Synthesis, Characterization, Herbicidal and Biological Activities of Glyphosate Derivatives

Thesis Submitted to

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DOCTOR OF PHILOSOPHY
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DECLARATION

I hereby declare that the thesis entitled**," Synthesis, Characterization, Herbicidal and Biological Activities of Glyphosate derivatives"** submitted for Ph.D. Chemistry Degree to Department of Chemistry, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for award of any other degree.

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CERTIFICATE

This is to certify that **Mrs. Jatinder Pal Kaur Gill** has completed the Ph.D. Chemistry titled **,** **" Synthesis, Characterization, Herbicidal and Biological Activities of Glyphosate derivatives"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma.

The thesis is fit for the submission for the partial fulfillment of the condition for the award of degree of Ph.D. in Chemistry.

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ABSTRACT

The present work deals with the synthesis, characterization, herbicidal, anti-microbial, microbial degradation, insecticidal and toxicological studies of glyphosate derivatives. The research work has been divided into two main parts. The first part includes synthesis and characterization of glyphosate derivatives while the second part comprises of all the biological studies of these synthesized derivatives (herbicidal activity, anti-microbial, microbial degradation, insecticidal and toxicological studies).

1) Synthesis and Characterization of glyphosate derivatives: 36 different derivatives of glyphosate were synthesized by undergoing different organic reactions on the glyphosate molecule. Seven different types of glyphosate derivatives were synthesized and their characterization was done using FTIR, NMR and ESI-MS. A series of ester derivatives of glyphosate were synthesized by using thionyl chloride and corresponding alcohol. It is an effective and efficient method used for the synthesis of alkyl substituted ester derivatives of glyphosate. Four different ester derivatives of glyphosate; **Methyl ester of glyphosate** (Methyl 2-{[(dimethoxyphosphoryl)methyl]amino}acetate hydrochloride), **Ethyl ester of glyphosate** (Ethyl 2-{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride), **Isopropyl ester of glyphosate** (Isopropyl 2-({[diisopropoxyphosphoryl]methyl}amino)acetate hydrochloride), **Butyl ester of glyphosate** (Butyl 2{[(dibutoxyphosphoryl)methyl]amino} acetate hydrochloride) were synthesized by using different alcohols. Second type of glyphosate derivatives are the amide derivatives of glyphosate, they were synthesized by condensation of glyphosate with required amine separately using mixed anhydride method of coupling with Isobutyl chloroformate as a coupling reagent in presence of inert solvent. Three amide derivatives were synthesized namely **Methyl amide of glyphosate** ({[2-(methylamino)-2-oxo ethyl amino} methyl) phosphonic acid, **Isopropyl amide of glyphosate** ({[2-oxo-2-(propan-2-ylamino) ethyl] amino} methyl) phosphonic acid), **Butyl amide of glyphosate** ({[2-(butylamino)-2-oxo ethyl] amino} methyl) phosphonic acid). Another derivative of glyphosate was synthesized by Boc-Protection of glyphosate in which di-tert-butyl dicarbonate was used as a protecting group. The derivative prepared was **Boc-Protected glyphosate** (2-((tert-butoxycarbonyl)(phosphono methyl) amino) ethanoic acid). Another derivative **Boc-Protected guanidilated glyphosate** (2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl) guanidino) ethanoic acid) was synthesised

by treating glyphosate with guanidinylating reagent 1,3- Bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea in dioxane as a solvent. Next derivative of glyphosate synthesized was **Guanidilated glyphosate** (2-(1-phosphonomethyl) guanidino)ethanoic acid). It was synthesized by acidolytic cleavage of Boc groups using Trifluoroaceticacid (TFA). A series of thioxylated ester derivatives of glyphosate were prepared, by refluxing the ester derivatives of glyphosate with phosphorus pentasulphide in dioxane as a solvent. Four thioxylated ester derivatives of glyphosate were synthesized **Thioxylated methyl ester of glyphosate** (*O* -methyl {[(dimethoxy phosphorothioyl)methyl]amino}ethanethioate), **Thioxylated ethyl ester of glyphosate** (*O*-ethyl{[(diethoxyphosphorothioyl)methyl]amino}ethanethioate), **Thioxylated isopropyl ester of glyphosate** (*O*-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl}amino) ethane thioate and **Thioxylated butyl ester of glyphosate** (*O*-butyl{[(dibutoxyphosphorothioyl) methyl] amino}ethanethioate). Apart from these, metal complexes of glyphosate and ester derivatives of glyphosate (25 total) were also synthesized. Five essential metal ions Zn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+} and Fe^{3+} were used to prepare these metal complexes. The formation of different derivatives of glyphosate was confirmed from spectroscopic studies.

2) <u>Herbicidal activity and other biological activities</u>: All the synthesized derivatives of glyphosate were screened for their herbicidal properties against two commonly grown weeds (*Parthenium hysterophorus* and *Cyperus rotundus*) and wheat grass (*Triticum aestivum*) Three different test concentrations [1X (recommended dose 441g/L per acre), 0.5X (half of recommended dose) and 0.25X (one-fourth of recommended dose)] of the synthesized derivatives were applied on the plants. They have shown excellent to moderate herbicidal effects on this weed. Out of all the thirty six derivatives of glyphosate, Ethyl 2-{[(diethoxyphosphoryl) methyl] amino}acetate hydrochloride and *O*-ethyl {[(diethoxy phosphorothioyl) methyl]amino} ethanethioate have shown the excellent results. All the synthesized derivatives of glyphosate have effectively reduced the chlorophyll content of the weeds and this decrease was more pronounced with increase in the concentration of the derivatives.

Anti-microbial activity of all the 36 derivatives of glyphosate was checked against two plant growth-promoting rhizo bacteria (PGPR) *Pseudomonas fulva* (Gram negative) (Accession Number- MF 782684), *Pseudomonas putida* (Gram negative) (Accession Number- MF 782681) and two fungal species *Aspergillus fumigatus* (NCIM-902), *Candida albicans* ().Very few derivatives have shown antibacterial and antifungal activity against these microbes.

Microbial degradation of the two synthesized derivatives of glyphosate was carried out using *Actinomyces* sp. (Gram positive) (Accession Number-KJ 854403.1). Only two derivatives, Ethyl 2-{[(diethoxyphosphoryl) methyl] amino}acetate hydrochloride and *O*-ethyl {[(diethoxy phosphorothioyl) methyl]amino} ethanethioate were taken to carried out their degradation studies. Both these synthesized derivatives have been successfully degraded to methyl phosphonic acid; m/z 97 and metaphosphonic acid; m/z 79.

Insecticidal effects of the synthesized derivatives of glyphosate was checked on *Drosophilla melanogaster* (Commonly known as Fruit fly). Three different concentrations (250ppm, 500ppm and 1000ppm) of glyphosate and its synthesized derivatives were used and the activity was monitored for 24hrs, 48 hrs and 72 hrs . Most of the synthesized derivatives remain ineffectual against *Drosophila melanogaster* at all the three test concentrations. Out of 36, only two synthesized derivatives namely (2-((tert-butoxycarbonyl)(phosphono methyl) amino) ethanoic acid and 2-(1-phosphonomethyl) guanidino)ethanoic acid have shown minor toxicity. To check the toxicological impact of the synthesized derivatives of glyphosate, their toxicity evaluation was done on Earthworms (*Esenia fetida*). Mortality of earthworms was taken as the criterion to analyze toxicological effects of the synthesized derivatives of glyphosate. Majority of the synthesized derivatives of glyphosate have neither shown any sign of toxicity nor killed any earthworm. Thus, the synthesized derivatives of glyphosate have demonstrated good herbicides properties and no sign of toxicity on microorganisms, insects and earthworms has been observed.

CHAPTER-1

INTRODUCTION

1.1) EMERGENCE OF GLYPHOSATE AS AN HERBICIDE: (HISTORY)

Agrochemicals have become global necessity to increase crop productivity in agricultural fields. Nowadays they play a pivotal role in controlling not only the pests and rodents but also many microbial infections. There are several types of herbicides, insecticides and pesticides that are in use in the modern cultivation of lands. Sadly, the surge in human needs and the greed for enhanced production of food yields has resulted in excessive consumption of these agrochemicals. Astonishingly, the initial use of pesticides began along with the ‘agricultural evolution’ of mankind.

According to definition of U.S. Environmental Protection Agency, pesticide is any substance proposed for repelling, destroying, preventing, regulating or controlling pests[1]. Originally, natural and organic pesticides were used for pest control. However, after World War II, there was starvation all around and in order to boost the fight against hunger and malnutrition there was an urgent need to augment the crop productivity. This excessive demand from the contemporary agricultural infrastructure motivated the scientific fraternity to invent many synthetic chemicals which could shoot up the crop productions by manifolds. The Need of hour and the accomplishment of modern pesticide industry persuaded the widespread recognition of these synthetic chemicals around the world and it led to subsequent dependence on them. Various synthetic herbicides like 2,4-dichlorophenoxyacetic acid[2] and 2,4,5trichlorophenoxyacetic acid[3] were used to control weeds in the farms, gardens and horticultural site.

The invention of glyphosate (N-(phosphonomethyl) glycine) was a big breakthrough in that era. Ever since its discovery in the year 1970, it is still the most commonly used herbicide all over the world. Henri Martin of a Swiss pharmaceutical company (Cilag) is considered to be the pioneer synthesizer of glyphosate molecule. Later on the herbicidal activity of glyphosate was analyzed by chemist John. E. Franz of Monsanto company in 1974[4].

It is a systemic, unselective, wide array and post-emergent weed killer employed to manage periodical and everlasting plants. Glyphosate based herbicides are used to kill unwanted weeds from farmlands but along with them, they also quell all the plants which are not genetically resistant to them[5].

1.2) WORLDWIDE CONSUMPTION OF GLYPHOSATE:

Glyphosate is the commonly used herbicide throughout the globe. Earlier, in agricultural lands it was applied before planting the main crop and was also used in weed eradication in grazing lands and wild territories.

U.S.A is the largest consumer of glyphosate[6]. In 1987, 6-8 million pounds of glyphosate was used by U.S. farmers and ranchers[7]. However, 20 years later in 2007 a report by EPA (Environmental Protection Agency) showed that the glyphosate's use had significantly elevated to 81.6–83.9 million kilograms[8].

Among other countries like Germany and Denmark 35-39% of the agriculture depends on glyphosate[9]. In Argentina, as well, glyphosate is the most frequently used herbicide, with annual usage of 180-200 million litres[10]. Also in U.K glyphosate is the regularly used herbicide in the arable food crops and commercial fruits orchards[11]. In European countries like France, Romania and Hungary 50-60% of sunflower crops are annually treated with glyphosate[12].

Agiculture plays a vital role in Indian economy. More than 70% of population in India depends upon agriculture. Glyphosate is also very commonly used in India. According to a report, the annual usage of glyphosate in India in 2015 was 35 kilo tons and it is predicted to increase to 75 kilo tons by 2024[13].

However, with the evolution of glyphosate- resistant crops, the farmers all around the world have been forced to increase the use of this herbicide many folds[14,15]. From various surveys conducted to determine the quantum of usage of glyphosate, it has been found that by 2014, annual consumption of glyphosate has increased to 240 million pounds. The annual consumption of glyphosate in last two decades has increased substantially[16] (Fig. 1)

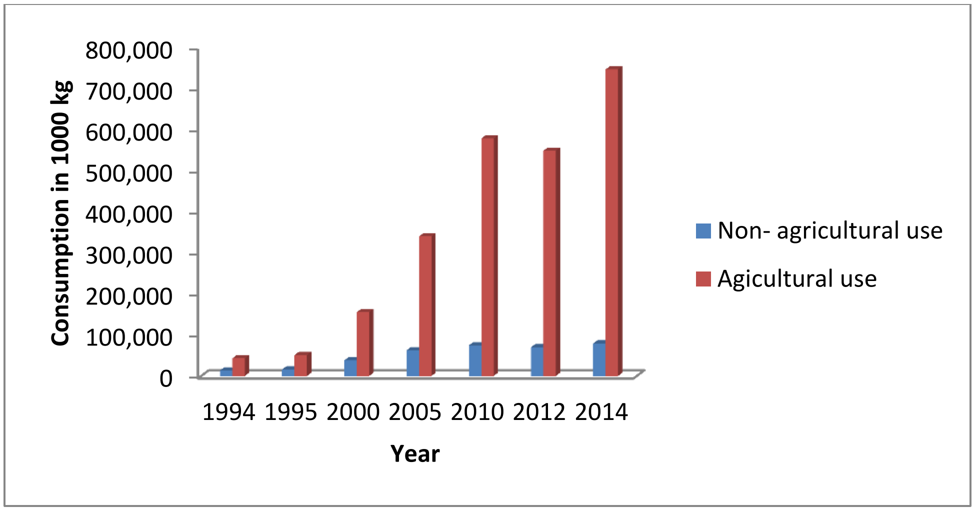


Figure1.1 The annual consumption of glyphosate throughout the world in the last two decade (from 1994-2014)

1.3) THE COMMON FORMULATIONS OF GLYPHOSATE USED WORLDWIDE:

Glyphosate is sold under numerous trade names having different formulations. Table1. enlists some common formulations of glyphosate with their trade names.

