UNIVERSITI PUTRA MALAYSIA

RESIDUAL ACTIVITY OF METSULFURON-METHYL AND TRICLOPYR APPLIED IN COMBINATION WITH GLYPHOSATE-IPA IN BEKENU SERIES SOIL

OOI LOO LING

RESIDUAL ACTIVITY OF METSULFURON-METHYL AND TRICLOPYR APPLIED IN COMBINATION WITH GLYPHOSATE-IPA IN BEKENU SERIES SOIL

OOI LOO LING

FACULTY OF AGRICULTURE AND FOOD SCIENCES
UNIVERSITI PUTRA MALAYSIA BINTULU CAMPUS

2007

Dedicated to:

My dearest father (Ooi Hai Siew),

mother (Ong Yoke Yoong),

brother and sisters,

and friends.

Thank you very much for all the support given.

ABSTRACT

An experiment was conducted in a netted-greenhouse located at the University Share Farm, UPMKB, to study the residual activity of metsulfuron-methyl and triclopyr applied in combination with glyphosate-IPA in Bekenu series soil. Herbicide treatments were metsulfuron-methyl plus glyphosate applied at three rates (15 g/ha + 0.5 kg/ha; 30 g/ha + 1.0 kg/ha and 60 g/ha + 2.0 kg/ha), and triclopyr plus glyphosate applied at three rates (0.15 kg/ha + 0.5 kg/ha, 0.30 kg/ha + 1.0 kg/ha and 0.60 kg/ha + 2.0 kg/ha) and one untreated control. Seeds of three bioassay species, rice *(Oryza sativa* var. ARC2*)*, tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) were sown into germination trays at each planting date from 1 day after herbicide treatment to several weeks after treatment. The percent inhibition, root and shoot lengths were recorded. There were significant differences in shoot and root lengths of the three bioassay species only at 1 day after treatment. Overall, the results indicated that triclopyr 0.60 kg/ha + glyphosate 2.0 kg/ha showed the highest percent inhibition on cucumber, tomato and rice shoot lengths compared to the other treatments. Metsulfuron-methyl plus glyphosate at all rates also had some effects but the inhibitory effects were less. Cucumber was the most suitable bioassay species compared to tomato and rice to monitor the carry-over effect of both triclopyr and metsulfuron-methyl.

ABSTRAK

Satu kajian telah dijalankan di rumah berjaring yang terletak di Ladang Kongsi, UPMKB, bagi mengkaji kesan kombinasi racun rumpai metsulfuron-methyl, triclopyr dan glyphosate-IPA pada tanah bersiri Bekenu. Kombinasi racun rumpai metsulfuron-methyl dengan glyphosate disemburkan pada kadar (15 g/ha + 0.5 kg/ha; 30 g/ha + 1.0 kg/ha and 60 g/ha + 2.0 kg/ha), dan kombinasi triclopyr dengan glyphosate pada kadar (0.15 kg/ha + 0.5 kg/ha, 0.30 kg/ha + 1.0 kg/ha and 0.60 kg/ha + 2.0 kg/ha) dan satu kawalan tanpa rawatan. Biji benih bagi padi *(Oryza sativa* var. ARC2*)*, tomato (*Lycopersicon esculentum*) dan timun (*Cucumis sativus*) ditanam dalam dulang percambahan pada satu hari hingga beberapa minggu selepas semburan racun rumpai dan peratus perencatan pertumbuhan akar, dan pucuk dicatat. Keputusan menunjukkan terdapat perbezaan bererti bagi ukuran panjang akar dan pucuk untuk ketiga-tiga tanaman hanya pada satu hari selepas semburan racun rumpai. Secara keseluruhan, keputusan bagi kombinasi triclopyr 0.60 kg/ha + glyphosate 2.0 kg/ha menunjukkan peratus perencatan yang paling tinggi terhadap panjang akar dan pucuk timun, tomato dan padi berbanding dengan kombinasi racun rumpai yang lain. Metsulfuron-methyl pada semua kadar memberi kesan tetapi tidak sebanyak triclopyr. Timun merupakan tanaman yang paling sesuai berbanding dengan tomato dan padi untuk mengenalpasti kesan sisa-baki racun rumpai triclopyr and metsulfuron-methyl.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Associate Professor Dr. Rajan Amartalingam for his advice, guidance, patience and unwavering encouragement throughout the period of this project.

Sincere appreciation is also extended to Dr. Osumanu Haruna Ahmed for his advice regarding the technique for the analysis of soil texture.

I would like to thank all the staff of TPU Student's Share Farm especially to Mr. Haidil and Mr. Nicholas for their help and assistance during the project.

Finally, great thanks to my parents, sisters, brother and all my friends who had given me great support throughout this project.

I certify that this research project report entitled "Residual Activity of Metsulfuron-methyl and Triclorpyr Applied in Combination with Glyphosate-IPA in Bekenu Series Soil" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

Assoc. Prof. Dr. Rajan Amartalingam

Faculty of Agriculture and Food Sciences

Universiti Putra Malaysia Bintulu Campus

(Supervisor)

Prof. Dato' Dr. Nik Muhamad Nik Ab. Majid

Dean

Faculty of Agriculture and Food Sciences

Universiti Putra Malaysia Bintulu Campus

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

a.i	=	Active ingredient
DAT	=	Day after herbicide treatment
ELISA	=	Enzyme-linked immunosorbent assay
GC	=	Gas chromatography
HPLC	=	High performance liquid chromatography
R	=	Recommended rate
WAT	=	Week after treatment
w/w	=	Weight per weight

CHAPTER 1

INTRODUCTION

Herbicides are used to kill weeds or unwanted plants. Herbicides are widely used in agriculture and in landscape turf management. Selective herbicides will kill specific target weeds leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weeds based on different modes of action. Herbicides used to clear waste ground are usually nonselective which kill all plant material with which they come into contact.

Factors that affect the movement of herbicides in the environment include (1) Plant factors, (2) Environmental factors (3) Soil factors, and (4) Chemical factors (Carter, 2000). Plant factors include branching habit, plant surface characteristics, maturity and plant species and variety. Environmental factors include temperature, humidity, rainfall, wind and light. Soil factors include soil-water stress, soil pH, organic matter and clay type. Chemical factors include herbicide concentration, solution pH, chemical structure and surfactants (Carter, 2000).

Metsulfuron-methyl (methyl-2-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benzoate) is in the sulfonylurea group of herbicides and has high herbicidal activity even at low rates (Yu *et al.,* 2005). It is widely used to control broadleaved weeds and some grasses (Hollaway *et al.*, 1999; Li *et al.*, 2005).

Triclopyr [(3,5,6-trichloro-2-pyridinyloxy]acetic acid) is a systemic herbicide that is used to control woody and herbaceous broadleaved plants (Begley and Foulger, 1988). Triclopyr will control target weeds by mimicking the plant auxin, causing uncontrolled and disorganized plant growth that leads to plant death.

Glyphosate (N-(phosphonomethyl)-glycine ($C_3H_8NO_5P$) is a systemic herbicide often used for controlling perennial weeds through application after harvest of field crops. In plantation crops, it is frequently used to kill weeds at postemergence.

Persistency of herbicides in the soil is the length of time that the herbicide remains active or persists in the soil. Persistence is important in soil-applied herbicides in order to give more effective weed control. However, persistence and high activity due to repeated or high application rates may result in damage or injury to existing crops. Further, if the herbicide residue remains too long in the soil, it may become injurious to subsequent crops.

Different herbicides have different modes of action and different residual activity. Therefore it is very important to know how long it will be active in the soil and how will it affect the environment, or contaminate food crops and affect sensitive crops planted in rotation.

There are various methods used in monitoring residues in harvested crops, in the soil and water. It can be monitored by using microbial activity assay, chemical methods such as enzyme-linked immunosorbent assay, high performance liquid chromatography (HPLC), gas chromatography (GC) and also seedling bioassay (Hollaway *et al.*, 1999).

Seedling biossays are simple, fast, inexpensive and direct methods to determine residue levels of herbicides in the soil. Reliable bioassay detection of residues in the soil will provide information on re-cropping intervals to growers (Hollaway *et al.*, 1999).

Tomato (*Lycopersicon sp.*) and cucumber (*Cucumis sativus*) are two examples of plant species that are very sensitive to broadleaved herbicides and are widely used as bioassay species. Ismail and Azlizan (2002) studied the persistence and bioactivity of metsulfuron-methyl in three soils using cucumber (*Cucumis sativus*) as the bioassay species. The range of bioassay plant species include peas, oat, maize, sugar beet and lupin (Stork and Hannah, 1995).

In Malaysia, combinations of metsulfuron-methyl, triclopyr and glyphosate-IPA are widely used for weed control (Ismail and Azlizan, 2002). Repeated use may result in residue problems or carry-over effects especially in mixed cropping systems. Although chemical analysis will provide accurate amounts of herbicide residues, but it does not indicate residues are at levels that may be damaging to the rotation crop. Therefore, a simple and quick bioassay method to determine residues in the soil will be more useful for management decisions on rotational crops.

Objectives:

The main objective of the study was to develop a suitable bioassay technique to study soil residual activity of metsulfuron-methyl and triclopyr applied in combination with glyphosate-IPA. The specific objectives were:

1. To compare residual soil activity of metsulfuron-methyl and triclopyr applied in combination with glyphosate-IPA using three bioassay species (cucumber, tomato, rice).
2. To determine the most suitable bioassay species to monitor carry-over effects of metsulfuron-methyl and triclopyr applied in combination with glyphosate-IPA.
3. To determine the safe interval for sowing of sensitive crops in a rotation.

CHAPTER 2

LITERATURE REVIEW

Herbicides are used to kill weeds or unwanted plants. Over 4000 herbicides have been discovered and developed in the past 55 years. There is almost no weed problem that cannot be solved by herbicides (Rao, 2000). Herbicides are widely used in agriculture and in landscape turf management. Selective herbicides will kill specific target weeds leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weeds based on different modes of action. Herbicides used to clear waste ground are usually nonselective which kill all plant material with which they come into contact. Herbicides are classified on the basis of method of application, chemical affinity and mode of action (Ashton and Crafts, 1973).

2.1 Soil Residual Activity

Soil is affected by weed control practices in many important ways. It is the anchoring medium and supplier of minerals and water for both weeds and crops, and it is the reservoir for large number of weed seeds and vegetative reproductive structures. Weed control measures such as tillage and applying herbicides to the soil actively involve the soil (Ross and Lembi, 1999).

The persistency of an herbicide in the soil is the length of time that the herbicide remains active or persists in the soil. The persistence of soil-applied herbicides is important for effective weed control. Herbicides that degrade rapidly are less desirable in these situations as they cannot be very effective on late emerging weeds. On the other hand, herbicides that have longer persistence of activity are not suitable as their residues may injure the sensitive crops grown in rotation (Rao, 2000).

