorter maize perhaps due to increased competition for below and above ground resources and the combined effects of both crops in attracting striga, intensifying severity on maize. Striga is reported to reduce maize and rice crop growth rates. The findings also showed that *Zea mays* (LONGE 6H) was more susceptible to striga attack than *Oryza sativa* (NERICA 1). This was associated with the reported higher genetic resistance and tolerance in NERICA 1 to striga parasitic weed. Intercropping rice with cymbopogon gave the highest rice grain yield amongst intercropping systems and the highest land equivalent ratio. The study indicates that a farmer would need 1.56 acres of land area planted with sole rice to achieve equal productivity from 1.0 acre of rice intercropped with cymbopogon and this clearly indicated the benefits from rice + cymbopogon intercropping systems.

The high rice growth and yields due to application of hand hoeing twice, hand hoeing thrice and Butanil (PRE) + hand hoeing once were attributed to possible lower interferences with weeds exerted on the rice crop. This condition improved rhizosphere nutrient uptake, reduced competition for crop growth resources such as light, water and nutrients due to effective weed control. Mulched treatments without a post mulch weed control operation produced the lowest percent filling of panicles per plant. The reduced filling of panicles was attributed to chemical effects of compounds in the mulches on physiological processes of rice development and competition for water, nutrients and solar radiation

Hand roguing increased rice growth and number of panicles relative to hand hoeing and this could be attributed to increased availability of soluble putative allelochemicals with inhibitory effects to the rice crop from the decomposed trash with hand hoeing relative to hand roguing. The higher inhibitory effects by different mulches on weeds were attributed to potency of the bio-active compounds with probable synergistic effects of mixed putative molecules in the compounds identified in maize, rice, mucuna, maize and cymbopogon under the study. The number of weeds and weed weight significantly reduced when the leaf, stem and root (LSR) powder concentrations were increased from 15 to 45 percent w/w. The increased weed control was attributed to increased effects of the phytotoxic compounds on the physiological processes of the target weeds. Increased weed seed germination and growth by rice/cymbopogon/desmodium and maize/cymbopogon/desmodium extracts were observed and attributed to hormesis.

## **8.2 Conclusions**

I identified bio-active compounds in *C. nardus*, *D. uncinatum*, *M. pruriens*, *O. sativa* (NERICA 1) and *Z. mays* (LONGE 6H) root exudates and stover. Cymbopogon, rice and desmodium commonly released two terpenoids namely Tert-Amylbenzene and 1-Sec-butyl-4-methylbenzene besides an alkane identified as 2,3-Dimethylundecane in root exudates. Rice, desmodium and maize released 2-n-Pentylfuran as a common furan in the root exudates and one terpenoid named Pentamethylbenzine was only exudated in the roots of cymbopogon and rice crops. Naphthalene terpenoid was similarly exudated by mucuna and cymbopogon crops only while rice and maize produced 1-Methyl-2-(2-propenyl) benzene terpenoid in their root exudates.



Rice, desmodium, maize and mucuna plant materials produced 3 common compounds identified as 1,4-Eicosadiene, 2,5-di-tert-butylphenol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol. Butylated Hydroxytoluene and (9Z)-9-Icosen-1-ol bio-compounds were extracted from rice, desmodium and maize plant stover and 1,2,3-Trimethyl-4-[(1E)-1-propenyl] naphthalene terpenoid was identified from the stover of rice, desmodium and maize. Three compounds namely 2,2'-Diethylbiphenyl, 1-Ethyl-2-(1-phenylethyl) benzene and 3,4-Diethyl-1,1'-biphenyl were produced only by rice and desmodium stover. Rice exclusively released Hexadecanoic acid ester as mucuna produced Hexa-hydro-farnesol phenol. The compounds identified as (9Z)-9-Icosen-1-ol; Butylated Hydroxytoluene and 3,4-Diethyl-1,1'-biphenyl were distinctively found in the maize stover. Ten terpenoids named Citronellal, -Citral, cis-Geraniol, trans-Carane, Eugenol, Geraniol acetate, -Elemen, Caryophyllene, -Gurjunene, -Cadinene and one ester called Citronellyl butyrate were only identified in cymbopogon stover. The profiled compounds from cymbopogon, desmodium, rice, mucuna and maize could be responsible for some of the negative allelopathic effects expressed by the study crops in natural and agricultural ecosystems, hence, the potential for synthesis and development of herbicides.