TABLE 1.1 Common formulations (Trade names) of glyphosate used worldwide:

S.No	Brand Name	Active Ingredients
1.	Roundup® Renew	360g/L Glyphosate
2.	Watson weed killer 360 concentrate	Glyphosate isopropyl amine salt (360g/L)
3.	McGregor's Weedout	Glyphosate isopropylamine salt (48%)
4.	Agpro Glyphosate 360	Glyphosate isopropylammonium salt (360g/L)
5.	Dow Glyphosate 360	Glyphosate isopropylamine salt (360g/litre)
6.	Clearout 180	Glyphosate iso-propyl ammonium salt
7.	Clearout 360	Glyphosate iso-propyl ammonium salt
8.	Cobra	Glyphosate iso-propyl ammonium salt
9.	Erase	Glyphosate iso-propyl ammonium salt
10.	Glygran SG	Glyphosate ammonium salt
11.	Glyphofix	Glyphosate isopropyl ammonium salt
12.	Glyphogan	Glyphosate isopropyl ammonium salt
13.	Glyphosate WSG	Glyphosate-sodium
14.	Kalach	Glyphosate-sodium
15.	Mamba	Glyphosate isopropyl ammonium salt
16.	Mamba MAX	Glyphosate isopropyl ammonium salt
17.	Nexus Glyphosate	Glyphosate isopropyl ammonium salt
18.	Rondo	Glyphosate-sodium

19.	Roundup	Glyphosate isopropyl ammonium salt
20	Roundup Bio-dry	Glyphosate-sodium
21.	Roundup CT	Glyphosate isopropyl ammonium salt
22.	Roundup Turbo	Glyphosate isopropyl ammonium salt
23.	Roundup Ultra	Glyphosate isopropyl ammonium salt
24.	Slash Turbo	Glyphosate isopropyl ammonium salt
25.	Sting	Glyphosate isopropyl ammonium salt
26.	Touchdown	Glyphosate + trimesium
27.	Touchdown plus	Glyphosate + trimesium
28.	UAP Glyphosate	Glyphosate-sodium
29.	Back draft	Glyphosate+ imazaquin
30.	Expert	Glyphosate+ S-metolachlor+ atrazine
31.	Extreme	Glyphosate+ imazethapyr
32.	Flexstar GT	Glyphosate+ fomesafen
33.	Sequence	Glyphosate+ S-metalachlor

1.4) CHEMISTRY OF GLYPHOSATE:

IUPAC Name: 2-(phosphonomethylamino) acetic acid

Molecular Formula: $C_3H_8NO_5P$

Molecular Weight: 169 g/mol

Physical State: White solid

Density: 1.74g/mL

Stability: Stable for 32 days at 25°C and pH 5, 7 or 9. Negligible volatility and decomposition begins at 215°C

Melting Point: 189.5°C

Odour: Odourless

Amphoteric Nature And Dissociation Constants:

Glyphosate is a complex amphoteric, polyprotic molecule[17] with four ionisable functional groups $pKa_1 = 0.78$ (1st Phosphoric acid group, $H_2O_3P^+$), $pKa_2 = 2.29$ (Carboxylate group, COO^-), $pKa_3 = 5.96$ (2nd Phosphoric acid group, $H_2O_3P^+$) and $pKa_4 = 10.98$ (amino group,-NH_2)[18].

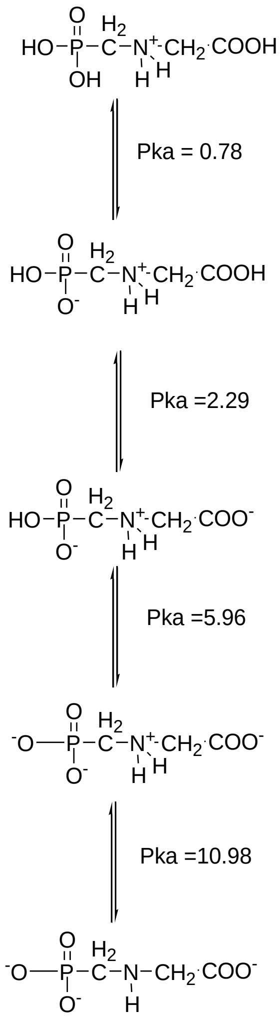


Figure 1.2 Ionisation species of glyphosate

In solid crystalline state, it exists as a zwitterion (Figure 1.3). A hydrogen ion from the first phosphonic acid group ($H_2O_3P^+$) is segregated and get connected with the amino group to form a dipolar molecule[19]. Zwitterionic form of glyphosate is the predominant form in which glyphosate can prevail.

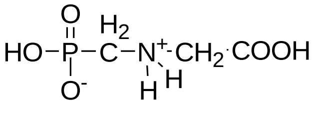


Figure 1.3 Zwitterionic form of glyphosate

SOLUBILITY: Glyphosate is soluble in water. Its solubility in water is 15700mg/L (pH 7 at 25°C)[20]. However glyphosate's solubility in organic solvents is negligible. Its solubility in acetone, xylenes and ethyl acetate is 0.6mg/L each while its solubility in methanol is 10mg/L[21]. Different Alcohol-Water systems with different mole fractions of water were used to check the solubility of glyphosate. It has been found that the solubility of glyphosate in pure water and different water-alcohol systems increase with rise in temperature and reduces with decrease in mole fraction of water. This indicates that glyphosate is mostly soluble in water systems with large mole fractions of water[22].

1.5) CRYSTALLOGRAPHY OF GLYPHOSATE:

X-ray crystallography of glyphosate shows that it has monoclinic shape with $P2_1/c$ space group. Cell dimensions for monoclinic glyphosate crystal are as follows:

a = 8.682(5) Å, b = 7.973(8) Å, c =9.875(5) Å, β = 105.74(4)° and Z =4.

Different bond lengths (Å) in glyphosate molecule are measured as follows:

C-P bond length is equal to 1.823 Å

C-H and N-H bond lengths vary from 0.914to 1.118 Å

O-H bond lengths in phosphono ($-P(=O)OH_2$) and carboxyl group(COOH) are 0.711 and 0.743 Å respectively. Two O atoms of the phosphono group in glyphosate moiety are in resonance with each other as indicated by their bond lengths $P\text{-}O_1$ =1.500 Å and $P\text{-}O_2$= 1.501 Å. While the third P-O bond is a single bond with bond length = 1.576 Å. Different molecules of glyphosate are bonded to each other through H-bonds[23.]

1.6) MODE OF ACTION OF GLYPHOSATE:

Glyphosate is merely the weedkiller that exterminate the plant by restraining the synthesis of enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). EPSPS is a vital enzyme of Shikimate pathway[24]. Shikimate pathway is a series of seven metabolic steps (Figure 1.4) through which phosphoenol pyruvate ($C_3H_5O_6P$) and erythrose 4-phosphate ($C_4H_9O_7P$) are converted to chorismate ($C_{10}H_{10}O_6$). Chorismate is a predecessor for the biosynthesis of aromatic amino acids (Phenylalanine, tyrosine and tryptophan), auxin ($C_{18}H_{32}O_5$) , phytoalexins, folic acid, lignin, plastoquinones and numerous different auxiliary products[25]. Glyphosate ($C_3H_8NO_5P$) is considered as a competing cognate of phosphoenolpyruvate($C_3H_5O_6P$) which act as an active substratum for EPSPS. Glyphosate mimic the enzyme-substrate complex in its transitional state and blocks the binding site for phosphoenol pyruvate on EPSPS. Impediment of the enzyme

EPSPS by glyphosate perturbed the pathway. This enzyme is required for the synthesis of amino acids tryptophan ($C_{11}H_{12}N_2O_2$), tyrosine ($C_9H_{11}NO_3$) and phenylalanine ($C_9H_{11}NO_2$). These aromatic amino acids are necessary for various primary and secondary metabolisms of plants. Deficiency of ESPS makes the plant deficient of amino acids, hinders the metabolisms of the plants leading to the death of plant[5].

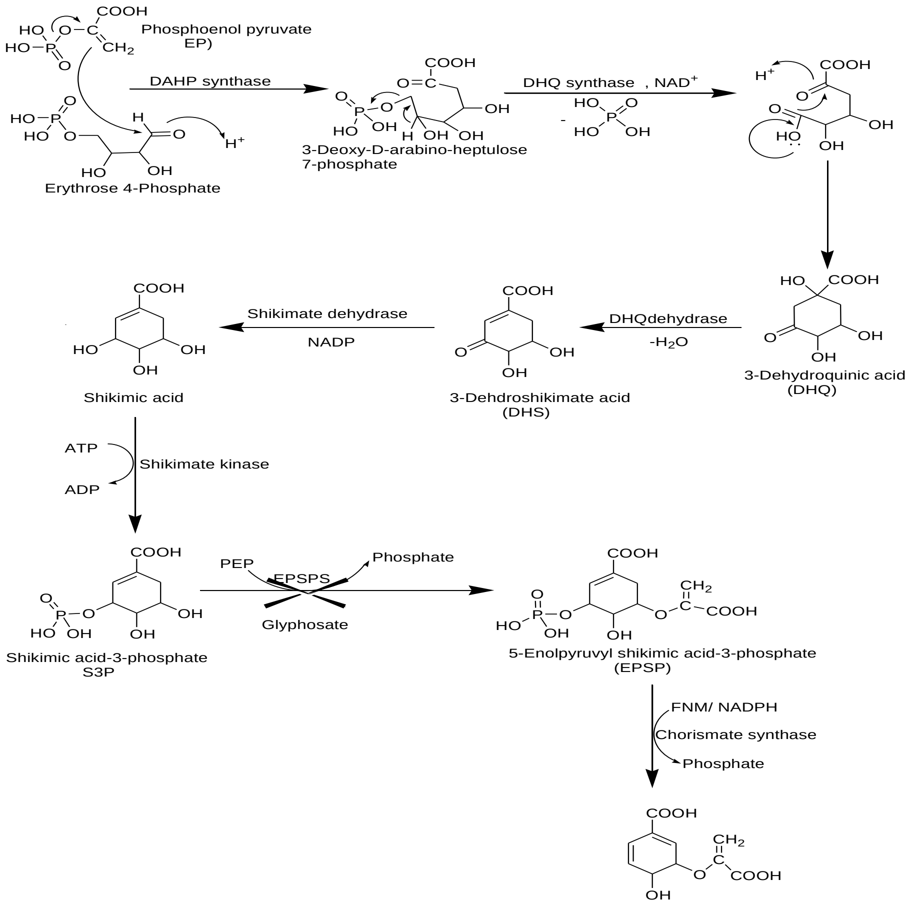


Figure 1.4 Shikimate Pathway

1.7) DEGRADATION OF GLYPHOSATE: Glyphosate is susceptible to both chemical and microbial degradation. Apart from this it also undergoes photodegradation.

1.7.1) Chemical Decomposition: When applied in fields, glyphosate is not easily oxidized or hydrolysed[26,27,28]. However the degradation of glyphosate in water is brought about by using oxidative advanced processes that showed that the decomposition of glyphosate persue the pseudo first–order dynamics[29]. Glyphosate and its common metabolite aminomethylphosphonic acid get degraded abiotically at 20°C with aqueous suspension of birnessite (A mineral of Mn along with Ca, K and Na present commonly in the soils). This abiotic degradation occurs on the surface of Mn oxide through the C-P bond breakage. Deprivation of N-phosphonomethyl glycine (glyphosate) with Mn oxide is faster and is elevated with rise in temperature. Though the existence of Cu^{2+} ions inhibited the degradation pathway. Cu ions co-ordinate strongly with glyphosate molecule and restricts the binding of glyphosate with the reactive oxidation sites at Mn oxide surface[30].