Herbicides reaching the soil become dissipated or removed depending on (1) uptake and metabolism by plants, (2) volatilization, (3) adsorption, (4) leaching, (5) surface runoff, (6) photo degradation, (7) microbial degradation and (8) chemical degradation (Devlin *et al.*, 1992; Monaco *et al.*, 2002).

2.2 Methods of Monitoring Residues

Various methods are used in monitoring residues in harvested crops, soil and water. Residues can be monitored by using microbial activity assay, enzyme-linked immunosorbent assay, high performance liquid chromatography, gas chromatography and plant bioassay (Hollaway *et al.*, 1999).

2.2.1 Microbial Activity Assay

Yu *et al.,* (2005) indicated that the microbial degradation of metsulfuron-methyl was 7.5 and 3.8 times faster with *Curvularia* sp. strain MD amended soils than in sterilized and fresh soil. The technique for monitoring the environment can be done by looking at the microbial activity (Andersen *et al.*, 2001). Ghani and Wardle (2001) studied the fate of 14C glucose and the herbicide metsulfuron-methyl in a plant-soil microcosm system and they reported that the presence of litter from killed nodding thistle (*Carduus nutans*) plants in the planted microcosm enhanced microbial biomass and its activity, and in turn had a major influence on the rate of metsulfuron-methyl degradation.

In studies by Ismail and Azlizan (2002), a significant degradation of metsulfuron-methyl was also observed in non-autoclaved soil compared with the autoclaved soil sample, indicating the importance of soil microorganisms in degradation of metsulfuron-methyl. Andersen *et al.,* (2001) indicated that the mineralization of metsulfuron-methyl, tribenuron-methyl and thifensulfuron-methyl in the upper profile was higher in the surface layer (5 – 35 cm) compared to soils at 70 – 75 cm.

2.2.2 Chemical Analysis

Chemical methods for monitoring residues include ELISA, HPLC and gas chromatography. Analysis of triclopyr residues using gas chromatography have been reported for fruits and vegetables (Ting *et al.*, 1995). Hollaway *et al.*, (1999) carried out comparative studies on sulfonylurea herbicide residues in the soil using bioassay, enzyme-linked immunosorbent assay (ELISA) and HPLC. In their studies ELISA was used to accurately measure residues in the range of 0.1 – 10 μg a.i. kg^{-1}, while HPLC quantified residues of between 3.0 – 10 μg a.i. kg^{-1} and the bioassay measured responses to biologically active residues from 0.1 – 1.0 μg a.i. kg^{-1}. Sarmah and Kookana (1999) analyzed residues in water and soil using HPLC. The dissipation of metsulfuron-methyl in soil had also been studied by HPLC (Bossi *et al.*, 1999; Sanyal *et al.,* 2006). Bossi *et al.*, (1999) indicated that the dissipation rate of metsulfuron-methyl in topsoil was very rapid, with a calculated half-life of 6.5 days. Chemical analysis can provide accurate information on herbicide levels in a soil. However, only a bioassay will indicate if levels present will injure a crop (Devlin *et al.*, 1992).

2.2.3 Plant Bioassay

Bioassay techniques for measuring soil persistence of a herbicide have an advantage over chemical methods in that they measure directly the bioactivity of residues that affect plant growth. Biossays are simple, fast, inexpensive and direct methods to determine residue levels of herbicides in the soil. Reliable bioassay detection of residues in the soil will provide information on re-cropping intervals to growers (Devlin *et al.*, 1992; Hollaway *et al.*, 1999).

Seiden *et al.,* (1998) reported the response of *Brassica napus* to metsulfuron-methyl and chlorsulfuron using a tissue culture bioassay method. Koger *et al.*, (2005) tested two rice varieties for response to drift rates of glyphosate. Noticeable visual injury and height reduction to both varieties were detected at 3 and 7 days after treatment (DAT). The effect of soil metsulfuron-methyl residues on the growth of rice (*Oryza sativa*) have been studied by Li *et al.*, (2005). Their results showed that the inhibition rate of root growth increased from 69.46 – 81.32 % to 85.18 – 95.97 % with the increasing of levels of bound residues of metsulfuron-methyl from 0.05 mg kg^{-1} to 0.5 mg kg^{-1}.

2.3 Bioassay Species

Tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) are two examples of plant species that are very sensitive to broadleaved herbicides and are widely used as bioassay species (Eastin, 1971; Santos *et al.*, 2006). Ismail and Azlizan (2002) reported persistence and bioactivity of metsulfuron-methyl in three soils using cucumber (*Cucumis sativus*) as the bioassay species. Rice had also been used as a bioassay species (Li *et al.*, 2005; Dhareesank *et al.*, 2005). Li *et al.*, (2005) showed that the number of rice roots could be taken as a sensitive index to screen the rice varities endurable to bound residues of metsulfuron-methyl in soil and to predict the potential hazards of bound residues of metsulfuron-methyl in soil to rice.

2.4 Metsulfuron-methyl

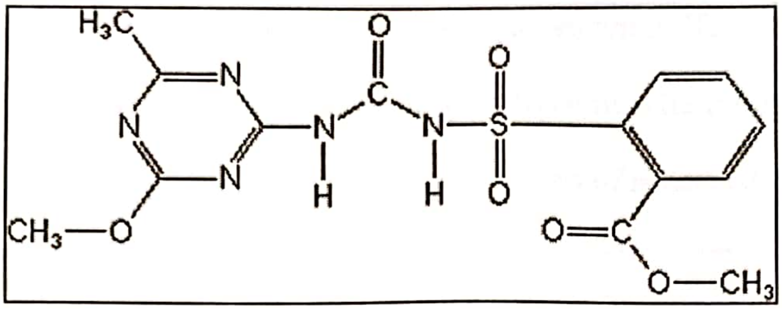

Figure 2.1: Chemical structure of metsulfuron-methyl

Metsulfuron-methyl (methyl-2-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] benzoate) (Figure 2.1) is a white to pale white solid with a sweet ester like odour; having a vapor pressure of 2.5 × 10-12 mm Hg at 25°C; and a water solubility of 548 mg/L (ppm) at 25°C at pH 5 and 2790 mg/L (ppm) at pH 7; and oral LD50 (rat) of 5000 mg/kg. (Monaco *et al.,* 2002). The herbicidal properties of metsulfuron-methyl were first reported in 1983 and were first marketed as ALLY (Rao, 2000). Metsulfuron-methyl is a selective and systemic herbicide from the sulfonylurea group of herbicides and has high levels of herbicidal activity even at low application rates (Yu *et al*., 2005). It is widely used to control broadleaved weeds such as *Brassica spp.* and *Cirsium arvense* and some grasses in many crops (Hollaway *et al*., 1999; Li *et al*., 2005).

Sulfonylurea group herbicides move in the xylem and phloem in plants and inhibit acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS) (Figure 2.2), the first common enzymes in the biosynthesis of branched amino acids such as valine, leucine, and isoleucine (Monaco *et al.,* 2002; Li *et al*., 2005). Some other herbicide families such as the imidazolinones, triazolopyrimidines, and pyrimidinyloxybenzoate herbicides also inhibit this enzyme (Tanaka *et al.*, 2006). They are potent and rapid inhibitors of plant cell division. The inhibition of growth is rapid in the growing tips of both the roots and shoots of sensitive plants.

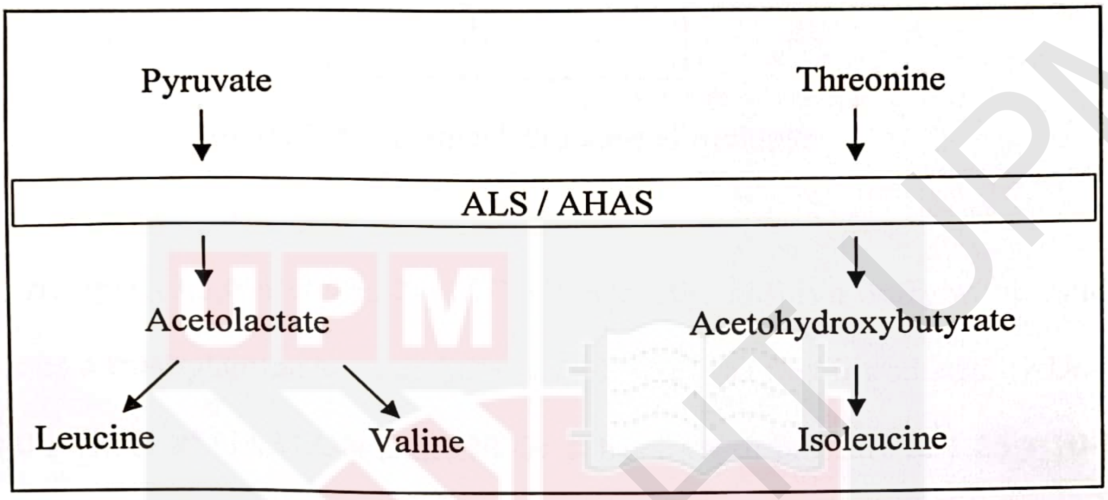

Figure 2.2: Biosynthetic pathway of branched chain amino acids

The behavior and residual fate of metsulfuron-methyl in soil have been studied (Ghani and Wardle, 2001; Sanyal *et al.*, 2006). Metsulfuron-methyl was moderately persistent in the soil with a soil half-life ranging from 40 – 180 days. Metsulfuron-methyl is basically degraded by photolysis. Chemical hydrolysis also plays an important role in the degradation particularly in soils with low pH. Moreover, soil moisture and temperature will also have considerable influence on the dissipation of metsulfuron-methyl (Rao, 2000; Andersen *et al.*, 2001).

2.5 Triclopyr

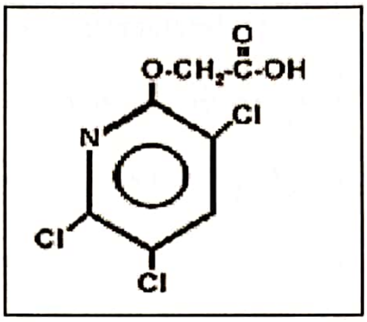

Figure 2.3: Chemical structure of triclopyr

Triclopyr[(3,5,6-trichloro-2-pyridinyloxy)acetic acid] is a fluffy white solid, available as a triethylamine salt and butoxyethyl ester was first introduced by Dow Chemical in 1975 as GARLON (Rao, 2000). It has a vapor pressure of 1.26 × 10-6 mm Hg at 25°C and a water solubility of 430 mg/L (ppm) for acid, 23 mg/L (ppm) for ester, and 2,100,000 mg/L (ppm) for the amine salt at 25°C and an oral LD50 (rat) of 713 mg/kg (Monaco *et al.,* 2002).

Triclopyr which is in the pyridinecarboxylic acid group (Figure 2.3) is a systemic herbicide that is used to control woody and herbaceous broadleaved plants (Lickly and Murphy, 1987; Begley and Foulger, 1988). It is absorbed by foliage and roots and readily translocated in plants. Triclopyr controls target weeds by mimicking the plant auxin (indole acetic acid), and when administered at effective doses, causes uncontrolled and disorganized plant growth that leads to plant death.