In the current study, I determined the allelopathic potential of bio-active compounds in rice, desmodium, maize and mucuna. The growth of *A. conyzoides*, *G. parviflora* and *B. pilosa* weeds reduced when supplied with root leachates from mucuna desmodium maize and rice. High concentrations of mixed rice, desmodium, maize and mucuna leachates increased the mean germination time for mucuna, desmodium, maize and rice seeds but reduced the seed germination indices. Rice/desmodium leachate reduced the maize dry biomass. Potting rice or maize with cymbopogon, mucuna and desmodium influenced the uptake of nutrients and reserves for example potting rice with cymbopogon, mucuna and desmodium significantly

increased the N, P and K uptake by rice while potting rice with maize recorded the lowest N, P and K reserves at harvest. Allelopathic properties of crops should be considered in selection of components crops under intercropping systems since they affect nutrient uptake, growth and development of crops.

Intercropping rice with mucuna, desmodium and cymbopogon, significantly reduced the striga weed count relative to sole rice crop. Intercropping rice with cymbopogon gave higher rice grain yield and combined land equivalent ratio amongst the intercropping systems. The yield parameters were lowest in rice intercropped with maize and mucuna. Application of Butanil (PRE), hand hoeing twice, hand hoeing thrice and maize/mucuna mulches increased rice plant height, number of tillers, leaf number, leaf length and width, grain yield and returns on Uganda shilling investment relative to the rice/cymbopogon mulches which were the most effective mulch formulations in controlling upland rice weeds including the noxious striga. Increasing the leaf, stem and root powder concentrations significantly reduced the number of sown *B. pilosa*, volunteer weeds and weed weight at 30 days after application of the powders. Application of the rice/cymbopogon and maize/mucuna powder and aqueous formulations most effectively reduced the density and biomass of weeds. Cymbopogon/desmodium mixed powders expressed the lowest inhibitory effects on weed seed germination and growth for both the powder and aqueous formulations. Maize, rice, cymbopogon and mucuna have the potential to produce bio-herbicides for weed control.

### **8.3 Recommendations**

1. Many compounds were identified in crop root exudates. Further studies should be conducted to determine the allelopathic potential of each of these compounds.

2. Direct properties of bio-active compounds on plant growth and development were determined in this study. Their potential indirect actions on soil properties, plant nutrient status and population (or activity) of micro-organisms and nematodes are recommended for further investigations.
3. Desmodium and cymbopogon intercrops reduced the striga weed population. Further studies to identify the bio-compounds responsible for inhibition of striga attachment on cereal crops should be conducted.
4. Rice root growth reduced when the crop was potted with desmodium, maize, mucuna and cymbopogon. Further field studies should be conducted on effects of allelopathic cultivars of rice and maize in succession or under intercropping systems.
5. Rice/cymbopogon and maize/mucuna based formulations most effectively controlled weeds with high rice grain yields. Further investigations should be conducted on the potential production of the herbicides from the crops.
6. Herbicidal hormesis was expressed when maize was potted with mucuna, cymbopogon and desmodium and when cymbopogon/desmodium water extracts were applied on weeds. Further studies should be conducted to establish and exploit potential benefits from this phenomenon.
7. Tolerance to weed populations is likely to occur if the same allelochemicals are continuously applied to the same weed population by allelopathic rice varieties such as NERICA 1 being grown in Eastern Uganda. Strategies to minimize these risks have to be developed.
8. There is need to create awareness to the rice growing farmers about the potential positive and negative effects of allelopathy in common crops grown in Eastern Uganda through mass media and extension services.

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