1.7.2) Microbial Degradation: Glyphosate can be easily sorbed by organic matter, minerals and soil. Glyphosate's sorption is guarded by cation-exchange capacity, clay, organic carbon content and pH of the soil. It has been found that glyphosate is sorbed due to the complex formation between its phosphonate group and soil exchanged polyvalent cations[31]. Glyphosate becomes strongly bound to the soil and has low desorption rate (about 5–24 % of the initially adsorbed glyphosate); only a little glyphosate is left available in the soil for microbial degradation, plant uptake and interaction with trace metals of the soil[32]. Degeneration of glyphosate with microbes is slow in the soils with greater adsorption ability. Its average half-life is two to ten weeks in water and two months in soil . Microbial degradation of glyphosate is also influenced by the type of community of microbes of each soil[5]. Glyphosate is easily degraded by microbial enzymatic systems through the cleavage of C-P bond. This type of metabolic pathway was reported in a Pseudomonas PG2982 strain that degraded glyphosate into phosphorous[33,34]. This pathway was also reported in other microorganisms like Agrobacterium radiobacter, Arthrobacter GLP-1 strain, Rhizobium meliloti and other Rhizobium strains[35,36,37,38]. A mutant of Arthrobacter strain GLP-1, named Arthrobacter GLP-1/ Nit-1, used glyphosate as a nitrogen source also[39]. Also Streptomyces spp. utilises glyphosate as a source of both phosphorous and nitrogen[40]. Small amount of glyphosate is left in the soil which gets easily degraded by the microorganisms. The soil containing the glyphosate is an accommodating substrate for various soil microorganisms. The presence of phosphorus in glyphosate makes it more susceptible to microbial degradation because phosphorus is an essential nutrient of these microorganisms[41]. The foremost metabolite of glyphosate, aminomethylphosphonic acid (AMPA) obtained during microbial degradation is non- toxic in nature and it is further degraded even slower than the parent glyphosate.

1.7.3) Photo degradation: Glyphosate is liable to photo degradation. Long-waved light doesn't have any photo degrading effect on glyphosate. However UV-light has capability to degrade glyphosate into aminomethylphosphonic acid (AMPA). AMPA is stable to photo degradation[42,43]. It has been found that the half-life of N-phosphonomethyl glycine in deionized water is four days under UV light[42].

1.8) GLYPHOSATE RESISTANCE IN PLANTS AND WEEDS:

Higher plants are not naturally resistant to glyphosate, but they show variable vulnerability towards it. Some plants are highly susceptible than others depending upon their physiological and biochemical mechanisms. For example, weeds like bermudagrass (Cynodon dactylon (L.)Pers.) and field bindweed (Convolvulus arvensis L.) are more resistant towards glyphosate[44,45,46]. With the repeated and large scale use of glyphosate worldwide, several plants and weed species are becoming resistant to this herbicide. Glyphosate resistance was firstly noted in annual ryegrass (Lolium rigidum) in crops of Australia[47]. In large region of the USA, Brazil and Argentina, crops having glyphosate-resistance are widely cultivated which has further resulted in the breakthrough of few weeds having glyphosate-resistant in them . For example, the first ever found glyphosate-resistant ragweed (Ambrosia artemisiifolia), glyphosate-resistant horseweed (Conyza) and common lambsquarters (Chenopodium album) have become very common in USA[48].

1.9) GLYPHOSATE-RESISTANT CROPPING SYSTEMS:

Due to large-scale use of glyphosate in the farming, crops having glyphosate-resistant were commenced in the market in 1996[49]. Glyphosate-resistant soybeans, cotton and corn crops are cultivated all over the world[32]. Later on with the advancements in biotechnology, CP4 gene of Agrobacterium sp. was used to encode glyphosate-resistant ESPS. These CP4 genes along with some promoters were introduced into the genome of various crops which show high levels of glyphosate resistance. Also a gene from Ochrobactrum anthropi was used to incorporate glyphosate resistance in canola[50]. In case of maize, mutagenesis of maize genes had been done to provide glyphosate-resistant 5-

enolpyruvylshikimate-3-phosphate synthase[51].The advent of these modified transgenic crops in the farmlands has encouraged the excessive use of glyphosate by many folds. This has lead to deterioration in the cropping systems. The unwanted plant species (weeds) have become more and more resistant to this herbicide, and therefore, it has become difficult to suppress them. Since it has been used in larger amounts over the years, it has become a strong component of most of the soil systems and the water bodies. Such an over use of glyphosate resulted in the presence of glyphosate and its metabolite AMPA in many food crops at their harvesting as well as in their progressed food[6]. The overuse of glyphosate is not only causing the development of resistant crops, passing over of residues in food materials but is also creating a significant toxic impact over a wide plethora of organisms in the environment.

1.10) GLYPHOSATE TOXICITY:

Gratuitous use of glyphosate is not only distressing the weed species but is also causing severe threat to several other non-target organisms found in the environment[52]. It affects the growth and other metabolic functions of many unicellular as well as multicellular organisms found in both soil and water[53].

Overuse of glyphosate had seriously affected the population of earthworms in the soil by decreasing their mean body weight, rate of reproduction and loss of biomass[54], Apart from this glyphosate also decreased the cocoon viability in the earthworms[55]. It has also caused serious morphological and genetic varations in them. Morphological changes (like elevation in the body, coiling and curling) and genetic varations like DNA damage and lysosomal damage are also observed in the earthworms found in the glyphosate treated soils[56]. Excessive use of glyphosate in the soils also resulted in the reduced surface casting activity of the earthworms[57]. Not only in soil systems glyphosate has also shown its devastating effects in aquatic systems. Many lower invertebrates are also directly affected by the lethal nature of glyphosate. Overuse of glyphosate in soil and its leaching in aquatic systems had reduced the egg laying capacity and have hampered the hatching process in snails and sea urchins[58].

Excessive use of glyphosate has also affected the population of bees all over the world. It kills the potential food source for honey bees by destroying the non-crop plants. Apart from this, it exterminates the beneficial bacteria found in the gut of honey bees[59] and caused many toxicological effects on them. In aquatic ecosystems glyphosate has posed several toxicological effects on lower vertebrates. Fish, the most important component of marine systems, is also severely affected by the overuse of this herbicide. It affected the liver, heart, kidney, develop epithelial hyperplasia and sub epithelial edema in the gills of the fish[60].

Not only lower vertebrates but humans are also severely exaggerated by this herbicide. Roundup is found to be potent endocrine disruptor in human beings. It has caused serious damage to placental cells with the decrease in the activity of enzyme aromatase[61]. It caused DNA damage, plasma damage and epithelial cell damage in humans[62]. Surfactant polyethoxylated tallowamine showed harmful effects on placental, hepatic and embryonic cell lines. Therefore, it can be concluded that the extreme use of glyphosate has caused toxic effects on non-target organisms found in soil and water. It has affected almost all organisms of animal kingdom. This is a serious concern as it had affected the whole food chain and produced many unwanted changes in it. Glyphosate has reduced the availability of weeds which may be an important food source for many species[63]. Thus, certain sustainable agricultural practices are needed to be adopted by farmers so as to maintain the interactions between living and non-living components of ecosystems to get the ecological balance and to save the food webs.

1.11) NEED FOR NEW DERIVATIVES:

Overuse of glyphosate in the agricultural fields and its seepage into ground water is responsible for the presence of its metabolites in water and soil systems. As a result it poses serious threats to the various non-target organisms present in soil and water. Along with it, many weed species have also become resistant to this herbicide which decreases the crop productivity. Very few different structural derivatives of glyphosate have been synthesized so far. Glyphosate is a trifunctional and have three polar functional groups –OH, -NH and-COOH so it is difficult to convert these functional groups into other groups without undergoing the reactions on other groups[64]. Thus, very less is known about the chemical reactivity of this molecule.

Apart from this, derivatization of glyphosate is mostly done for enhancing its stability or delectability with different detectors. Since the excessive use of glyphosate is responsible for the presence of its metabolites in the water and soil systems, so its detection has been achieved only by derivatizing the glyphosate with chemical reagents. These reagents have made the glyphosate easily accessible to the different detectors used in the process.

1.12) DERIVATISATION OF GLYPHOSATE:

Derivatization of glyphosate proceeds with the addition and replacement of different functional groups in the molecule. It was done mainly to detect the presence of metabolites of glyphosate in the soil and water. As glyphosate has a strong affinity for water and is highly insoluble in organic solvents, therefore it is very difficult to detect its presence in soil and water. Also it lacks fluorophore and chromophore groups. Hence to detect the presence of glyphosate and its metabolites in the soil and water at residual levels it is derivatised with some chromophoric groups. Various types of derivatising agents and techniques have been used.

Only few different types of glyphosate derivatives have been synthesized to check their herbicidal properties. Two photopolymerizable derivatives of glyphosate have synthesized. Acrylate and methacrylate derivatives of glyphosate have also showing herbicidal activity and were biologically active as the parent molecule[64].

Apart from this different metal complexed derivatives of glyphosate have also synthesized. Glyphosate was complexed with different divalent metal ions (Cu^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+},Ni^{2+}). Their complex formation was characterized by Infra red spectroscopy[65].

Herbicidal and toxicological studies of these metal complexed glyphosate derivatives were not evaluated.

Hence the objective of this work intends towards the modifications of the glyphosate molecule by further derivatizing it with different reagents to change its functional groups. The modified derivatives of glyphosate may solve the problem related to the resistive weeds and their seepage into soil and water systems. Such modified derivatives of glyphosate have shown good herbicidal activities and significantly decreased the chlorophyll content in the treated plants. Apart from this they are non- toxic to the non-target organisms present in the soil.

CHAPTER-2

REVIEW OF LITERATURE

2.1. DERIVATIZATION OF GLYPHOSATE FOR THE SCOPE OF DETECTION:

Excessive use of glyphosate in the agricultural fields has resulted in the presence of glyphosate metabolites in the land and aquatic systems. Thus, with the increasing concerns about the environmental aspects different researchers have detected its presence, by derivatizing it with different derivatizing agents. As glyphosate has a strong affinity for water[5] and is highly insoluble in organic solvents[66]; therefore, it is very difficult to detect its presence in soil and water. Also it lacks fluorophore and chromophore groups[67]. Hence, in order to analyse the existence of glyphosate and its metabolites in the land systems ,aquatic systems and food materials at residual levels, it is derivatized with some chromophoric groups[17]. Different types of derivatizing agents and techniques have been used by different researchers all over the world.

2.1.1) Detection of Glyphosate In Water:

Although glyphosate is highly soluble in water (1.01 g/ 100 mL at 20 °C), it binds the soil tightly and has minimum runoff in water bodies[68]. It is present in environmental water in residual levels and is determined by reacting it with chromophoric groups as listed below:

2.1.1.1) Isobutylchloroformate:

Kataoka et al. derivatized the glyphosate and its common metabolite AMPA with Isobutylchloroformate to detect its presence in the water samples. Water samples collected from the source were filtered and fortified with glyphosate and aminomethylphosphonic acid. To these water samples, 2-amino-4 phosphonobutyric acid was added (as internal standard); further, derivatising agent isobutylchloroformate was added at pH 10 (Figure 2.1). The reaction mixture was acidified and aquatic layer was concentrated with NaCl and was again separated with diethylether containing 10 % of 2-propanol solution. Solvents were evaporated under the stream of dry air and the derivatized product was inserted into gas chromatograph (GC) attached with a flame photometric detector (FPD) with fused silica capillary column having a cross-linked DB-1701. The detection limits were about 10pg for glyphosate and 15pg for (aminomethyl) phosphonic acid. The restoration of these compounds in drinkable water and stream water samples were 96.2–100.3 % [69].

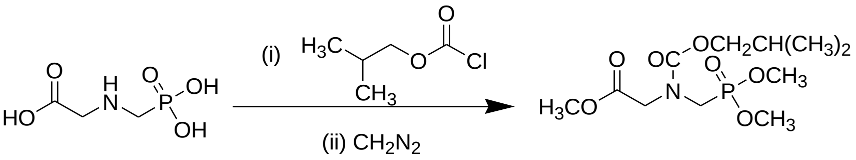


Figure 2.1 Derivatization of glyphosate using Isobutylchloroformate

2.1.1.2) Isopropylchloroformate:

Similar type of studies were further carried out for the investigation of glyphosate, glufosinate and AMPA in the samples collected from streams ,by derivatizing them with isopropylchloroformate using gas chromatography having flame photometer attached to it. The collected water samples were filtered and fortified with herbicide and its metabolites. Firstly, 2-amino-4 phosphonobutyric acid was mixed and the reaction mixture was reacted with isopropylchloroformate at pH of 10 as shown in figure 2.2. The pH of the mixture was then maintained to 2, and extraction was carried out. The aqueous layer was concentrated with NaCl and was again recovered with 20 % tert-butanol. Solvents were evaporated under a stream of dry air. The final derivatised product was then analysed with gas chromatograph using DB-1701 fused silica capillary column. Detection limits were 10pg for glyphosate and 15pg for aminomethylphosphonic acid. Overall recoveries of glyphosate, glufosinate and (aminomethyl) phosphonic acid from environmental samples were 91–106 % and relative standard deviations were 0.3–7.7 % [70].