Low concentrations of triclopyr can stimulate RNA, DNA, and protein synthesis leading to uncontrolled cell division and growth and destruction of the vascular tissue. While at high concentrations, triclopyr inhibits cell division and growth. Triclopyr is moderately persistent in soil, with a half-life of 30 days, ranging from 10 – 46 days depending on soil type, moisture content and temperature (Tu *et al.*, 2001). Triclopyr is rapidly degraded by soil microbes (Rao, 2000). It is non-toxic to bees and fish.

2.6 Glyphosate

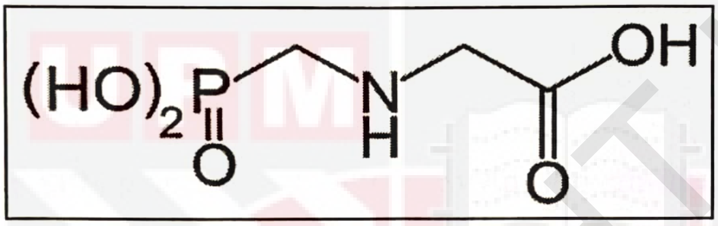

Figure 2.4: Chemical structure of glyphosate

Glyphosate (N-(phosphonomethyl)-glycine, $C_3H_8NO_5P$) is a white solid and a week organic acid derivative of phosphonic acid and the amino acid glycine (Figure 2.4). It has a vapor pressure of 1.84×10^{-7} mm Hg at 45°C and a water solubility of 900,000 mg/L (ppm) at 25°C and oral LD50 (rat) of 5000 mg/kg. It is generally formulated as salts and these are very temperature stable under normal conditions (-20 to 40°C) (Monaco *et al.*, 2002).

Glyphosate was discovered and developed as a herbicide by Monsanto Chemical Company in 1974. It is popularly sold under the trade name of Roundup (IPA-salt) and Touchdown (TMS salt). Glyphosate is a nonselective systemic herbicide often used for controlling annual and perennial weeds through application after harvest of field crops. In plantation crops, it is frequently used to kill weeds at postemergence. Selective control of weeds is possible with appropriate timing and selective placement and spot treatment. Glyphosate gives effective control of rhizomatous and deep-rooted weeds including *Imperata cylindrica, Cynodon dactylon, Paspalum conjugatum, Setaria palmifolia, Eleusine indica, Mikania micrantha, Digitaria sanguinalis, Panicum repens, Echinochloa crus-galli, Pteridium aquilinum, Cyperus rotundus* and many other species (Rao, 2000; William and Kangetsu, 2004).

Glyphosate kills plants by inhibiting growth through interference with the production of essential aromatic amino acids by inhibition of the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS). EPSPS is responsible for the biosynthesis of chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis. EPSPS catalyzes the reaction of shikimate 3-phosphate (S3P) and phosphoenolpyruvate (PEP) to form EPSP and phosphate (Williams *et al*., 2000; Monaco *et al*., 2002). The aromatic amino acids are also used to make secondary metabolites such as folates, ubiquinones and naphthoquinones. The shikimate pathway is not present in animals (Williams *et al*., 2000).

Glyphosate binds tightly to soil clay particles so it does not end up passing through the soil and ending up in the aquifers (Monaco *et al*., 2002). Glyphosate is degraded in the soil by microbes to carbon dioxide.

Different herbicides have different modes of action and different residual activity. Therefore it is important to know the residual effects in the soil, in the environment, or as contaminants in food crops as well as their effects on sensitive crops planted in rotation.

In Malaysia and in many parts of the world, metsulfuron-methyl and triclopyr in combinations with glyphosate-IPA are widely used for weed control (Hemmanda *et al,*. 1994). Repeated use would result in residue problems or carry-over effects especially in mixed cropping systems. Altougth chemical analysis will indicate accurate amounts of herbicide residues, but it does not indicate residues are at levels that may be damaging to the rotation crop (James *et al.,* 1999). Therefore, a simple and quick bioassay method to determine phytotoxic levels of residues in the soil will be more useful for on-farm applications (Andersen, 2001). There is therefore a need to develop simple bioassay techniques to monitor residues in the local soil environment.

CHAPTER 3

MATERIALS AND METHODS

3.1 Germination Test of Bioassay Species to be Used in the Study

Three hundred seeds of the three test species were placed in plastic containers with the size of 14 cm diameter and 7 cm depth. Water was applied twice daily (morning and evening). After one week, the number of seeds germinating were counted and recorded.

3.2 Herbicide Treatments to be Tested for Soil Residual Activity

The experiment consisted of seven treatments: metsulfuron-methyl plus glyphosate applied at three rates (0.5, 1.0 and 2 times the recommended rate); triclopyr plus glyphosate applied at three rates (0.5, 1.0 and 2 times the recommended rate) and one untreated control (Table 1). The spray volume was 400 L/ha.

Table 3.1: Herbicide treatments used in the study

Herbicides Treatments	Rates (ai per ha)		
	0.5 × R	1.0 × R	2.0 × R
metsulfuron-methyl (20.0 % w/w)	15 g	30 g	60 g
+ glyphosate (41 % w/w)	0.5 kg	1.0 kg	2.0 kg
triclopyr (32.1 % w/w)	0.15 kg	0.30 kg	0.60 kg
+ glyphosate (41 % w/w)	0.5 kg	1.0 kg	2.0 kg

R= Recommended rate

3.3 Preparation of Bioassay Media and Herbicides Application

The experiment was conducted in germination trays placed in a netted-greenhouse located at the University Share Farm. A 40g sample of Bekenu soil was placed into each of 104 compartments in germination trays of size 55 × 36 cm. There were a total of 42 germination trays, with a total of 4368 compartments and each compartment was filled to 4 cm soil depth. Herbicides were applied using a RB15 manually operated knapsack sprayer with a 8004 fan nozzle. The compartments within each tray were allocated into three groups, one for each of the three bioassay species to be sown.

The germination trays were laid out in a randomized complete block design with 7 herbicide treatments in 6 replicates (Table 3.1).

3.4 Bioassay Test

Thirty seeds of each test species, rice *(Oryza sativa* var. ARC2*)*, tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) were sown into each of the treated trays at each planting date from 1 day after herbicide treatment (DAT) to several weeks after treatment (WAT). For tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) the seeds were sown at 1 DAT, 2 WAT, 4 WAT and 6 WAT, while for rice (*Oryza sativa* var. ARC2) the seeds were sown at 1 DAT, 3 WAT, 5 WAT and 7 WAT. After the seeds were sown, the soil was drenched with 4g of thiram (80.0%) fungicide in 2L of water. The fungicide was applied by spraying with a OSATU manually operated knapsack sprayer fitted with a cone nozzle. Simple rain gauges were placed at two locations at the experimental site to monitor daily rainfall during the study.

Trays were watered twice daily (morning and evening). Seedlings were harvested at 9 or 13 days after each sowing for measurement of data as described in section 3.6.

3.5 Control of Insect Pests

Malathion (57.0% a.i) was sprayed over all trays once every week to prevent damage by insects. A spray mixture containing 40 ml of the malathion in 4 L of water was sprayed onto the germination trays and areas surrounding the trays using a OSATU manually operated knapsack sprayer fitted with a cone nozzle.

3.6 Parameters Measured

The percent inhibition, root and shoot lengths of cucumber (*Cucumis sativus*) were recorded at 9 days after sowing, while for tomato (*Lycopersicon esculentum*) and rice (*Oryza sativa* var. ARC2) measurements were recorded at 13 days after sowing (Li *et al*., 2005).

3.6.1 Shoot and Root Length Measurements

For tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) the shoot length was measured from the base of the shoot to the junction of the first leaf. The root length was measured from the top of the root until the tip of the taproot. For rice (*Oryza sativa* var. ARC2) the shoot length was measured from base of the shoot until the tip of the topmost visible node. The root length was measured from the base of the culm to the tip of the longest fibrous root. All measurements were made with a standard 30 cm ruler.

3.6.2 Percent Inhibition

Percent inhibition in root or shoot length was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Length (Untreated)} - \text{Length (Treated)}}{\text{Length (Untreated)}} \times 100$$

All data were statistically analyzed using the Statistical Analysis System (SAS). The data were subjected to analysis of variance (ANOVA) and differences were tested using the Tukey's Test.

3.7 pH Analysis

Ten g of air-dried soil samples were placed into each of six Bijoux bottles. Twenty-five ml of distilled water was added into each of three Bijoux bottles and 25 ml of 0.01M $CaCl_2$ was added into each of the remaining three Bijoux bottles. All six Bijoux bottles were shaken at 180 rpm for 15 minutes. After 15 minutes, the pH for the three Bijoux bottles that contained 0.01M $CaCl_2$ was determined using a pH meter. The other three Bijoux bottles that contained distilled water were left for 24 hours before the pH was determined.

3.8 Soil Texture Analysis

The soil texture was analyzed using the hydrometer method to determine total sand, silt and clay contents. Triplicate of 50 g air-dried soil sample were weighed and placed into a blender cup. Then, it was filled with distilled water to 10 cm from the top and 10 ml of 2M NaOH solution was added. The cup was then attached to the blending machine and was mechanically blended for 15 minutes. After 15 minutes of blending, the soil suspension was transfer into a measuring cylinder and distilled water was added to a total volume of 1130 ml.

A stirring rod was used to stir up the sediments at the bottom of the cylinder. After 40 seconds, the stirring was stopped and a hydrometer was placed into the cylinder and readings recorded. At the same time the temperature was also recorded. The hydrometer was removed and rinsed. The suspension was stirred again and the analysis was repeated after 40 seconds. The average of both readings was recorded. The suspension was left to settle for 2 hours and the final hydrometer reading and temperature were recorded. The percentage of sand, silt and clay were then determined from the hydrometer readings.

CHAPTER 4

RESULTS

4.1 Effect of Treatments on Shoot Lengths of Seedlings

4.1.1 Cucumber Bioassay

The results showed that the effects of metsulfuron-methyl and triclopyr on cucumber shoot length were only significant at 1 DAT (Table 4.1, Figure 4.1), while the shoot lengths for 2, 4 and 6 WAT did not showed any significant differences. This indicates that the treatments were only active in the soil at one day after spraying. At 1 DAT, shoot length of cucumber for treatments metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha), metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha), metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha) and triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) were comparable to the untreated control treatment. Treatments with triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha) and triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha) significantly reduced shoot lengths of cucumber. The results showed that triclopyr at the recommended rate and twice the recommended rate will have an effect on the cucumber if the seeds were sown at less than 2 weeks after spraying the triclopyr.