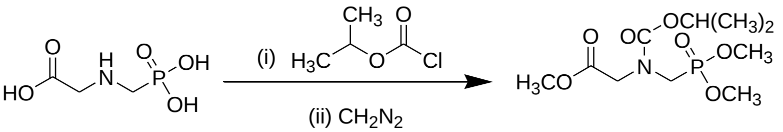


Figure 2.2 Derivatization of glyphosate using Isopropylchloroformate

2.1.1.3) Mixture Of Trifluoroacetic Acid, Trifluoroacetic Anhydride And Trimethyl ortho Acetate:

Detection of glyphosate, aminomethylphosphonic and N-methylaminomethylphosphonic acids in river water was done by using trifluoroacetic acid, trifluoroacetic anhydride and trimethyl ortho acetate as derivatising agent. Samples of river water were filtered. To the water samples, trifluoroacetic acid (TFA), trifluoroacetic anhydride (TFAA) and trimethyl orthoformate (TMOA) were incorporated and the mixture was blended at 100°C (Figure 2.3). The derivatised product was analysed using 31P NMR, gas chromatograph having chemical ionisation mass spectrometer attached to DB-17 capillary column. The detection limits of glyphosate, aminomethylphosphonic acid and N-methylaminomethylphosphonic acids were calculated. The average recovery of glyphosate was 96.8–97 %, aminomethylphosphonic acid was 97–97.5 % and N-methylaminomethylphosphonic acid was 97.4–97.7 %[71].

Figure 2.3 Derivatization of glyphosate using TFA,TFAA and TMOA

2.1.1.4) 9-Fluorenylmethylchloroformate:

Incidence of glyphosate and its metabolites (glufosinate, AMPA) in ecological water samples was determined by using derivatising agent 9-fluorenylmethylchloroformate (Figure 2.4). Two approaches were used, a multi-residue method, in which simultaneous analysis of three analytes in water samples was done up to 1 lg/L and a single residue method, in which analysis of single analyte was done to sub-lg/L level. The analytes present in the environmental water samples capitulate fluorescent derivatives which were determined by coupled column liquid chromatography with fluorescence detector using reverse-phase C18 column (C-1) coupled to a weak ion-exchange column (C-2). Glyphosate, glufosinate and aminomethylphosphonic acid were recovered from water samples at 0.50–10 lg/L level[72].

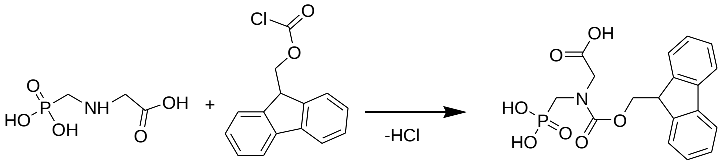


Figure 2.4 Derivatization of glyphosate using 9-Fluorenylmethylchloroformate

In another work, a magnetic particle immunoassay was used for quick, precise and accurate part-per-trillion monitoring of glyphosate by derivatizing it with 9-

fluorenylmethylchloroformate (Figure 2.5) in water matrixes, in combination with a solid-phase removal chased by liquid chromatography (LC) linked with mass spectrometry. The magnetic particle immunoassay was used for the detection of about 140 specimens of groundwater. The amount of glyphosate was more than the range of evaluation in 41 % of the samples with concentrations as soaring as 2.5lg/L with a mean concentration of 200ng/L[73].

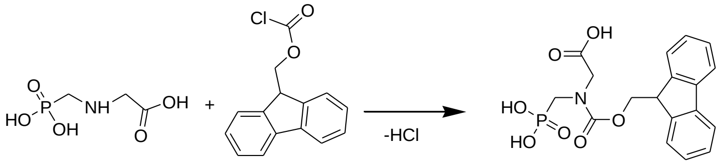


Figure 2.5 Derivatization of glyphosate using 9-Fluorenylmethylchloroformate

Another very efficient technique for the analysis of glyphosate in the water specimen was developed in which aquatic samples were collected, filtered and mixed with glyphosate and aminomethylphosphonic acid. These samples were then derivatized with 9-fluorenylmethyl chloroformate and 0.3 mL of pH 9 borate buffer at normal temperature (Figure 2.6). The derivatized samples were injected into HPLC using single polymeric amino column having liquid phase containing mixture of 55 % (v/v) acetonitrile (C_2H_3N) and 50 mM phosphate buffer ($Cl_2H_3K_2Na_3O_8P_2$). This method was validated by analyzing fortified samples of rain water at a level of 1 lg/L. The average revival was 94 % with relative standard deviation of 5.4 % and detection limit of 0.16µg/L[74.]

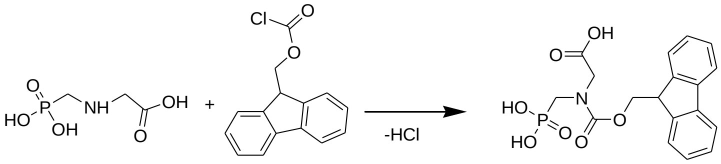


Figure 2.6 Derivatization of glyphosate using 9-Fluorenylmethylchloroformate

2.1.1.5) 4-Methoxybenzenesulfonyl Fluoride:

A low-cost and highly sensitive technique for the scrutiny of glyphosate and AMPA in water was developed which includes derivatization of glyphosate and AMPA with 4 methoxybenzenesulfo-nyl fluoride (Figure 2.7). Water samples from the discharge of pesticide industry were collected, filtered and concentrated through evaporation. The contents were extracted with dichloromethane, and the organic layer was rejected.. To the aquatic layer, disodium hydrogen phosphate and 4-methoxybenzenesulfonyl fluoride were added. All the contents were reacted at pH 10.5 and 45°C for 10 min. After the completion of reaction, products formed were detected using reverse-phase liquid chromatography having ultraviolet detector attached to it. The limits of detection for glyphosate and its metabolite in real water samples were found to be 0.1 lg L^{-1} [75].

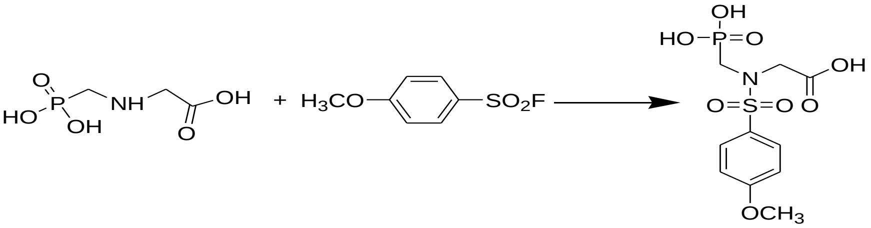


Figure 2.7 Derivatization of glyphosate using 4-Methoxybenzenesulfonyl Fluoride

2.1.1.6) O-Phthalaldehyde in Presence of Mercaptoethanol:

Another method for the analysis of glyphosate in aquatic samples was done by means of high-performance liquid chromatography (HPLC) followed by post-column reactor with attached fluorescence detector. Water samples were collected and mixed with glyphosate and amino methyl phosphonic acid. These samples were then extracted with dichloromethane to remove the organic compounds. The extracted samples were concentrated by rotary evaporator and were passed through a strong anion-exchange cartridge. Glyphosate and aminomethylphosphonic acid were then eluted with citrate buffer (pH = 5). Glyphosate was oxidized with calcium hypochlorite $(Ca(ClO)_2$ to form glycine $(C_2H_5O_2)$ which was then treated with o-phthalaldehyde $(C_6H_4(CHO)_2$ in presence of mercaptoethanol (C_2H_6OS) (Figure 2.8) to make it fluorophore and was detected with fluorescence detector (λ_{ex} = 340 nm, λ_{em} = 455 nm). For both the components, detection limit was < 2µg/L and the average recovery was >85 % [66].

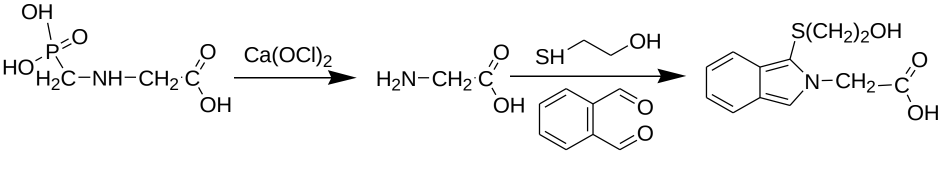


Figure 2.8 Derivatization of O-Phthalaldehyde in presence of mercaptoethanol

2.1.1.7) 4-Chloro-3, 5-Dinitrobenzotrifluoride:

Another research group also proposed an efficient method for the analysis of glyphosate in water specimens using HPLC technique. The technique includes the pre-column derivatization of glyphosate using 4-chloro-3,5-dinitrobenzotrifluoride for 30 min at 60°C in pH 9.5 by using H_3BO_3-$Na_2B_4O_7$ media (Figure 2.9). The resulting solution was filtered through 0.45 nylon filament. The labelled glyphosate was passed through Kromasil C18 column at normal temperature. The ultraviolet detection was done at 360 nm. The limit of detection was 0.009 mg L^{-1}. This method was successfully applied in environmental water samples, and recoveries of 91.80–100.20 % were obtained with relative standard deviation of 2.27–6.80, depending on the sample investigated[76].

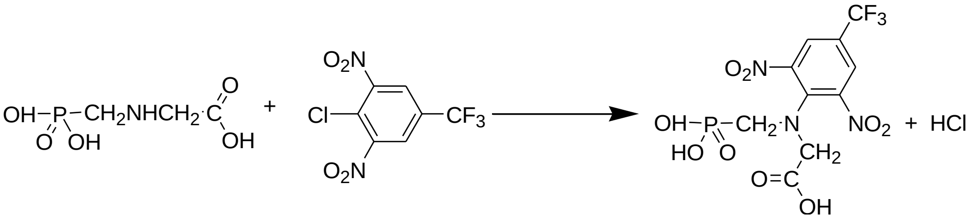


Figure 2.9 Derivatization of glyphosate with 4-Chloro-3, 5-Dinitrobenzotrifluoride

2.1.1.8) 2,2-Dihydroxyindane-1,3-Dione (Ninhydrin):

A very trouble-free and proficient technique for the inspection of glyphosate was developed in which ultraviolet spectrophotometric technique was applied to find the presence of glyphosate in water specimens . Glyphosate was reacted with a chromogenic reagent 2,2-dihydroxyindane-1,3-dione (ninhydrin) in the presence of sodium molybdate in neutral aqueous medium at 100°C (Figure 2.10). Ruhemann's purple coloured product with maximum absorption at 570 nm was obtained. Beer's law was followed at the concentration limit of 0.1–3.5µgmL^{-1}. Glyphosate's complex with ninhydrin showed the molar absorption of 3.2816 ×10^{-4}L$mol^{-1}cm^{-1}$ with limit of detection of 0.04 µg mL^{-1} and relative standard deviation (RSD) of 1.74 %. This method was very authentic and efficient[77].

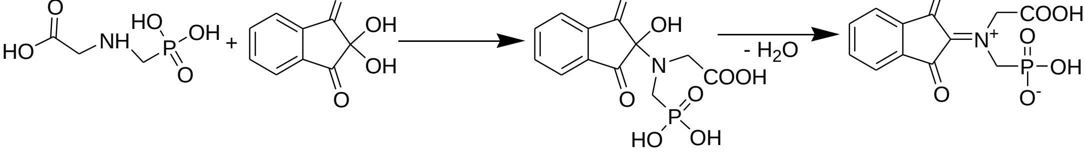


Figure 2.10 Derivatization of glyphosate with Ninhydrin

2.1.1.9) Carbon Disulphide:

A very simple and selective method was developed by another research group to determine the presence of glyphosate in environmental water samples. In this method, glyphosate was derivatised with 1 % solution of carbon disulphide to form dithiocarbamic acid. This derivative was then converted to copper complex by reacting with 1 mL ammonical copper nitrate solution (Figure 2.11). The reaction mixture was then shaken vigorously for 3 min, and the solution was allowed to stand. Yellow coloured derivatised complex was formed, and the absorbance of this copper dithiocarbamate complex was calculated at 435 nm with molar absorptivity of 1.864 $\times 10^{-3} L mol^{-1} cm^{-1.}$ Glyphosate was determined successfully with limit of detection and quantification of 1.1 and 3.7 µg mL^{-1}, respectively. This technique was used to environmental water specimens and revival values were 85.0 ± 0.68 to 92.0 ± 0.37 %[78].