Table 4.1: Effect of treatments on shoot length of cucumber bioassay

Herbicide Treatments, (kg ai/ha)	Cucumber Shoot Length (mm)			
	Days / Weeks After Treatment (DAT / WAT)			
	1 DAT	2 WAT	4 WAT	6 WAT
metsulfuron-methyl 15 g + glyphosate 0.5 kg	12.67 a	14.74 a	12.73 a	16.35 a
metsulfuron-methyl 30 g + glyphosate 1.0 kg	12.04 a	12.46 a	13.65 a	18.92 a
metsulfuron-methyl 60 g + glyphosate 2.0 kg	11.68 a	12.11 a	13.33 a	17.06 a
triclopyr 0.15 kg + glyphosate 0.5 kg	13.31 a	13.28 a	13.32 a	16.29 a
triclopyr 0.30 kg + glyphosate 1.0 kg	8.39 b	11.87 a	13.53 a	16.47 a
triclopyr 0.60 kg + glyphosate 2.0 kg	5.05 c	12.85 a	13.37 a	17.20 a
Untreated Control	14.36 a	13.27 a	14.41 a	18.50 a
Standard Error of Means	0.7393	1.2226	0.5877	0.7266

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

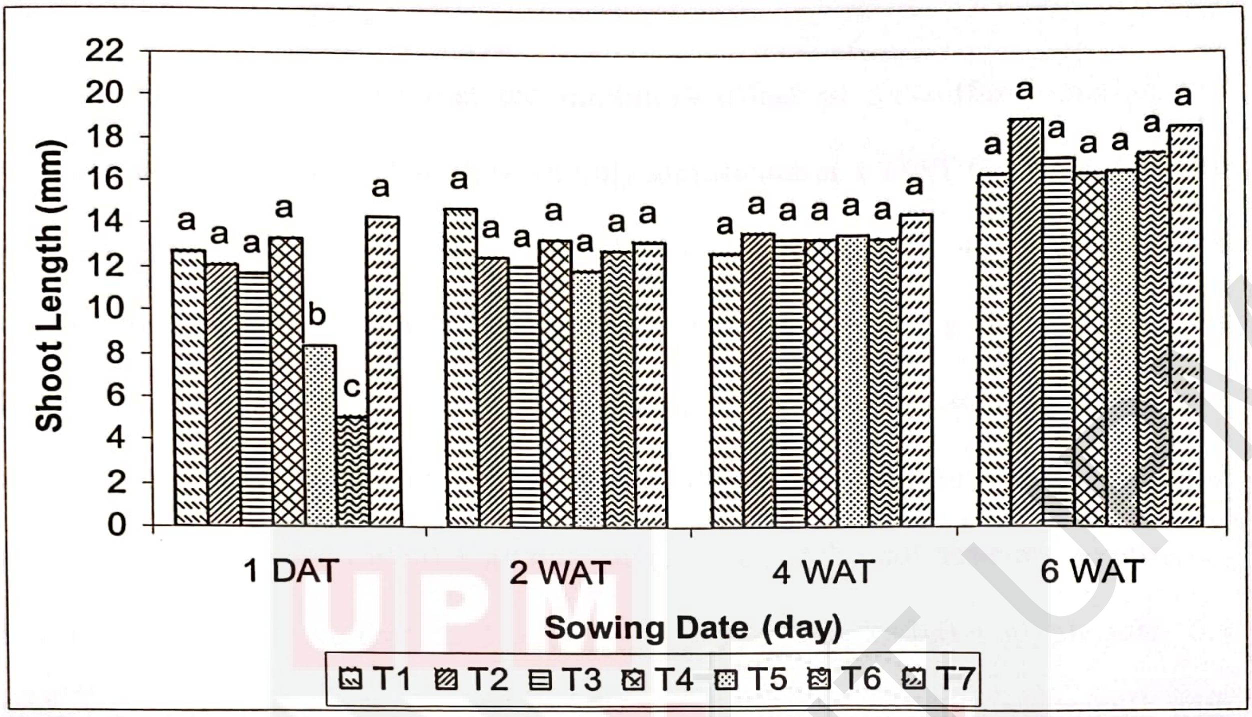

Figure 4.1: Effect of treatments on shoot length of cucumber bioassay

(DAT = day after treatment; WAT = weeks after treatment; T1 = metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha); T2 = metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha); T3 = metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha); T4 = triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha); T5 = triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha); T6 = triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha); T7 = untreated control)

4.1.2 Tomato Bioassay

The results showed that the inhibitory effect of metsulfuron-methyl and triclopyr on tomato shoot length were only significant at 1 DAT (Table 4.2).There were no significant differences between the shoot lengths for 2, 4 or 6 WAT. This showed that the herbicides were only active in the soil at one day after spraying. At 1 DAT, shoot length of tomato for treatments metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha), metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha) and metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha) did not have any significant effect compared to the untreated control. Triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) showed some effects on the shoot length of tomato while treatments with triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha) and triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha) had the most effect on the shoot length of tomato. This indicated that metsulfuron-methyl at all rates did not have any significant effects on the tomato shoot length. While triclopyr at all rates gave an effect towards the tomato shoot length. Higher rates of triclopyr had higher reduction of tomato shoot length.

Table 4.2: Effect of treatments on shoot length of tomato bioassay

Herbicide Treatments, (kg ai/ha)	Tomato Shoot Length (mm)			
	Days / Weeks After Treatment (DAT / WAT)			
	1 DAT	2 WAT	4 WAT	6 WAT
metsulfuron-methyl 15 g + glyphosate 0.5 kg	14.61 abc	13.54 a	13.76 a	18.55 a
metsulfuron-methyl 30 g + glyphosate 1.0 kg	13.22 bc	15.32 a	13.58 a	18.76 a
metsulfuron-methyl 60 g + glyphosate 2.0 kg	15.07 ab	11.96 a	15.71 a	18.36 a
triclopyr 0.15 kg + glyphosate 0.5 kg	11.27 cd	12.81 a	14.70 a	15.88 a
triclopyr 0.30 kg + glyphosate 1.0 kg	8.87 d	10.84 a	14.20 a	16.50 a
triclopyr 0.60 kg + glyphosate 2.0 kg	7.58 d	15.13 a	14.41 a	17.66 a
Untreated Control	17.22 a	16.97 a	14.74 a	16.01 a
Standard Error of Means	0.9239	1.6801	0.8970	0.7256

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

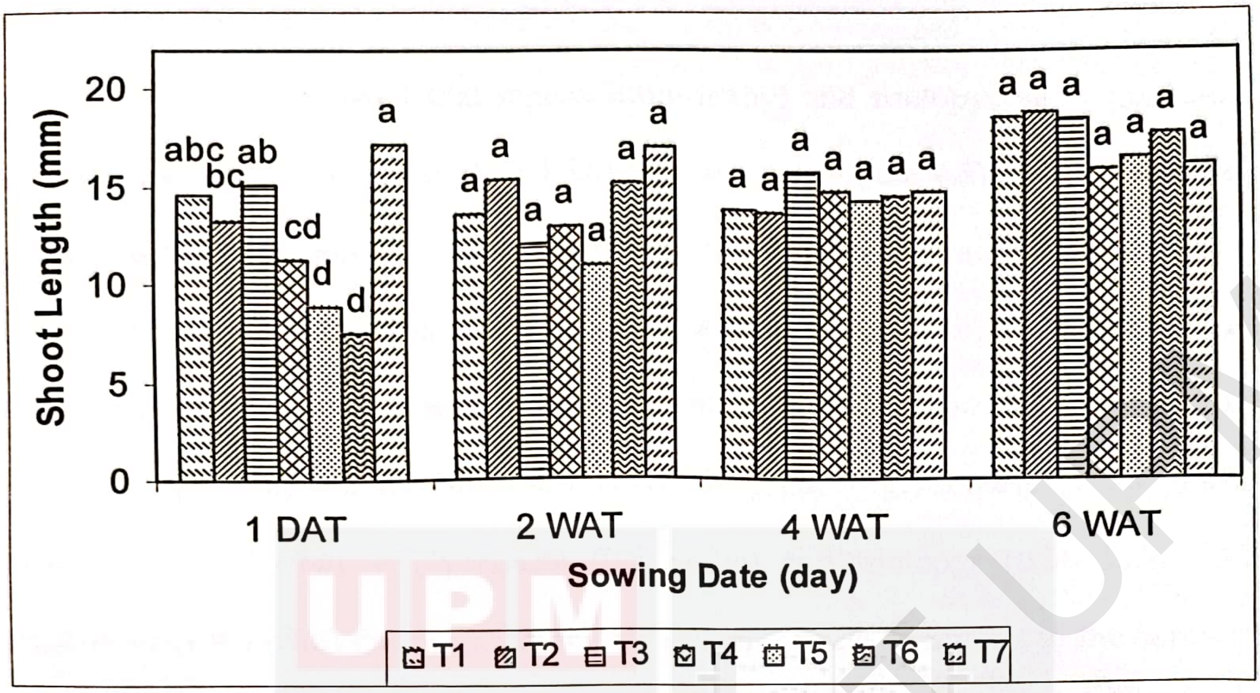

Figure 4.2: Effects of treatments on shoot length of tomato bioassay

(DAT = day after treatment; WAT = weeks after treatment; T1 = metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha); T2 = metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha); T3 = metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha); T4 = triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha); T5 = triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha); T6 = triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha); T7 = untreated control)

4.1.3 Rice Bioassay

The results showed that metsulfuron-methyl and triclopyr had significant effects towards rice shoot length at 1 DAT (Table 4.3, Figure 4.3). But, there were no significant differences in rice shoot lengths in all treatments at 3, 5 and 7WAT. This showed that the herbicides were only active in the soil at one day after spraying. At 1 DAT, rice shoot length for treatment metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha), metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha), triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) and triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha) did not have any significant effects compared to the control treatment. Treatments with metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha) and triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha) showed some effects on the rice shoot length. This indicates that metsulfuron-methyl and triclopyr were inhibitory at double the recommended rate.