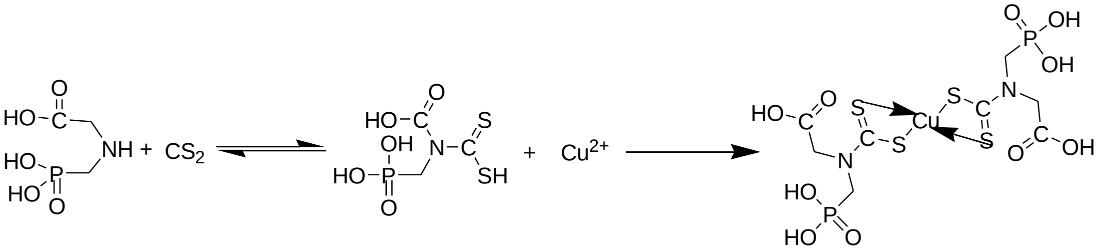


Figure 2.11 Derivatization of glyphosate with Carbon disulphide

2.1.1.10) 4-Chloro-7-Nitrobenzofurazan:

Another very efficient and economical technique to determine glyphosate and metabolites of glyphosate in water was developed in which compounds were made fluorescent by derivatising them with 4-chloro-7-nitrobenzofurazan in basic medium. To the solutions containing glyphosate and aminomethylphosphonic acid, 0.2M borax solution added and the pH of the solution was maintained at 9.7mL then $3.8 x 10^{-3}$M methanolic solution of chloro-7-nitrobenzofurazan was mixed and the solutions were heated at 90°C for 5 min, placed in ice water and then 0.6 mL of 12M HCl was added. The fluorescent derivatives were then accurately determined using fluorescence spectroscopy. For concentrations ranging from 100–600 µg L^{-1}, analytical recovery for glyphosate was 83–94 % and for aminomethylphosphonic acid was 104–120 %[79].

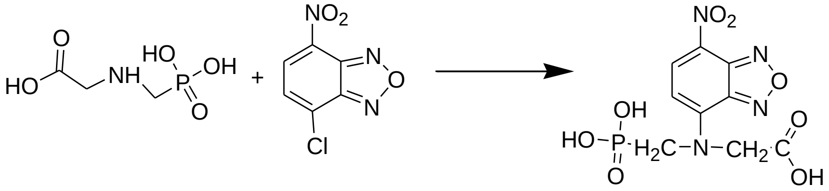


Figure 2.12 Derivatization of glyphosate with 4-Chloro-7-Nitrobenzofurazan

2.1.1.11) Naphthalene-2,3-Dicarboxaldehyde:

In another technique for the analysis of glyphosate in river water, ion-exchange solid-phase extraction technique was used. Water samples from river were collected and were spiked with glyphosate. The water samples were pumped through BS-9000-8 syringe pump using a clean-up cartridge followed by a resin pre-concentration tip. The glyphosate was converted into glycine by using calcium hypochlorite at pH 10.4. Further fluorescent labelling was done by using 20 µL of 10 mM sodium cyanide along with 20µL of 2.5 mM of naphthalene-2,3-dicarboxaldehyde (Figure 2.13). Fluorescent labelling made the sample suitable for micellar electrokinetic chromatography (MEC) separation and laser-induced fluorescence (LIF) detection. From this method, detection limit of glyphosate was 0.04 nM in standard solutions and 1.6 nM in spiked river water [80].

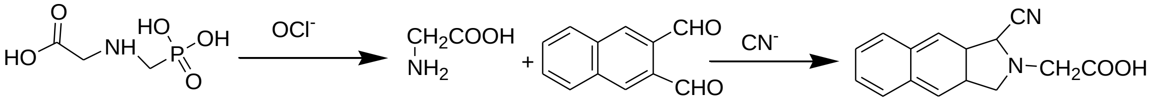


Figure 2.13 Derivatization of glyphosate with Naphthalene-2,3-Dicarboxaldehyde

2.1.1.12) Fluorescein Isothiocyanate:

Microchip electrophoresis system (MES) with LIF detection was employed for the study of glyphosate and glufosinate remains in water. For this method, disposable cyclic olefin copolymer microchips and laser-induced fluorescence detector were used. Samples were derivatised with fluorescein isothiocyanate. Water samples were collected and filtered and then fluorescein isothiocyanate was added in it. To this solution, 10 mmol/L sodium tetraborate buffer with pH 9.2 was added (Figure 2.14). The reaction mixture was mixed in a micro-centrifuge tube. The tube was wrapped with a piece of alumina foil to avoid exposure to light. The contents of the tube were heated at 55°C in water bath for 20 min. The resulting mixture was used for detection.

Under the specific conditions, glyphosate and glufosinate were determined from sample matrices. Derivatization at lower concentrations (<10µg/L) was also examined, detection limit for glyphosate was 0.34µg/L and for glufosinate was 0.18µg/L. Recoveries of glyphosate and glufosinate spiked in these samples were 84.0–101.0 % and 90.0–103.0 %, respectively [81].

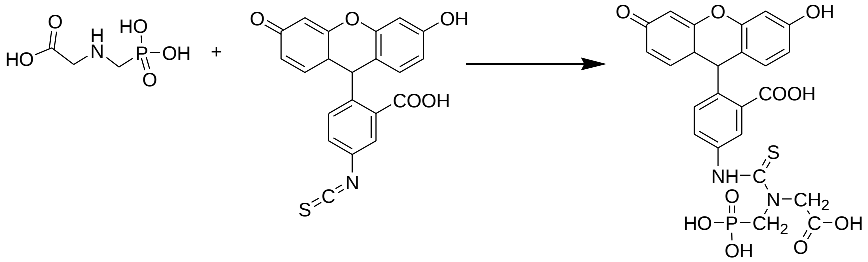
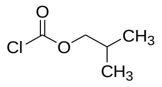
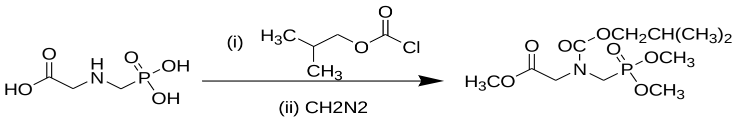
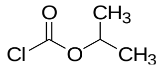
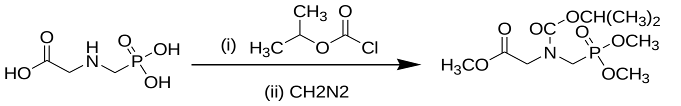
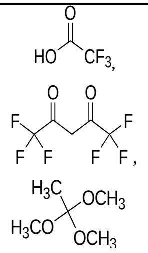
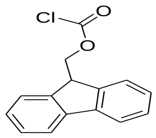
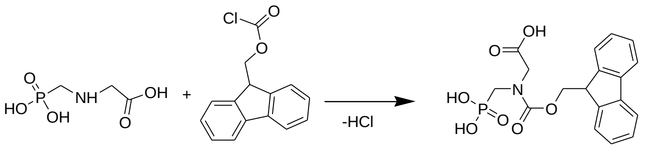
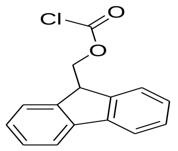
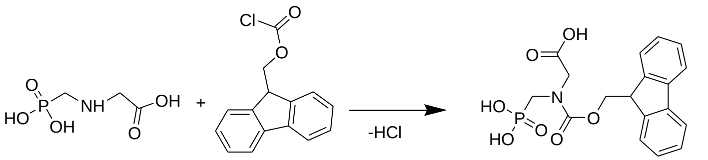
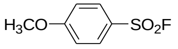
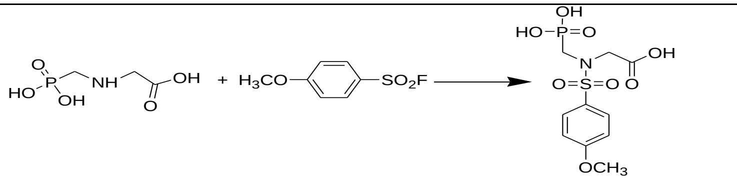
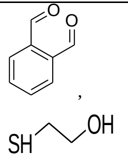
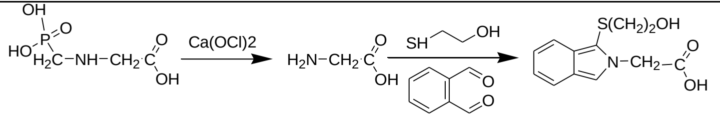
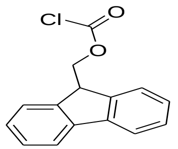
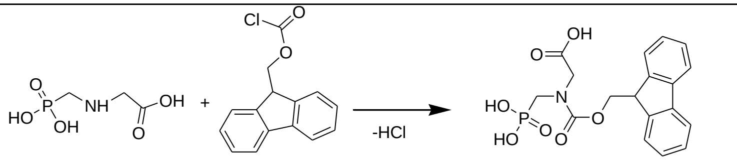
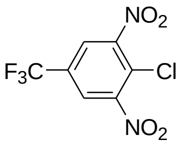
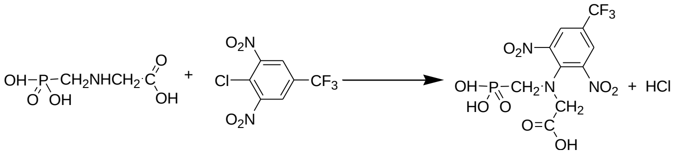
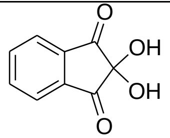
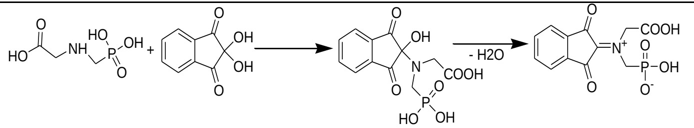
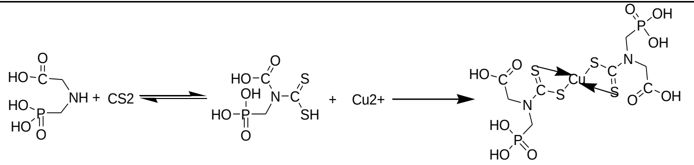
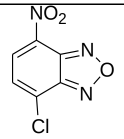
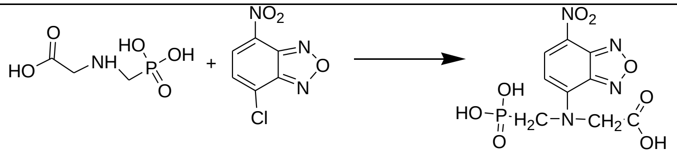
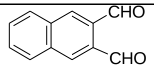
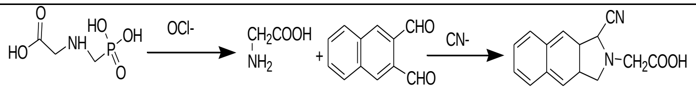
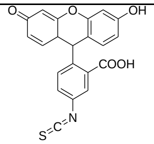
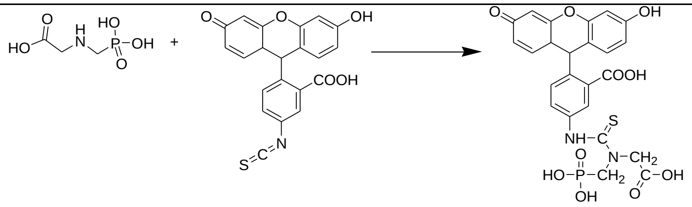


Figure 2.14 Derivatization of glyphosate with Fluorescein Isothiocyanate

TABLE 2.1: Derivatizing agents and spectroscopic techniques used for detection of glyphosate in water

SNo.	Derivatising Agent used	Chemical structure	Chemical reaction involved	Spectroscopic technique and column used for detection	Solvent used	Reference
1.	Isobutylchloroformate			GC with Flame photometer detector. Fused silica capillary column with cross-linked DB-1701	Ethyl acetate	69
2.	Isopropylchloroformate			GC with Flame photometer detector. Fused silica capillary column with cross-linked DB-1701	Ethyl acetate	70
3.	Mixture of Trifluoroacetic acid, trifluoroacetic anhydride, trimethylorthoacetate			GC with flame ionisation detector. DB-17 capillary column	Benzene	71
4.	9-Fluorenylmethylchloro-formate			LC with fluorescence detector. Reversed phase C-18 column and Amino,5μm column	Ammonium acetate and methanol	72

5.	9-Fluorenylmethylchloroformate			LC with Mass spectrometry. C-18 Reversed phase column	Acetonitrile and (0.05M Phosphate pH5.5) in water (35: 65, v/v)	73
6.	4-Methoxybenzenesulfonylfluoride			LC. Reversed phase ODS column	Phosphate buffer (50mM,pH 3) and Methanol	75
7.	o-Phthalaldehyde in presence of mercaptoethanol			HPLC with fluorescence detector. cation-exchange column	0.005 M KH_2PO_4 buffer	66
8.	9-Fluorenylmethylchloroformate			HPLC. Polymeric amino column.	Acetonitrile-50mM phosphate buffer (pH10) as first mobile phase and Acetonitile-water (60:40, v/v) as second mobile phase.	74
9.	4-Chloro-3,5-dinitrobenzotrifluoride			HPLC and UV detection. Reversed –phase ODS C18 column.	Acetonitrile-0.01M Cetyltrimetylammonium bromide solution (5:5v/v), phosphate buffer (50mM, pH2.5)	76

10.	Ninhydrin in presence of sodium molybdate			UV Spectroscopy	-------NA-------	77
11.	Carbondisulphide	CS_2		UV-Visible spectroscopy	------NA-------	78
12.	4-Chloro-7-nitrobenzo-furazan			Fluorescence spectroscopy	-------NA-------	79
13.	Naphthalene-2,3-dicarboxaldehyde			Laser-induced fluorescence detection	-------NA-------	80
14.	Fluorescein isothiocyanate			Microchip electrophoresis system with laser induced fluorescence detector	-------NA-------	81

2.1.2) DETECTION OF GLYPHOSATE IN SOIL:

There is extensive global use of glyphosate for controlling many annual and perennial weeds. Consequently, aminomethylphosphonic acid (the major metabolite of glyphosate) and glyphosate itself have entered into metabolic machinery of plants and into the soil as well[73]. Thus, it has become very important to determine the presence of glyphosate in soil and to achieve this aim, different spectroscopic techniques along with different derivatizing agents were used.

2.1.2.1) Isopropyl Chloroformate:

A technique was employed to detect the presence of glyphosate, glufosinate and AMPA in soil by derivatizing it with isopropyl chloroformate. Glyphosate and its metabolite were extracted from soil with 0.2 M sodium hydroxide solution by shaking. The extracts were mixed with isopropyl chloroformate (Figure 2.15). The solution was mixed vigorously with vortex mixer. Diethylether was added, and the ethereal extracts were methylated with diazomethane. The solvent was dissipated to dryness and the derivatised output was injected into gas chromatography–flame photometric detector using DB-1701 capillary column. The quantitative limit of aminomethylphosphonic acid in soil was 8 ng/g and for glyphosate and glufosinate these limits were 12 and 20 ng/g, respectively. The overall recovery of these herbicides in soil was 91–106 % and relative standard deviations were 0.3–7.7 %[70].

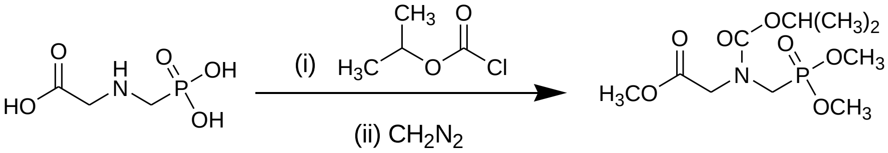


Figure 2.15 Derivatization of glyphosate with Isopropyl Chloroformate

2.1.2.2) Mixture of Trifluoroacetic Anhydride And Trifluoroethanol:

Another research group developed a method to determine glyphosate residues in soil by using trifluoroacetic anhydride and trifluoroethanol as derivatizing agents (Figure 2.16) with capillary gas chromatography having nitrogen phosphorus detector attached to it. The residues were extracted from soil with 2M ammonium hydroxide solution. To the sample, mixture of water/methanol/HCl (160:40:27, v/v) was added followed by addition of concentrated phosphoric acid. The contents of the flask were kept at normal temperature for 1 hour and then centrifugation of sample was carried out and the supernatant was collected. To the supernatant trifluoroacetic anhydride was added followed by addition of trifluoroethanol. The mixture was held for 60 min on oil bath at 100°C. The solution was extracted in separatory funnel using methylene chloride. Solvent was evaporated and the derivatised product was injected into gas chromatograph–nitrogen phosphorus detector. The limit of detection was 9×10^{-12} g. The quantity of glyphosate found in the specimen was found to be 0.01 mg/kg. The average recovery was 84.4–94 %, while the relative standard deviation was 8.1–13.7 % [82].

Figure 2.16 Derivatization of glyphosate with Mixture Of Trifluoroacetic Anhydride And Trifluoroethanol

2.1.2.3) Acetic Anhydride And Triethylamine:

In another method, glyphosate and its biodegradable products were derivatised using the mixture of acetic anhydride and triethylamine (10:1) (Figure 2.17). After adding the derivatising agent into the compound, the bottle containing reaction mixture was closed and kept at normal temperature for 30 min. The resulting products was placed in a vacuum desiccator connected with oil pump. The precipitates formed were dissolved in 0.01 N ammonium sulphate solution. The n-acyl derivatives of glyphosate were formed which were separated using ion-exchange liquid chromatography with ultraviolet detector. The detection limit (at wavelength 210 nm) for glyphosate was 30µg and for aminomethylphosphonic acid was 45µg. Glyphosate and its biodegradable products were further separated by thin-layer chromatography (TLC) using silica gel plates by isopropanol/5 % ammonia solution in 1:1 ratio[83].

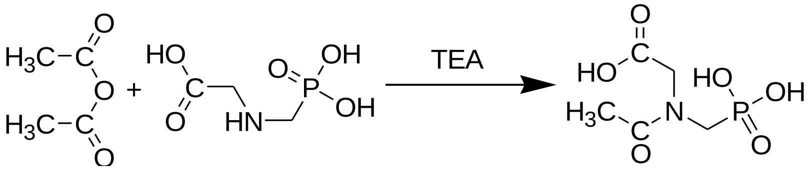


Figure 2.17 Derivatization of glyphosate with Acetic Anhydride And Triethylamine

2.1.2.4) 9-Fluorenyl Methylchloroformate:

In another method, detection of glyphosate and AMPA in soil was done by derivatizing it using 9-fluorenylmethylchloroformate (Figure 2.18) followed by liquid chromatography–multistage mass spectroscopy analysis. Soil samples were extracted with KOH. The sample extract was collected and derivatised with 9-fluorenylmethylchloroformate with borate buffer. The contents were placed at normal temperature for 12–15 hours. The derivatised extracts were cleaned through 0.45µm nylon filter and acidified with HCl to pH 1.5 and left stand for 1 h. Then, the centrifuged extract was injected into liquid chromatography–electrospray ionisation/ multistage mass spectroscopy system. The limit of quantification for glyphosate is 0.05 mg/kg and 0.03 mg/kg for aminomethylphosphonic acid, while the limit of detection was 0.02 mg/kg for glyphosate and 0.01 mg/kg for aminomethylphosphonic[84].

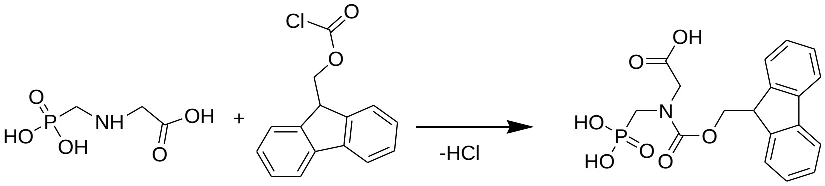


Figure 2.18 Derivatization of glyphosate with 9-Fluorenyl Methylchloroformate

An effective extraction and analysis process for the scutiny of glyphosate, glufosinate and AMPA in field was developed for soil samples. Extraction was done through fast solvent extraction and ultrasonic extraction by using water as a solvent. After extraction, analysis was done by precolumn derivatization using 9-fluorenylmethyl chloroformate by using borate buffer and attached magnetic stirrer for 1 hour at normal temperature (Figure 2.19). The excess reagent was removed by liquid–liquid extraction. The aqueous layer was collected and was injected into HPLC system having a 300 mm C-18 column with fluorescence detector attached to it. This method is proficient and sensitive for a clay loamy soil with limit of detection of 103µg kg^{-1} for glyphosate, 15µg kg^{-1} for glufosinate and 16µg kg^{-1} for AMPA in soil samples[85].

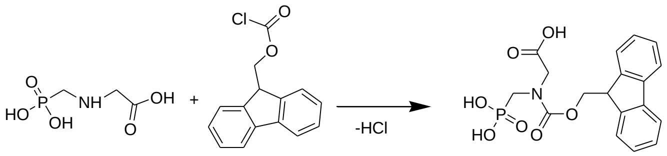


Figure 2.19 Derivatization of glyphosate with Fluorenyl Methylchloroformate

2.1.2.5) 1-Fluoro-2, 4-Dinitrobenzene:

In another technique for the evaluation of glyphosate metabolites in soil, derivatization was done by using 1-fluoro-2,4-dinitrobenzene (Figure 2.20). Compounds from the soil were extracted by using triethylamine. The extracted solution was vapourised to dryness. The residue was mixed in saturated solution of sodium tetraborate. 2mL of 3.75 mg 1-fluoro-2,4-dinitrobenzene and 1 mL of 96 % ethanol was added to it and the reaction was carried out in dark at normal temperature for 1 h. To the reaction mixture 0.1 M sodium phosphate buffer with pH 3.2 was added. Then sodium chloride was added to it at pH 5 and the solution was separated with ethyl acetate. Aqueous layer was then collected and solvent was dried. The residue which was left was collected and derivatized compounds were then quantified with ion-pair high pressure liquid chromatography. Minimum detectable quantities were 0.05µg g^{-1} for glyphosate and 0.1µg g^{-1} for its metabolite[86].

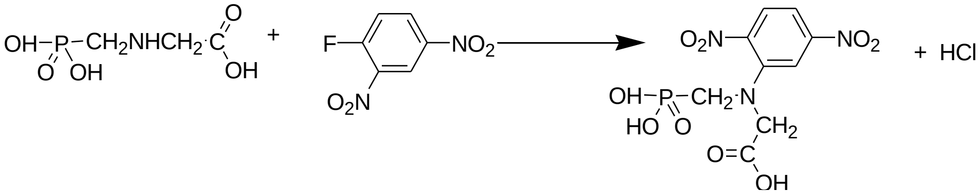


Figure 2.20 Derivatization of glyphosate with 1-Fluoro-2, 4-Dinitrobenzene

2.1.2.5) Carbon Disulphide:

Another research group developed a easy spectrophotometric method for the detection of glyphosate in environmental specimens. In this technique, glyphosate was reacted with 1 % carbon disulphide to form dithiocarbamic acid which was further complexed with 1 mL ammonical copper nitrate solution (Figure 2.21). Yellow coloured complex was formed whose absorbance was measured at 435 nm. Its molar absorptivity was found to be 1.864×10^{3} $Lmol^{-1}$ cm^{-1}. Glyphosate was determined with limit of detection and quantification of 1.1 and 3.7µg mL^{-1}, respectively. Recovery value of glyphosate in soil was 80.0 ± 0.46 to 87.0 ± 0.28 %[78] .

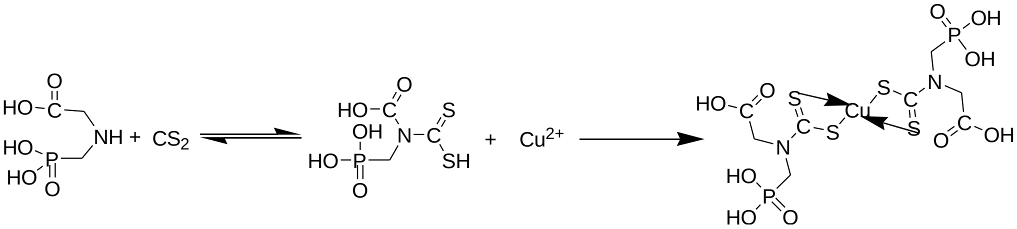
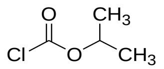
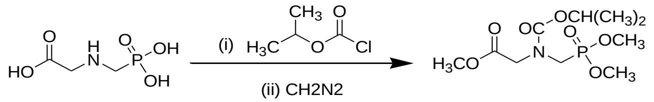
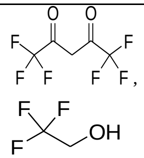
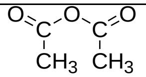
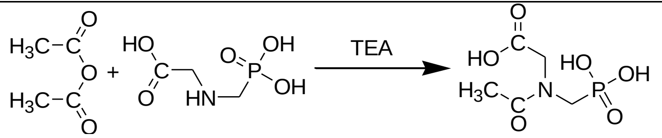
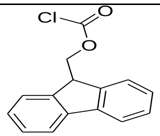
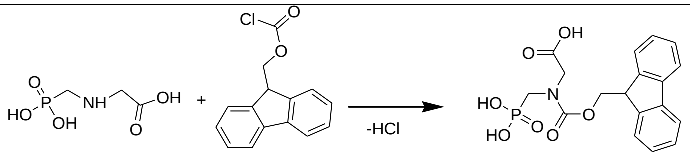
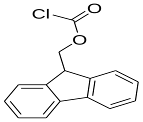
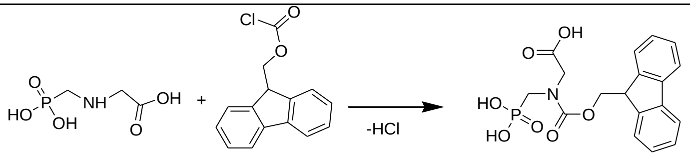
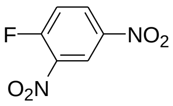
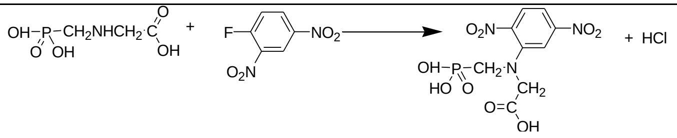
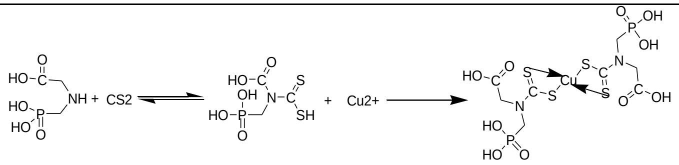


Figure 2.21 Derivatization of glyphosate with Carbon disulphide

TABLE 2.2: Derivatizing agents and spectroscopic techniques used for detection of glyphosate in soil.