Table 4.3: Effect of treatments on shoot length of rice bioassay

Herbicide Treatments, (kg ai/ha)	Rice Shoot Length (mm)			
	Days / Weeks After Treatment (DAT / WAT)			
	1 DAT	3 WAT	5 WAT	6 WAT
metsulfuron-methyl 15 g + glyphosate 0.5 kg	79.14 ab	73.25 a	85.87 a	89.11 a
metsulfuron-methyl 30 g + glyphosate 1.0 kg	70.43 abc	59.78 a	93.40 a	70.64 a
metsulfuron-methyl 60 g + glyphosate 2.0 kg	53.98 bc	55.72 a	89.19 a	74.28 a
triclopyr 0.15 kg + glyphosate 0.5 kg	84.73 a	77.42 a	96.13 a	79.16 a
triclopyr 0.30 kg + glyphosate 1.0 kg	73.25 abc	55.61 a	94.62 a	70.05 a
triclopyr 0.60 kg + glyphosate 2.0 kg	49.98 c	54.28 a	74.18 a	70.78 a
Untreated Control	84.55 a	64.11 a	85.46 a	76.64 a
Standard Error of Means	6.9607	6.0185	7.9964	6.7699

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

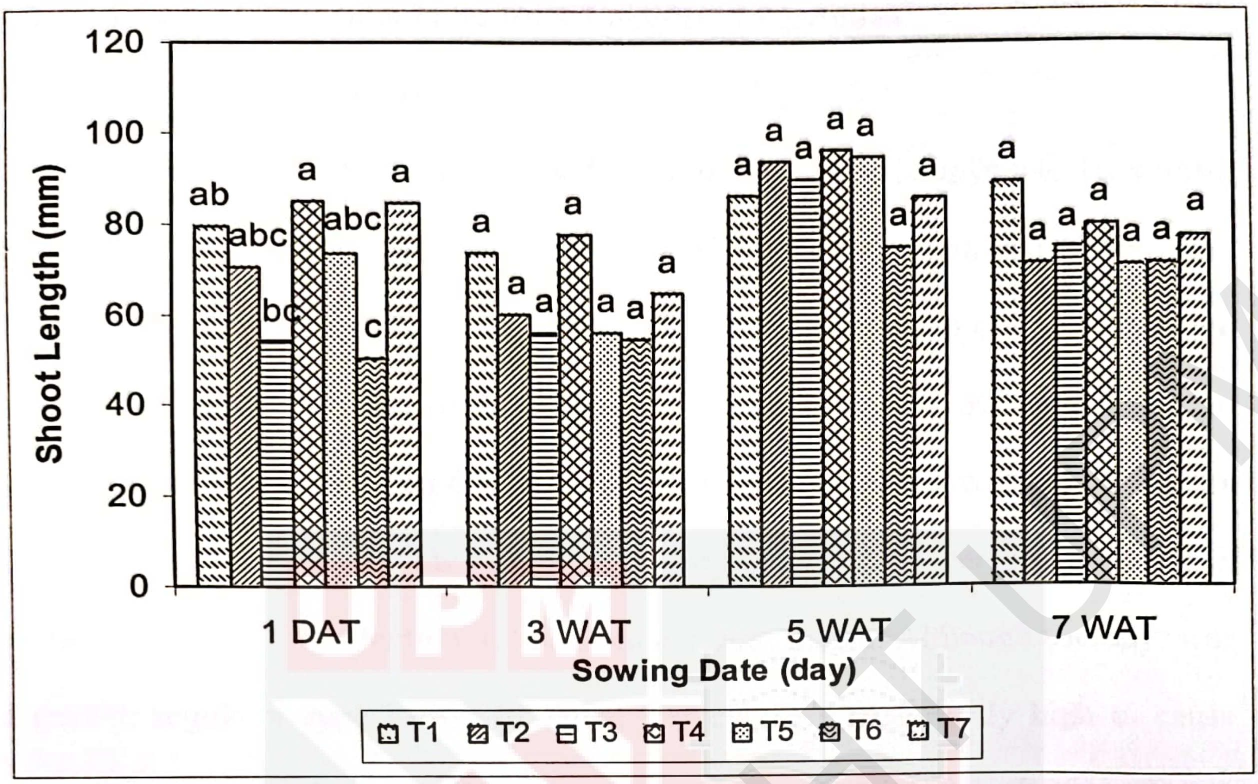

Figure 4.3: Effect of treatments on shoot length of rice bioassay

(DAT = day after treatment; WAT = weeks after treatment; T1 = metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha); T2 = metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha); T3 = metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha); T4 = triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha); T5 = triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha); T6 = triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha); T7 = untreated control)

4.2 Effect of Treatments on Root Lengths of Seedlings

4.2.1 Cucumber Bioassay

Results showed that the metsulfuron-methyl and triclopyr had significant effects only at 1 DAT (Table 4.4, Figure 4.4). There were no significant differences between shoot lengths at 2, 4 and 6 WAT. The herbicides were only active in the soil at one day after spraying. At 1 DAT, the cucumber root length for all treatments were significantly different compared to the control treatment. All rates of metsulfuron-methyl significantly reduced the cucumber root length. Triclopyr at all rates also showed reduction in the cucumber root length. Although triclopyr was a growth regulator type herbicide, but the levels were sufficiently high to cause reduction in cucumber root lengths. Treatments with triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha) and triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha) had stronger inhibitory effects compared to treatment with triclopyr (15 kg/ha) + glyphosate (0.5 kg/ha). Triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) was intermediate in cucumber root length reduction.

Table 4.4: Effect of treatments on root length of cucumber bioassay

Herbicide Treatments, (kg ai/ha)	Cucumber Root Length (mm)			
	Days / Weeks After Treatment (DAT / WAT)			
	1 DAT	2 WAT	4 WAT	6 WAT
metsulfuron-methyl 15 g + glyphosate 0.5 kg	11.88 c	39.07 a	41.83 a	50.28 a
metsulfuron-methyl 30 g + glyphosate 1.0 kg	11.85 c	31.04 a	35.12 a	58.50 a
metsulfuron-methyl 60 g + glyphosate 2.0 kg	12.44 c	29.83 a	34.33 a	53.22 a
triclopyr 0.15 kg + glyphosate 0.5 kg	38.10 b	36.77 a	47.37 a	49.29 a
triclopyr 0.30 kg + glyphosate 1.0 kg	13.28 c	42.63 a	44.32 a	48.41 a
triclopyr 0.60 kg + glyphosate 2.0 kg	7.39 c	30.21 a	44.09 a	53.61 a
Untreated Control	59.31 a	44.00 a	47.08 a	60.56 a
Standard Error of Means	2.5557	3.4889	3.3469	4.5436

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

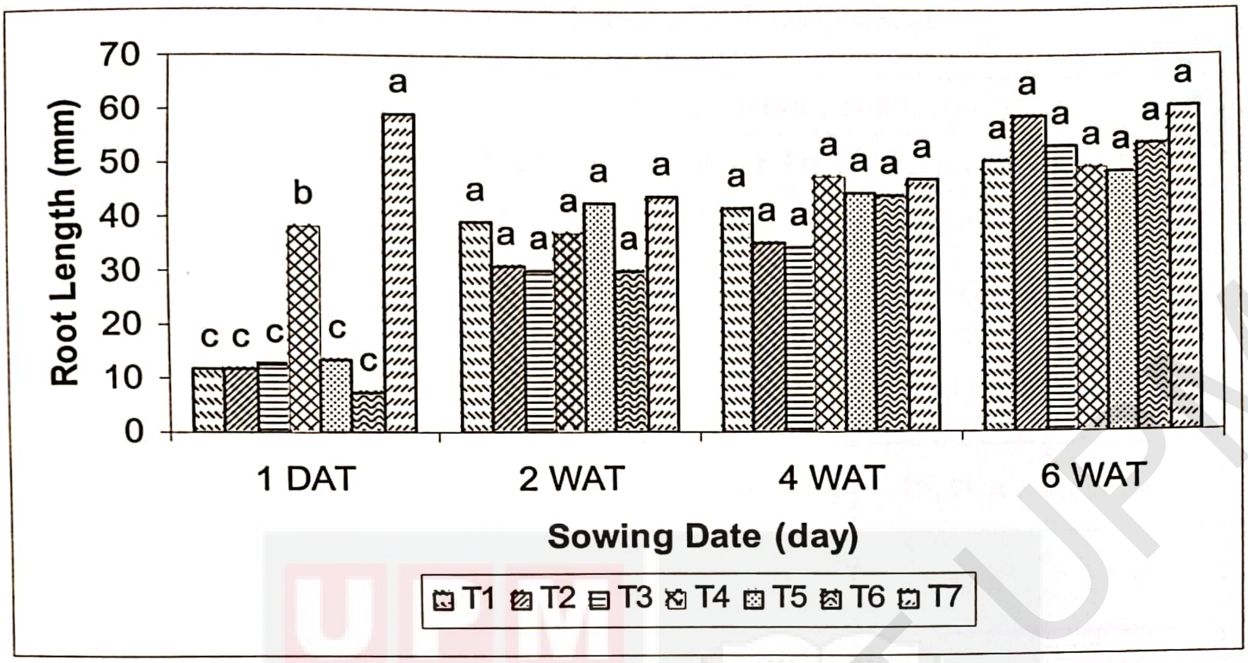

Figure 4.4: Effect of treatments on root length of cucumber bioassay

(DAT = day after treatment; WAT = weeks after treatment; T1 = metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha); T2 = metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha); T3 = metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha); T4 = triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha); T5 = triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha); T6 = triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha); T7 = untreated control)

4.2.2 Tomato Bioassay

The results showed that the effects of metsulfuron-methyl and triclopyr on tomato root length were only significant at 1 DAT (Table 4.5, Figure 4.5). There were no significant differences between treatments at 2, 4 and 6 WAT. This showed that the herbicides were only active in the soil at one day after spraying. At1 DAT, the tomato root length in all treatments was significantly reduced compared to control treatment. This showed that both the metsulfuron-methyl and triclopyr had reduction effects on the tomato root length. Triclopyr at double the recommended rate gave the highest reduction in tomato root length.

Table 4.5: Effect of treatments on root length of tomato bioassay

Herbicide Treatments, (kg ai/ha)	Tomato Root Length (mm)			
	Days / Weeks After Treatment (DAT / WAT)			
	1 DAT	2 WAT	4 WAT	6 WAT
metsulfuron-methyl 15 g + glyphosate 0.5 kg	6.94 bc	9.15 a	14.10 a	18.38 a
metsulfuron-methyl 30 g + glyphosate 1.0 kg	6.61 bc	10.52 a	13.85 a	16.24 a
metsulfuron-methyl 60 g + glyphosate 2.0 kg	8.29 bc	7.54 a	15.31 a	15.36 a
triclopyr 0.15 kg + glyphosate 0.5 kg	8.76 b	7.91 a	15.22 a	15.82 a
triclopyr 0.30 kg + glyphosate 1.0 kg	5.78 bc	8.67 a	16.60 a	16.88 a
triclopyr 0.60 kg + glyphosate 2.0 kg	4.64 c	9.55 a	15.42 a	14.77 a
Untreated Control	14.34 a	12.61 a	14.27 a	14.66 a
Standard Error of Means	0.9233	1.2511	1.8961	1.5352

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

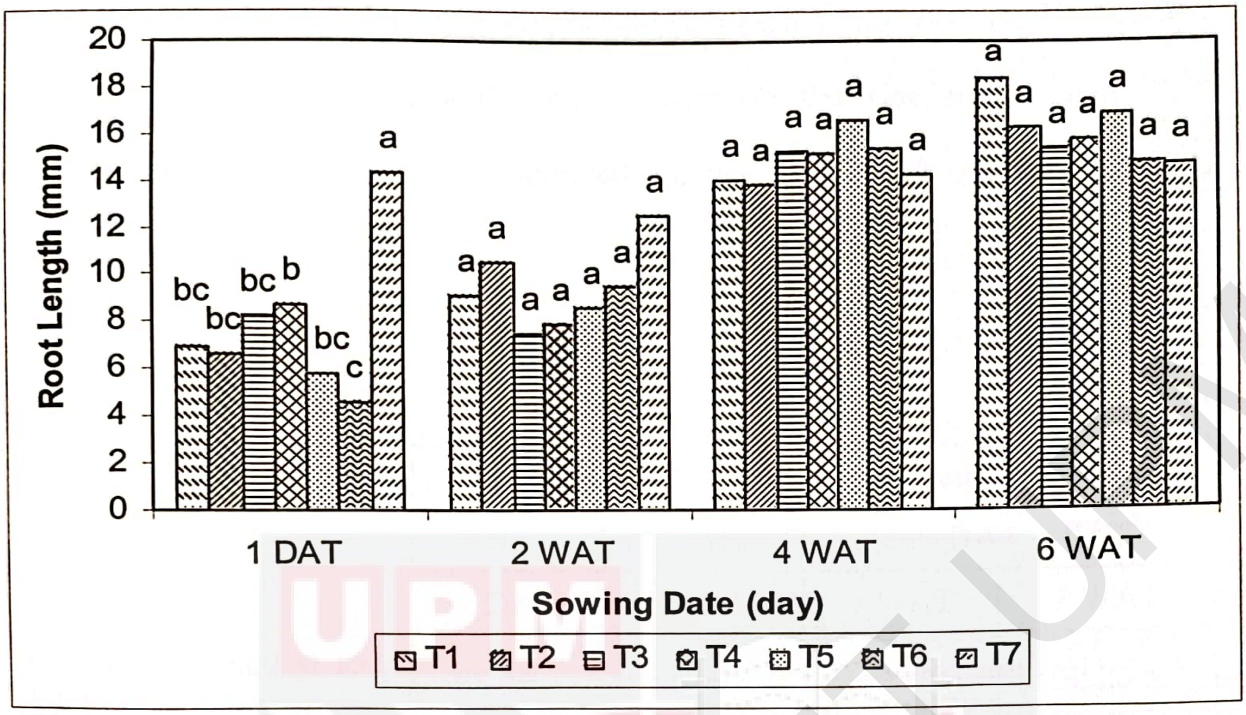

Figure 4.5: Effect of treatments on root length of tomato bioassay

(DAT = day after treatment; WAT = weeks after treatment; T1 = metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha); T2 = metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha); T3 = metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha); T4 = triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha); T5 = triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha); T6 = triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha); T7 = untreated control)

4.2.3 Rice Bioassay

The results showed that metsulfuron-methyl and triclopyr had significant effects on rice root length at 1 DAT (Table 4.6, Figure 4.6). There were no significant differences between all the treatments at 3, 5 and 7 WAT. This showed that the herbicides were only active in the soil at one day after spraying. At 1 DAT, rice root length for treatment metsulfuron-methyl (15 kg/ha) + glyphosate (0.5 kg/ha), metsulfuron-methyl (30 kg/ha) + glyphosate (1.0 kg/ha), triclopyr (15 kg/ha) + glyphosate (0.5 kg/ha) and triclopyr (30 kg/ha) + glyphosate (1.0 kg/ha) did not have any significant effects compared to control treatment. But treatments with metsulfuron-methyl (60 kg/ha) + glyphosate (2.0 kg/ha) and triclopyr (60 kg/ha) + glyphosate (2.0 kg/ha) showed some effects on the rice root length. Both metsulfuron-methyl and triclopyr reduced the rice root length only at double the recommended rate.

Table 4.6: Effect of treatments on root length of rice bioassay

Herbicide Treatments, (kg ai/ha)	Rice Root Length (mm) Days / Weeks After Treatment (DAT / WAT)			
	1 DAT	3 WAT	5 WAT	7 WAT
metsulfuron-methyl 15 g + glyphosate 0.5 kg	88.29 a	66.00 a	106.12 a	92.25 a
metsulfuron-methyl 30 g + glyphosate 1.0 kg	79.07 ab	52.50 a	108.68 a	86.65 a
metsulfuron-methyl 60 g + glyphosate 2.0 kg	49.71 bc	51.17 a	105.44 a	88.44 a
triclopyr 0.15 kg + glyphosate 0.5 kg	95.55 a	76.81 a	108.03 a	87.89 a
triclopyr 0.30 kg + glyphosate 1.0 kg	79.03 ab	60.33 a	113.58 a	84.54 a
triclopyr 0.60 kg + glyphosate 2.0 kg	38.43 c	51.03 a	88.38 a	88.03 a
Untreated Control	98.23 a	68.06 a	96.64 a	91.46 a
Standard Error of Means	8.6000	7.0120	10.0768	9.8493

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

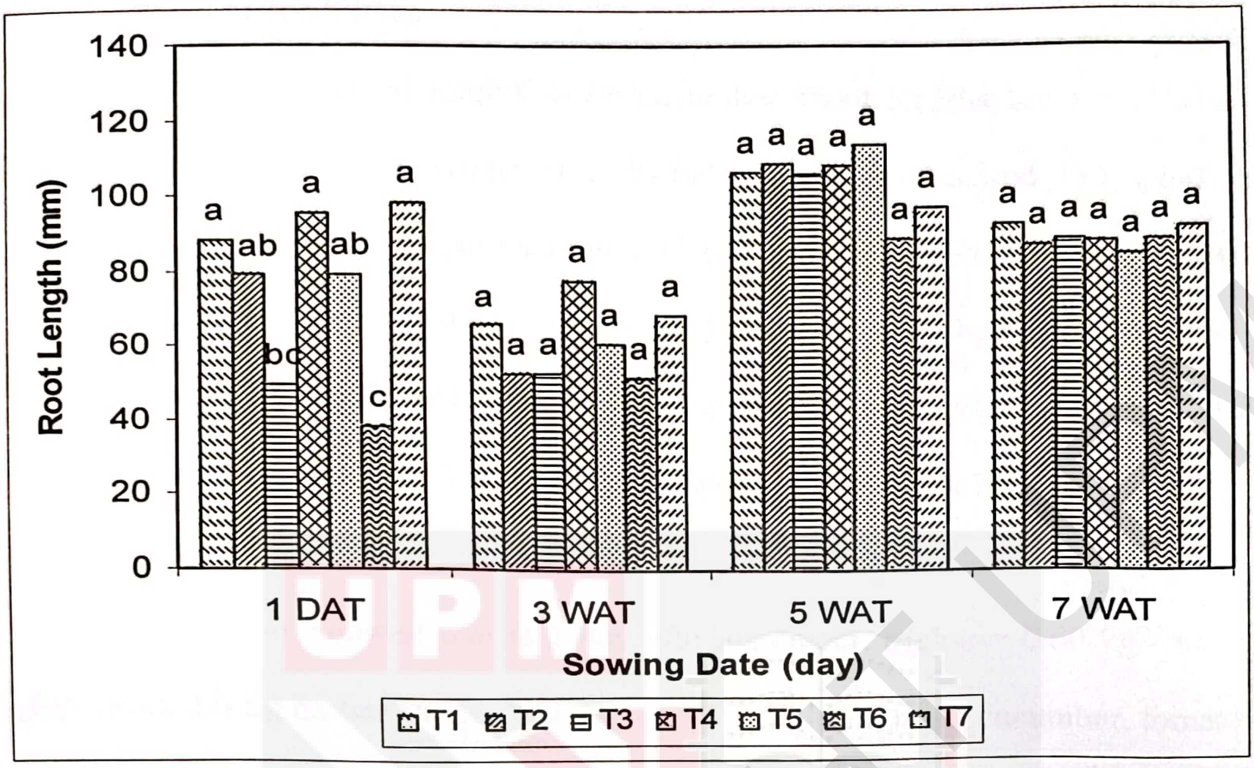

Figure 4.6: Effect of treatments on root length of rice bioassay

(DAT = day after treatment; WAT = weeks after treatment; T1 = metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha); T2 = metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha); T3 = metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha); T4 = triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha); T5 = triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha); T6 = triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha); T7 = untreated control)

4.4 Percent Inhibition

The percentage of inhibition on cucumber shoot lengths for metsulfuron-methyl (15 g/ha) + glyphosate (0.5 kg/ha), metsulfuron-methyl (30 g/ha) + glyphosate (1.0 kg/ha), metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha), triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha), triclopyr (0.30 kg/ha) + glyphosate (1.0 kg/ha) and triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha) were 11.84 %, 16.16 %, 18.66 %, 7.31 %, 41.57 % and 64.83 % respectively (Table 4.7).

The results showed that at 1 day after treatment, triclopyr 0.60 kg / ha + glyphosate 2.0 kg/ha has the highest percentage of inhibition on cucumber, tomato and rice shoot lengths compared to the other treatments.

The highest rate of triclopyr inhibited the shoot length of cucumber, tomato and rice by 64.83 %, 55.98 % and 40.89 %, respectively. Whereas, triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) had the lowest percentage of inhibition (7.31 %) on cucumber shoot length. Metsulfuron-methyl (60 g/ha) + glyphosate (2.0 kg/ha) had the lowest percentage of inhibition (12.49 %) on tomato shoot length and triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) had the lowest percentage of inhibition on rice shoot length.

Table 4.7: Effect of treatments on shoot length of three bioassay species at 1 day after treatment

Herbicide Treatments, (kg ai/ha)	Cucumber		Tomato		Rice	
	Shoot Length (mm)	% Inhibition	Shoot Length (mm)	% Inhibition	Shoot Length (mm)	% Inhibition
metsulfuron-methyl 15 g + glyphosate 0.5 kg	12.66 a	11.84	14.61 ab	15.16	79.14 a	6.40
metsulfuron-methyl 30 g + glyphosate 1.0 kg	12.04 a	16.16	13.22 ab	23.23	70.43 ab	16.70
metsulfuron-methyl 60 g + glyphosate 2.0 kg	11.68 a	18.66	15.07 ab	12.49	53.98 b	36.16
triclopyr 0.15 kg + glyphosate 0.5 kg	13.31 a	7.31	11.27 b	34.55	84.73 a	-0.21
triclopyr 0.30 kg + glyphosate 1.0 kg	8.39 b	41.57	8.87 c	48.49	73.25 ab	13.36
triclopyr 0.60 kg + glyphosate 2.0 kg	5.05 c	64.83	7.58 c	55.98	49.98 b	40.89
Untreated Control	14.36 a		17.22 a		84.55 a	
Standard Error	0.6749		1.1486		6.3542	

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

Triclopyr 0.60 kg / ha + glyphosate 2.0 kg/ha had the highest percentage of inhibition on root length of cucumber (87.54 %), tomato (67.64 %) and rice (60.88 %) (Table 4.8). Whereas, triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) had the lowest percentage inhibition on root length of cucumber (35.76 %), tomato (38.91 %) and rice (2.73 %).