SNo.	Derivatising Agent used	Chemical structure	Chemical reaction involved	Spectroscopic technique and column used for detection	Solvent used	Reference
1.	Isopropylchloroformate			GC with Flame photometer detector. Fused silica capillary column with cross-linked DB-1701	Ethyl acetate	70
2.	Trifluoroacetic anhydride and trifluoroethanol			GC with N-P detector. Capillary column supelcoequity-5	Ethyl acetate	82
3.	Acetic anhydride and triethylamine			Ion exchange LC with UV detector. Repro-Gel H column	H_2SO_4 (0.0I N)	83
4.	9-Fluorenylmethylchloro-formate			LC-ESI-MS/MS. Analytical column C-18	A -(Water 5Mm, acetic acid/ ammonium acetate pH4.8) B- Acetonitrile	84
5.	9-Fluorenylmethylchloro-formate			HPLC with fluorescence detector. Reverse phase C-18 column	H_3PO_4 (0.2%)/ Acetonitrile	85

6.	1-fluoro-2,4-dinitrobenzene			HPLC. C-18 Radial-PAK cartridge	0.02 M tetraethylammonium bromide, 0.05 M sodium dihydrogen phosphate-Acetonitrile	86
7.	Carbondisulphide	CS_2		UV-Visible spectroscopy	------NA--	78

2.1.3) DETECTION OF GLYPHOSATE IN FOOD MATERIALS:

2.1.3.1) Isopropyl Chloroformate:

A very perceptive and quick method was intended to resolve the presence of glyphosate, AMPA and glufosinate in carrot samples by using isopropylchloroformate as derivatizing agent. The carrot samples were homogenized in water by adding 4-aminobutylphosphonic acid as internal standard with ultra-disperser and centrifuged. The supernatant was diluted and fortified with herbicides and was used for analysis. Extracted supernatant was reacted with isopropyl chloroformate at pH 10 to convert the herbicides present in it into their N-isopropoxycarbonyl methyl ester derivatives. Extraction was done using ether and aqueous layer was collected. Solvents were then evaporated under the steam of dry air. The derivatised product was injected into gas chromatograph having flame photometric detector using DB-1701 capillary column. The quantitative limit of AMPAin carrot was 8 ng/g and for glyphosate and glufosinate these limits were 12 and 20 ng/g respectively. The overall recovery of these herbicides in carrots was 91–106 %, and relative standard deviation was 0.3–7.7 %[70].

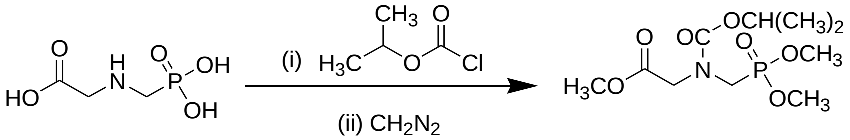


Figure 2.22 Derivatization of glyphosate with Isopropyl Chloroformate

2.1.3.2) Carbon Disulphide:

Simple spectrophotometric method for the analysis of glyphosate in wheat grains was developed in which glyphosate was reacted with carbon disulphide (CS_2) to form dithiocarbamic acid which was then complexed with Cu in the presence of NH_3 (Figure 2.23). Wheat samples were cleaned with distilled water and known amount of glyphosate was mixed with them. The samples were extracted with ammonium hydroxide solution. Extracted sample solution was reacted with 1 % carbon disulphide solution to form dithiocarbamic acid which was further reacted with ammonical copper nitrate solution. Yellow coloured complex was formed whose absorbance was measured at 435 nm. Its molar absorptivity was found to be 1.864×10^3 $Lmol^{-1}cm^{-1}$. Glyphosate was determined with limit of detection and quantification of 1.1 and 3.7µgmL^{-1}, respectively. Recovery value of glyphosate in wheat grains was from 95.0 ± 0.88 to 102.0 ± 0.98 % [78].

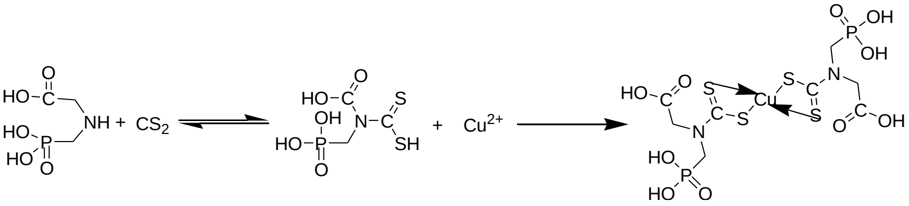


Figure 2.23 Derivatization of glyphosate with Carbon disulphide

2.1.3.3) 9-Fluorenylmethyl Chloroformate:

A very efficient technique for the analysis of glyphosate in grass was developed. Grass samples were collected and spiked with glyphosate and aminomethylphosphonic acid. They were then flash-frozen with liquid nitrogen and homogenized. Sample aliquots were mixed with deionized water and were blended for 5 min. These contents were then extracted using methylene chloride. These plant extracts were then derivatised with 9-fluorenylmethyl chloroformate at normal temperature (Figure 2.24). The derivatized samples were injected into high-performance liquid chromatography using single polymeric amino column with mobile phase containing mixture of 55 % (v/v) C_2H_3N and 50 mM phosphate buffer. This method was validated by analyzing grass samples spiked at the level of 1 mg/kg. The average recovery was 82.4 % with relative standard deviation of 10.3 % and the detection limit of 0.3 mg/kg [74].

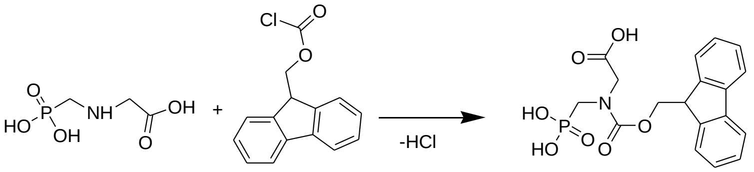


Figure 2.24 Derivatization of glyphosate with 9-Fluorenylmethyl Chloroformate

2.1.3.4) Fluorescein Isothiocyanate:

Another method was developed in which detection was done for the discovery of glyphosate and glufosinate remains in broccoli and soya bean. Specimens were derivatized with fluorescein isothiocyanate (Figure 2.25) and were detected using microchip electrophoresis system with LIF detector attached with it. For this method, disposable cyclic olefin copolymer microchips and laser-induced fluorescence detector were used. Soybean samples were grinded and mixed with water and then vacuum-filtered. The precipitates were washed and the filtrate was collected. Acetonitrile was added and the contents were rotated for 10 minutes at normal temperature. The supernatant was collected. Similar type of method was used for broccoli with water. To both the sample supernatants, fluorescein isothiocyanate and sodium tetraborate buffer (10 mmol/L and pH 9.2) were mixed in 500µL microcentrifuge tube. The tubes were wrapped with aluminium foil and were heated in a water bath at 55 °C for 20 min. Under the selected condition, glyphosate and glufosinate were derivatised and were redetermined from food samples with a buffer having 10 mmol/L borax and 2.0 % (m/v) hydroxypropyl cellulose at pH 9. Recoveries of glyphosate and glufosinate spiked in these samples were 84–101.0 % and 92.0–102.0 %, respectively[81].

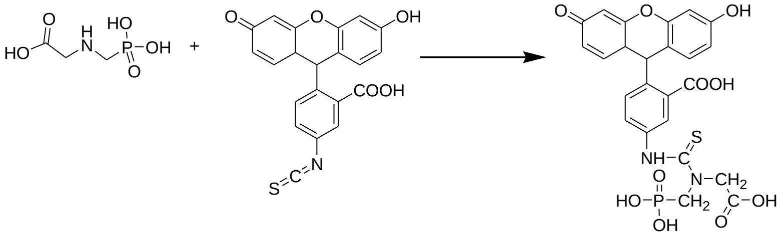
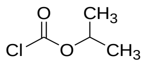
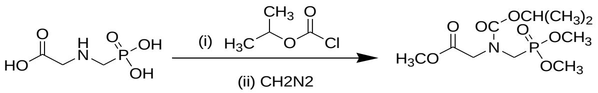
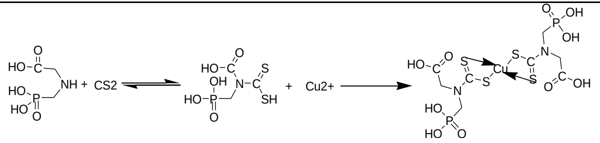
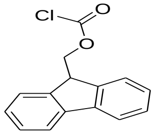
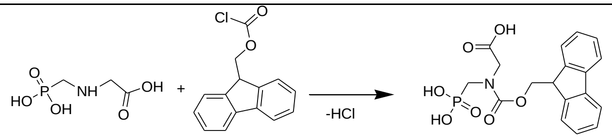
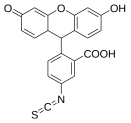
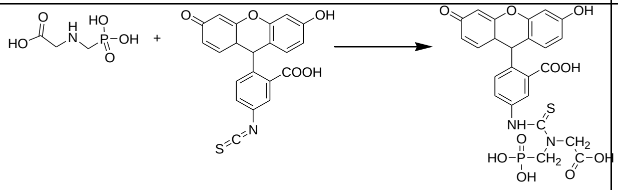


Figure 2.25 Derivatization of glyphosate with Fluorescein Isothiocyanate

TABLE 2.3: Derivatizing agents and different spectroscopic techniques used for detection of glyphosate in food materials:

SN o.	Derivatising Agent used	Chemical structure	Chemical reaction involved	Spectroscopic technique and column used for detection	Solvent used	Reference
1.	Isopropylchloroformate			GC with Flame photometer detector. Fused silica capillary column with cross-linked DB-1701	Ethyl acetate	70
2.	Carbondisulphide	CS_2		UV-Visible spectroscopy	------NA--	78
3.	9-Fluorenylmethylchloro-formate			HPLC. Polymeric amino column.	Acetonitrile-50mM phosphate buffer (pH10) as first mobile phase and Acetonitile-water (60:40, v/v) as second mobile phase.	74
4.	Fluorescein isothiocyanate			Microchip electrophoresis system with laser induced fluorescence detector	-------NA--	81

2.2 DERIVATIZATION OF GLYPHOSATE FOR THE SCOPE AS AN HERBICIDE:

Apart from synthesizing glyphosate derivatives for the detection in soil, water and food materials, few different derivatives of glyphosate have also been synthesized to check their herbicidal activity and to solve the problem of weed resistivity.

Mao et al. synthesized a series of Thiocarboxylate S-esters of glyphosate from N-benzyloxy carbonyl glycine using a methylene reagent (N-methylene-tert-butylamine) followed by nucleophilic phosphorylation with tris (trimethylsilyl) phosphate. N-benzyloxy carbonyl glycine (Z-glycine) activated with diphenylphosphoryl azide and mixed anhydride was reacted with preferred mercaptans to form corresponding thiocarboxylate S-esters. These thiocarboxylate esters were synthesized in good yields under controlled temperature conditions. All these thiocarboxylate-S-ester derivatives of glyphosate were showing excellent herbicidal properties as the parent molecule[87].