Table 4.8: Effect of treatments on root length of three bioassay species at 1 day after treatment

Herbicide Treatments, (kg ai/ha)	Cucumber		Tomato		Rice	
	Root Length (mm)	% Inhibition	Root Length (mm)	% Inhibition	Root Length (mm)	% Inhibition
metsulfuron-methyl 15 g + glyphosate 0.5 kg	11.88 c	79.97	6.94 bc	51.60	88.29 a	10.12
metsulfuron-methyl 30 g + glyphosate 1.0 kg	11.85 c	80.02	6.61 bc	53.91	79.07 a	19.51
metsulfuron-methyl 60 g + glyphosate 2.0 kg	12.44 c	79.03	8.29 bc	51.60	49.71 b	49.39
triclopyr 0.15 kg + glyphosate 0.5 kg	38.10 b	35.76	8.76 b	38.91	95.55 a	2.73
triclopyr 0.30 kg + glyphosate 1.0 kg	13.28 c	77.61	5.78 bc	59.69	79.03 a	19.55
triclopyr 0.60 kg + glyphosate 2.0 kg	7.39 c	87.54	4.64 c	67.64	38.43 b	60.88
Untreated Control	59.31 a		14.34 a		98.23 a	
Standard Error	4.6941		1.0257		7.8507	

Means within columns with same alphabets are not significantly different at $p < 0.05$ (Tukey's Test)

CHAPTER 5

DISCUSSION

5.1 Cucumber Bioassay

Triclopyr at the recommended rate (0.30 kg/ha) and twice the recommended rate (0.60 kg/ha) had significant effects on cucumber shoot lengths when sown one day after spraying the triclopyr onto the soil. The higher rate of triclopyr gave greater reduction in shoot length. But metsulfuron-methyl at all rates did not have any significant effect on cucumber shoot length.

Metsulfuron-methyl and triclopyr at all rates showed an average of 73 % reduction in cucumber root length. It was very clear that the higher concentration of triclopyr resulted in greater reduction in root length. Triclopyr at half of the recommended rate gave much less reduction in cucumber root length, but showed some growth regulator effects. Triclopyr at 0.60 kg/ha had the highest percent inhibition on cucumber shoot and root lengths.

5.2 Tomato Bioassay

Metsulfuron-methyl did not show any inhibitory effect on growth of tomato shoots, while triclopyr was inhibitory at all rates tested. Higher rates of triclopyr gave higher reduction on tomato shoot length. The shoot length with triclopyr at twice the recommended rate (0.60 kg/ha) was reduced by 56 %.

Both metsulfuron-methyl and triclopyr caused reduction in tomato root length. Triclopyr at twice the recommended rate had the highest reduction effect on tomato root length. Root length reductions range from 30 – 60 %. Triclopyr at 0.60 kg/ha also had the highest percent inhibition on tomato shoot and root lengths.

5.3 Rice Bioassay

Both metsulfuron-methyl and triclopyr at twice the recommended rate gave significant reduction in both shoot and root lengths with around 40% reduction in shoot length and 50 % in root length compared to the untreated control treatment.

All three bioassay species showed significant differences between treatments only at 1 DAT. No significant differences were observed at 2, 4 and 6 WAT. The low pH which is around 4.51 in water and 3.84 in 0.01M $CaCl_2$ clay loam texture of the Bekenu series soil maybe responsible for more rapid degradation and reduced persistence in the soil (Pons and Baniuso, 1998). Besides that, the heavy rainfall received during the study period may have enabled faster degradation of the herbicides (James *et al.*, 1999).

The results suggest that both herbicides were biologically inactive in the soil at 2 weeks after treatment. Additional studies would be required to determine the loss in activity over the period between 1 DAT and 2 WAT.

CHAPTER 6

CONCLUSION

Through this analysis, it was found out that the effects of all treatments on shoot and root lengths of cucumber, tomato and rice were only significantly different at 1 DAT. This indicated that the herbicides were only active in the soil at one day after spraying.

Triclopyr (0.60 kg/ha) + glyphosate (2.0 kg/ha) had the most inhibitory effects on shoot and root length on all three bioassay species (cucumber, tomato and rice). Whereas triclopyr (0.15 kg/ha) + glyphosate (0.5 kg/ha) had the least inhibitory effect on shoot and root lengths of all three bioassay species. Metsulfuron-methyl at all rates had some effects on both shoot and root length of three bioassay species.

Cucumber was the more sensitive species and hence the most suitable bioassay species compared to tomato and rice to monitor carry-over effects of both triclopyr and metsulfuron-methyl. The studies indicated that it is safe to sow after two weeks after treatment. Further studies would be required to determine the loss in activity over the period between 1 DAT and 2 WAT to determine a more precise safe interval for sowing of sensitive crops in a rotation.

REFERENCES

Andersen, S.M., P.B Hertz, T. Holst, R. Bossi, C.S. Jacobsen. 2001. Mineralisation studies of 14C-labelled metsulfuron-methyl, tribenuron-methyl, chlorsulfuron and thifensulfuron-methyl in one Danish soil and groundwater sediment profile. *Chemosphere* **45**: 775 – 782.

Ashton F.M. and A.S. Crafts. 1973. Mode of action of herbicides. Wiley-interscience publication. New York. 504p.

Belio, R., J.E. Jensen, and P. Seiden. 2000. Applications of likelihood asymptotics for nonlinear regression in herbicide bioassays. *Biometrics* **56**: 1204 – 1212.

Begley, P. and B. E. Foulger. 1988. Detection of trace levels of triclopyr using capillary gas chromatography-electron-capture negative-ion chemical ionization mass spectrometry. *Journal of Chromatography* **435**: 45 – 53.

Bossi, R., P. Seiden, S.M. Andersen, C.S. Jacobsen, J.C. Streibig. 1999. Analysis of metsulfuron-methyl in soil by liquid chromatography/ tandem mass spectrometry. Application to a field dissipation study. *Journal of Agric Food Chemistry* **47**(10): 462 – 468.

Carter. 2000. Herbicide movement in soils: principles, pathways and processes. *Weed Research* **40**(1): 113 – 122.

Devlin, D.L., D.E. Peterson and D.L. Regehr. 1992. Residual herbicides, degradation and recropping intervals. Kansas State University Agricultural Experiment Station and Cooperative Extension Service 11p.

Dhareesank A., Kobayashi K., Usui K. 2006. Residual phytotoxic activity of pethoxamid in soil and its concentration in soil water under different soil moisture conditions. *Weed Biology and Management* **6**(1): 50 – 54.

Eastin, E.F. 1971. Movement and fate of fluorodifen-1'-^{14}C in cucumber seedlings. *Weed Research* **11**: 63 – 68.

Ghani, A., D.A. Wardle. 2001. Fate of 14C from glucose and the herbicide metsulfuron-methyl in a plant – soil microcosm system. *Soil Biology & Biochemistry* **33**: 777 – 785.

Hemmanda, S., M., Calmon, J.P. Calmon. 1994. Kinetics and hydrolysis mechanism of chlorsulfuron and metsulfuron-methyl. *Pestic. Sci.* 40: 71 – 76.

Hollaway K.L., R.S. Kookana, D.J. Mc Quinn, M.R. Moerkerk, D.M. Noy & M.A. Smal. 1999. Comparison of sulfonylurea herbicide residue detection in soil by bioassay, enzyme-linked immunosorbent assay and hplc. *Weed Research* **39**(5): 383 – 397.

Ismail B.S. and B.A. Azlizan. 2002. Persistence and bioactivity of metsulfuron-methyl in three soils. *Journal of Environment Science Health biology* **37**(4): 345 – 353.

James, T.K., P.T. Holland, A. Rahman and Y.R. Lu. 1999. Degradation of the sulfonylurea chlorsulfuron and triasulfuron in a high-organic-matter volcanic soil. *Weed Research* **39**: 137 – 147.

Jayakumar, R. and R. Jagannathan. 2003. Weed Science Principles. Kalyani Plublishers, India. p. 120 – 133, 136 – 137, 151 – 155, 250 – 285.

Koger, C.H., D.L. Shanner, L.J. Kurtz, T.W. Walker, N. Buehring, W.B. Henry, W.E. Thomas and J.W. Wilcut. 2005. Rice (*oryza sativa*) response to drift rates of glyphosate. *Pest Manag. Sci.* **61**: 1161 – 1167.

Li, Z.J., J.M. Xu, A. Muhammad and G.R. Ma. 2005. Effect of bound residues of metsulfuron-methyl in soil on rice growth. *Chemosphere* **58**: 1177 – 1183.

Lickly, T.D. and P.G. Murphy. 1987. The amount and identity of [14C] residues in bluegills (lepomis macrochirus) exposed to [14C] triclorpyr. *Environment International* **13**: 213 – 218.

Monaco, T.J., S.C. Weller and F.M. Ashton. 2002. Weed Science: principles and practices, John Wiley and Sons, Inc. New York. p. 98 – 108, 127 – 135, 303, 326 – 331, 341, 353 – 354.

Pons, N., E., Baniuso. 1998. Fate of metsulfuron-methyl in soils in relation to pedo-climatic conditions. *Pestic. Sci.* **53**: 311 – 323.

Rao, V.S. 2000. Principles of Weed Science. Second Edition. Science Publishers, Inc. USA. p.67, 97, 116 – 124, 141, 159 – 161, 185, 217 – 219, 232 – 272.

Ross, M.A and C.A. Lembi. 1999. Applied Weed Science. Second Edition. Prentice Hall. New Jersey. p.42, 76 – 80, 106 – 115, 126 – 143, 166 – 172.

Santos, B.M., J.P. Gilreath, C.E. Esmel and M.N. Siham. 2006. Effects of sublethal glyphosate rates on fresh market tomato. *Crop Protection* 3p.

Sanyal, N., S.K. Pramanik, R. Pal, A. Chowdhury. 2006. Laboratory simulated dissipation of metsulfuron methyl and chlorimuron ethyl in soils and their residual fate in rice, wheat and soybean at harvest. *Journal Zhejiang University Science Biology* **7**(3): 202 – 208.

Sarmah, A.K. and R.S. Kookana. 1999. Simultaneous analysis of triasulfuron, metsulfuron-methyl and chlorsulfuron in water and alkaline soils by high-performance liquid chromatography. *Journal of Environment Science Health* **34**(3): 363 – 380.

Seiden, Karpel and Streibig. 1998. Response of Brassica napus in tissue culture to metsulfuron-methyl and chlorsulfuron. *Weed Research* **38**(3): 221-228.

Stork, P. and M.C. Hannah. 1996. A bioassay method for formulation and residues studies of sulfonylurea and sulfonanylide herbicides. *Weed Research* **36**: 271 – 281.

Tanaka, Y., Y. Kajiwara,T. Kajiwara,M. Noguchi,N. Shimizu and K. Minami. 2006. Sulfonylurea derivatives with fused heterocycle moiety control sulfonylurea-resistant paddy weeds. Weed Biology and Management **6**: 115 – 119.

Ting, K.C.and C.S. Lee. 1995. Gas chromatographic determination of triclopyr in fruits and vegetables. *Journal of Chromatography A* **690**: 119 – 129.

Tu, M., C. Hurd, R. Robinson and J.M. Randall. 2001. Triclorpyr. Weed conrol methods handbook. *The Nature Conservancy*. 7k.1-7k.8.

Walker and Jurado-Exposido. 1998. Adsorption of isoproturon, diuron and metsulfuron-methyl in two soils at high soil solution ratios. *Weed Research* **38**(3): 229 – 238.

William, T.M. and H. Kangetsu. 2004. Comparison of commercial glyphosate formulations for control of prickly sida, purple nutsedge, morningglory and sicklepod. *Weed Biology and Management* **4**: 136–141.

Williams, G.M., R. Kroes, and I.C. Munro. 2000. Safety Evaluation and Risk Assessment of the Herbicide Roundup1 and Its Active Ingredient, Glyphosate, for Humans. *Regulatory Toxicology and Pharmacology* **31**: 117–165

Yu, Y.L., X. Wang, Y.M. Luo, J.F. Yang, J.Q. Yu, D.F. Fan. 2005. Fungal degradation of metsulfuron-methyl in pure cultures and soil. *Chemosphere* **60**: 460 – 466.

Appendices

Appendix 1: pH of soil used in the bioassay

Method	Bijoux bottles with 10g soil sample and 25ml distill water.	Bijoux bottles with 10g soil sample and 25 ml 0.01M $CaCl_2$
pH reading 1	4.51	3.84
pH reading 2	4.50	3.83
pH reading 3	4.53	3.86
Average pH	4.51	3.84

Appendix 2: Rainfall data collected at the experimental site

Day	Rain Collected
1	100.674
2	30.702
3	20.706
4	1.428
5	2.856
6	5.712
7	14.994
8	2.142
9	0
10	0
11	7.854
12	0
13	0
14	0
15	216.342
16	0
17	0
18	0
19	1.428
20	57.12
21	72.114
22	5.712
23	0
24	1.428
25	137.802

Day	Rain Collected
26	0
27	0
28	6.426
29	0
30	0
31	0
32	34.986
33	137.802
34	33.558
35	0
36	17.85
37	0
38	0
39	30.702
40	82.824
41	29.274
42	41.412
43	31.416
44	5.712
45	0
46	0
47	0
48	321.3
49	0
50	0

Day	Rain Collected
51	113.526
52	0
53	0
54	0
55	157.794
56	14.994
57	100.674
58	29.274
59	229.194
60	0
61	0

Appendix 3: ANOVA for effect of treatments on cucumber shoot length at 1 DAT

Source	dF	SS	MS	F	Prob.
Total	41	466.2004			
Rep	5	5.2753	1.0551	0.39	0.8544
Treat	6	378.9345	63.1558	23.11	<.0001
Error	30	81.9906	2.7330		

C.V. = 14.94%

Appendix 4: ANOVA for effect of treatments on cucumber shoot length at 2 WAT

Source	dF	SS	MS	F	Prob.
Total	41	324.7819			
Rep	5	67.3631	13.4726	1.80	0.1425
Treat	6	33.2145	5.5358	0.74	0.6211
Error	30	224.2042	7.4734		

C.V. = 21.12%

Appendix 5: ANOVA for effect of treatments on cucumber shoot length at 4 WAT

Source	dF	SS	MS	F	Prob.
Total	41	84.6675			
Rep	5	23.8146	4.7629	2.76	0.0364
Treat	6	9.0498	1.5083	0.87	0.5258
Error	30	51.8031	1.7268		

C.V. = 9.75 %

Appendix 6: ANOVA for effect of treatments on cucumber shoot length at 6 WAT

Source	dF	SS	MS	F	Prob.
Total	41	138.2810			
Rep	5	18.7854	3.7571	1.42	0.2444
Treat	6	40.3141	6.7190	2.55	0.0411
Error	30	79.1815	2.6394		

C.V. = 9.42 %

Appendix 7: ANOVA for effect of treatments on tomato shoot length at 1 DAT

Source	dF	SS	MS	F	Prob.
Total	41	579.2310			
Rep	5	15.5866	3.1173	0.73	0.6063
Treat	6	435.6005	72.6001	17.01	<.0001
Error	30	128.0439	4.2681		

C.V. = 16.48 %

Appendix 8: ANOVA for effect of treatments on tomato shoot length at 2 WAT

Source	dF	SS	MS	F	Prob.
Total	41	603.9558612			
Rep	5	16.5898686	3.3179737	0.24	0.9440
Treat	6	163.9414857	27.3235809	1.94	0.1073
Error	30	423.4245069	14.1141502		

C.V. = 27.23 %

Appendix 9: ANOVA for effect of treatments on tomato shoot length at 4 WAT

Source	dF	SS	MS	F	Prob.
Total	41	154.9441			
Rep	5	16.0329	3.2066	0.80	0.5605
Treat	6	18.2217	3.0369	0.75	0.6106
Error	30	120.6894	4.0229		

C.V. = 13.89 %

Appendix 10: ANOVA for effect of treatments on tomato shoot length at 6WAT

Source	dF	SS	MS	F	Prob.
Total	41	194.8635792			
Rep	5	60.60429103	12.12085821	4.60	0.0031
Treat	6	55.27499887	9.21249981	3.50	0.0096
Error	30	78.9842893	2.6328096		

C.V. = 9.33 %

Appendix 11: ANOVA for effect of treatments on rice shoot length at 1 DAT

Source	dF	SS	MS	F	Prob.
Total	41	14879.2271			
Rep	5	558.5088	111.7018	0.46	0.8019
Treat	6	7053.0991	1175.5165	4.85	0.0014
Error	30	7267.6190	242.2539		

C.V. = 21.96 %

Appendix 12: ANOVA for effect of treatments on rice shoot length at 2 WAT

Source	dF	SS	MS	F	Prob.
Total	41	10490.7387			
Rep	5	2009.0389	401.8077	2.22	0.0784
Treat	6	3048.3584	508.0597	2.81	0.0275
Error	30	5433.3414	181.1114		

C.V. = 21.40 %

Appendix 13: ANOVA for effect of treatments on rice shoot length at 4 WAT

Source	dF	SS	MS	F	Prob.
Total	41	13837.2593			
Rep	5	2197.9902	439.5980	1.37	0.2615
Treat	6	2047.8462	341.3077	1.07	0.4035
Error	30	9591.4229	319.7141		

C.V. = 20.23 %

Appendix 14: ANOVA for effect of treatments on rice shoot length at 6 WAT

Source	dF	SS	MS	F	Prob.
Total	41	9793.3892			
Rep	5	1259.9096	251.9819	1.10	0.3812
Treat	6	1658.6574	276.4429	1.21	0.3302
Error	30	6874.8222	229.1607		

C.V. = 19.97 %

Appendix 15: ANOVA for effect of treatments on cucumber root length at 1 DAT

Source	dF	SS	MS	F	Prob.
Total	41	14512.2319			
Rep	5	100.4999	20.1000	0.62	0.6889
Treat	6	13431.9679	2238.6613	68.55	<.0001
Error	30	979.7643	32.6588		

C.V. = 25.93 %

Appendix 16: ANOVA for effect of treatments on cucumber root length at 2WAT

Source	dF	SS	MS	F	Prob.
Total	41	3552.6581			
Rep	5	443.5686	88.7137	1.46	0.2329
Treat	6	1283.2128	213.8688	3.51	0.0094
Error	30	1825.8767	60.8626		

C.V. = 21.54 %

Appendix 17: ANOVA for effect of treatments on cucumber root length at 4 WAT

Source	dF	SS	MS	F	Prob.
Total	41	3154.9447			
Rep	5	451.4499	90.2899	1.61	0.1871
Treat	6	1023.2551	170.5425	3.04	0.0190
Error	30	1680.2395	56.0079		

C.V. = 17.81 %

Appendix 18: ANOVA for effect of treatments on cucumber root length at 6 WAT

Source	dF	SS	MS	F	Prob.
Total	41	4479.1536			
Rep	5	609.6371	121.9274	1.18	0.3416
Treat	6	772.8661	128.8110	1.25	0.3106
Error	30	3096.6503	103.2217		

C.V. = 19.02 %

Appendix 19: ANOVA for effect of treatments on tomato root length at 1 DAT

Source	dF	SS	MS	F	Prob.
Total	41	550.3912			
Rep	5	61.7182	12.3436	2.90	0.0300
Treat	6	360.7878	60.1313	14.11	<.0001
Error	30	127.8852	4.2628		

C.V. = 26.11 %

Appendix 20: ANOVA for effect of treatments on tomato root length at 2 WAT

Source	dF	SS	MS	F	Prob.
Total	41	376.0344			
Rep	5	34.2634	6.8527	0.88	0.5092
Treat	6	106.9708	17.8285	2.28	0.0626
Error	30	234.8002	7.8267		

C.V. = 29.69 %

Appendix 21: ANOVA for effect of treatments on tomato root length at 4 WAT

Source	dF	SS	MS	F	Prob.
Total	41	871.2064			
Rep	5	298.7589	59.7518	3.32	0.0166
Treat	6	33.1852	5.5309	0.31	0.9280
Error	30	539.2623	17.9754		

C.V. = 28.33 %

Appendix 22: ANOVA for effect of treatments on tomato root length at 6 WAT

Source	dF	SS	MS	F	Prob.
Total	41	573.8949			
Rep	5	158.9941	31.7988	2.70	0.0396
Treat	6	61.3527	10.2254	0.87	0.5298
Error	30	353.5481	11.7849		

C.V. = 21.43 %

Appendix 23: ANOVA for effect of treatments on rice root length at 1 DAT

Source	dF	SS	MS	F	Prob.
Total	41	32436.3189			
Rep	5	2459.1782	491.8356	1.33	0.2784
Treat	6	18883.2186	3147.2031	8.51	<.0001
Error	30	11093.9222	369.7974		

C.V. = 25.47 %

Appendix 24: ANOVA for effect of treatments on rice root length at 2 WAT

Source	dF	SS	MS	F	Prob.
Total	41	12588.0859			
Rep	5	1764.2445	352.8490	1.44	0.2404
Treat	6	3448.5696	574.7616	2.34	0.0569
Error	30	7375.2714	245.8424		

C.V. = 25.71 %

Appendix 25: ANOVA for effect of treatments on rice root length at 4 WAT

Source	dF	SS	MS	F	Prob.
Total	41	21740.6459			
Rep	5	3902.8787	780.5757	1.54	0.2081
Treat	6	2606.5102	434.4184	0.86	0.5382
Error	30	15231.2570	507.7086		

C.V. = 21.70 %

Appendix 26: ANOVA for effect of treatments on rice root length at 6 WAT

Source	dF	SS	MS	F	Prob.
Total	41	18297.9234			
Rep	5	3491.5663	698.3133	1.44	0.2389
Treat	6	254.9282	42.4880	0.09	0.9971
Error	30	14551.4289	485.0476		

C.V. = 24.89 %

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify I have no objection to publish the project entitled "Residual activity of metsulfuron-methyl and triclopyr applied in combination with glyphosate-IPA in Bekenu series soil" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

OOI LOO LING

Date: 2/5/07