Bogdanova et al. synthesized two photo polymerizable derivatives of glyphosate which were biologically active and were also showing herbicidal activity. Methacrylate was prepared by dissolving glyphosate in 10% NaOH solution with vigorous stirring. Then methacryloyl chloride was added drop wise in presence of argon. After stirring, the contents of reaction mixture were mixed with HCl and were concentrated using rota evaporater . The pH of reaction mixture was then increased by adding NaOH solution. White precipitates of methacrylate derivatives of glyphosate were obtained which were dried and weighed. Similarly acrylate was prepared by similar method using acryloyl chloride as the reagent. Both these derivatives of glyphosate were characterized by NMR and their herbicidal properties were checked. Both these derivatives were showing good herbicidal properties[64].

2.3 METAL-COMPLEXED DERIVATIVES OF GLYPHOSATE:

Glyphosate has the ability to bind with metal ions present in the soil. Due to this property it loses its herbicidal activity[88] and is easily degraded by micro organisms[26]. Glyphosate binds the soil through phosphonoate moiety[89]. Glyphosate molecule has many active donor sites, so it can make chelates and complexes with metal ions present in the soil. Cu and Zn ions get strongly complexed with glyphosate, whereas Iron, Calcium, Magnesium and Manganese ions get complexed in smaller amount[90]. At neutral pH, the carboxylate and phosphonate groups get deprotonated. Therefore glyphosate's ability to coordinate as tridentate and tetradentate ligand makes it a special herbicide[91]. All the metabolites of glyphosate should also form strong complexes with transition metal ions. Metal complexation of glyphosate was done to control the glyphosate contamination in groundwater and to study the properties of metal-complexes of glyphosate[65].

Insoluble metal-complexes of glyphosate with Ferric ,Cuporous, Calcium and Magnesium ions were synthesized at neutral pH by Subramanium et al. Glyphosate (0.010 mol) was dissolved in 0.02 mol solution of NaOH in 25mL of distilled water. Then 0.010 mol of metal salt solution was added to it. The reaction mixture was placed at normal temperature until the precipitates of the metal-complexed glyphosate were formed. The precipitates were washed, dried and recrystallized for spectroscopic analysis. From X-ray diffraction studies it was confirmed that in these complexes glyphosate can co-ordinate as a tetradentate ligand and from IR spectroscopic studies it was shown that in these complexes, glyphosate was co-ordinated to different metal ions through N-atom of amino group ($-NH_2$), O-atom of carboxylate group (COO^-) and O-atom of phosphonate group of glyphosate moiety. All these metal complexes of glyphosate were neutral complexes which were highly insoluble[65].

Sundram et al. synthesized six different glyphosate-metal complexes and calculated their solubility constants. Then the toxicity of these complexes was checked on tomato plants and white spruce seedlings. Metal complexes were synthesized by mixing 0.010 mole of glyphosate with 0.010 moles of metal salt solutions of (Mg^{2+}, Ca2+, $Cu^{2+,}$ Zn^{2+}, Mn^{2+}) and 0.020 mole of glyphosate with 0.020 moles of Fe^{3+} ions in KOH- based alkaline medium. The reaction mixture was kept at normal temperature till crystallization occured. The metal-glyphosate crystals were washed, dried and their spectroscopic analysis was done. Then the solubility product of all these complexes was calculated in distilled water having phosphate buffer added to it (pH~7.0). The solubility products (K_{sp}) for glyphosate-metal complexes dwindled in the order of Mg= Ca>

Mn>Zn>Cu>Fe. Also the toxicity of the synthesized metal complexes was checked and the plants were monitored for 12 days. It was found that the complexes with greater solubility caused the greater decline in the height of the plant, and the less soluble complexes had shown less inhibition in the growth of the plant[92].

Metal complexes of certain divalent metal ions with glyphosate were synthesized by R.L. glass. Two different procedures were used by them. In the first procedure, 25mL of different glyphosate solutions with concentrations varying from 20 to 800µg mL were mixed with 1g of pulverized copper (II) oxide. The mixtures were then shaked for 1 hour in polypropylene tubes at normal temperature. The solutions were centrifuged, and filtered. The residues were collected and dried for further spectroscopic analyses. The concentrations of Cu (II) ions in the liquid phases were determined by atomic absorption spectrometry and colorimetric method using 2,2'-bicinchoninic acid. UV-Visible analyses and IR analyses using KBr pellet was done on the residue. The concentration of metal ions and glyphosate in this case was 0.25-2.60 mM. In the second procedure 25ml of stock solutions of different metal salts ($Cu (NO_3)_2$, $Pb (NO_3)_2$, $CdSO_4$ and $ZnSO_4$) were prepared in 0.1M KCl and were mixed with weighed amount of glyphosate. The pH of the mixtures was maintained by adding dilute NaOH. The amount of metal ion and glyphosate was in the range of 1-50 mM. The metal complexes of glyphosate were characterized by differential pulse polarography using a dropping mercury electrode. Results from the polarographic study showed that glyphosate exhibits fairly strong complexing properties towards divalent metal ions[93].

A series of alkaline earth metal complexes of glyphosate with general formula $[M (HL).2H_2O]$ were synthesized by Sagatys et al.. The characterization of complexes was done by FTIR spectrophotometer using KBr pellet. Apart from this, Ba complex of glyphosate was characterized by single-crystal X-ray diffraction. For the synthesis of Sr and Ba complexes, 10 mmol of glyphosate was mixed in $25cm^3$ of water containing 12.5mmol of NaOH, 11mmol of $Sr (NO_3)_2$ or $Ba (NO_3)_2$ along with $10cm^3$ of ethanol and 8g of urea. The contents were thoroughly mixed and cloudy white precipitates were formed which were redissolved by adding conc. HNO_3. The resultant solution was mixed for 3 hours and was allowed to evaporate for a week. Colourless crystals were formed after a week which were filtered under vacuum. Ca-glyphosate complex was also prepared with the similar procedure. However Mg-glyphosate complex was synthesized by mixing 25cm^3 of aqueous solution having 10mmol of glyphosate and 0.8g of NaOH to 25cm^{3}of 10mmol solution of $Mg(NO)_{3.}$ The solution was kept at normal temperature which lead to the formation of white crystals. X-ray diffraction of Ba-glyphosate complex showed that it has polymeric structure with dimeric repeating units. The co-ordination of Ba with the glyphosate takes place through O-atom of carboxyl and O-atom of Phosphonate group and formed an 8-membered ring structure[94].

2.4 ANTI-MICROBIAL ACTIVITY AND TOXICITY EVALUATION OF GLYPHOSATE ON MICRO-ORGANISMS :

Antimicrobic activity of N-phosphonomethyl glycine is known ever since its discovery in 1970. It shows its activity on many bacterial and fungal species[95]. It can regulate the development of apicomplexan parasites, such as *Plasmodium falciparum*(malaria), *Toxoplasma gondii*, and *Cryptosporidium parvum* etc and acts as antimicrobial agent for mammals. Suppression of shikimate process with glyphosate is effectual in regulating the development of these parasites. Thus, glyphosate can act as a broad-spectrum antimicrobial agent which can show its effectiveness against bacterial and fungal species and can control apicomplexan parasites[96]. Antimicrobial action of glyphosate, its commercial formulations and its adjuvants have been studied by various researchers all over the world.

Clair et al. studied the effect of N-phosphonomethyl glycine and its commercial composition (Roundup) on the development and viability of certain food microbes (*Lactobacillus delbrueckii subsp. bulgaricus* , *Geotrichum candidum* and *Lactococcus lactis subsp. cremoris*) present in the food, used in conventional and engineering dairy. Results showed that Roundup suppressed the growth of all these microbes and acted as a microbicide at minimum concentrations (concentrations lower than the recommended doses). However glyphosate in the pure form had shown no significant effect on these micro-organisms. These results confirmed that the presence of surface active adjuvants have amplified the toxic effects of glyphosate on the microbial community[97].

Kurenbach et al. calculated the effects of viable formulations of weedkillers on the antibiotic susceptibility in *Salmonella enteric serovar Typhimurium* and *Escherichia coli*. Strains of *S. Typhimurium* and *E.coli* were exposed to the sublethal concentrations of the herbicides. Maximum herbicidal concentration for glyphosate used was ≤ 1240ppm which was below the MIC of glyphosate. In case of *Salmonella Typhimurium* exposed to the sub lethal concentration of Roundup, significant increase in the tolerance to antibiotics Kanamycin and Ciprofloxacin was observed. But it reduced the susceptibility to Ampicillin, chloramphenicol and Tetracycline. However in case of *E.coli* subjection to Roundup had augmented the tolerance to Kanamycin and Ciprofloxacin. It had no effect on the feedback or augmented the vulnerability to Ampicillin, chloramphenicol and Tetracycline[98].

Bonnet et al. examined the consequences of different weedicides (glyphosate and its main metabolite AMPA) on non-target organisms by using the microorganisms' *Tetrahymena pyriformis* and *vibrio fischeri*. Toxicity evaluation was done using Microtox test, *T. pyriformis* population growth impairement test and *T. pyriformis* non specific esterase activities test. Glyphosate and Aminomethyl phosphonic acid showed less toxic effects on *T. pyriformis* than on *vibrio fischeri* . However, in these cases glyphosate had caused more harm. It was found to be more toxic than its metabolite aminomethyl phosphonic acid[99].

Richardson et al analysed the consequences of glyphosate on single-celled *Euglena gracilis*. The test organism was treated with of 3×10^{-3} M of glyphoste. It was found that the use of glyphosate on this microbe has decreased the chlorophyll content from 21 to 69%. It has also reduced the rate of photosynthesis and respiration in *Euglena* by 20%[100].

Apart from this glyphosate has also shown a prominent reduction in the radial growth of all ectomycorrhizal fungal species like *Cenococcum geophilum* Fr., *Pisolithus tinctorius* (Pers.) Coker and Couch and *Hebeloma longicaudum* (Pers.). The growth of these ectomycorrhizal fungal species was completely inhibited when glyphosate was used at higher concentration of $\geq$ 5000 ppm[101].

Chakravarty and Sidhu analysed the toxic effects of glyphosate on the common mycorrhizal fungal species like *Hebeloma crustuliniforme*, *Laccaria laccata*, *Thelephora americana*, *T. terrestris* and *Suillus tomentosus*. It has been found that glyphosate reduced the growth of these fungal microorganisms at concentrations above 10 ppm[102].

Glyphosate shows negative impact on the growth of certain rhizospheric microbial communities. Bacteria like *Fusarium*, fluorescent *pseudomonads*, Mn-transforming bacteria, and indoleacetic acid-producing bacteria present in the rhizosphere soils of soybean were treated with glyphosate. Glyphosate increased the profusion of *Fusarium spp*. while it reduced the profusion of fluorescent *pseudomonads*, Mn-reducing bacteria and indole acetic acid-producing rhizobacteria[103].

Another research group also studied the consequences of glyphosate on soil rhizosphere-associated communities and found that the application of glyphosate increases the relative abundance of *proteobacteria* (particularly gammaproteobacteria). But the excessive use of glyphosate on glyphosate-immuned crops like corn and soybean reduced the relative profusion of *Acidobacteria*. Since *Acidobacteria* are also implicated in biogeochemical processes, the decline in the profusion of these bacteria could lead to considerable transformation in nutrient status of the rhizosphere and would also affect plant growth[104].

Further the effects of N-phosphonomethyl glycine on some common pathogens and useful members of poultry microbiota were studied in vitro, and it had been established that extremely pathogenic bacteria like *Salmonella gallinarum, Salmonella enteritidis, Salmonella typhimurium, Clostridium perfringens and Clostridium botulinum* are resistant to glyphosate. But the vulnerability of glyphosate toward the beneficial bacteria of poultry like *Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis* and *Lactobacillus spp.* varies from species to species. Some of these microorganisms are highly susceptible, while some are moderate. Out of these, *Campylobacter spp*. (which are responsible for gastrointestinal diseases in humans) are highly prone to glyphosate. Intake of glyphosate by the poultry reduces the beneficial bacteria present in the gastrointestinal tract, which could perturb the normal functions of bacterial community present in the gut of these birds[105].

2.5 MICROBIAL DEGRADATION OF GLYPHOSATE:

Glyphosate is the only herbicide known for its abrupt detoxification from the soil, due to its binding with the soil as well as its degradation with micro-organisms[106]. Two major degradation pathways have been suggested for the biodegradation of glyphosate. In the first pathway, glyphosate decompose through the breakage of C-N bond resulting in the formation of AMPA as the major degradation product. The studies carried out on the bacteria isolated from glyphosate waste water treatment plant suggested that the glyphosate is initially degraded to AMPA and glyoxylate. Enzyme oxidoreductase is responsible for this initial degradation step[107]. In the second pathway glyphosate's degradation proceeds through the cleaveage of C-P bond and produces inorganic phosphate and amino acid sarcosine. Majority of bacteria isolated from soil have shown to proceed through this pathway[35].

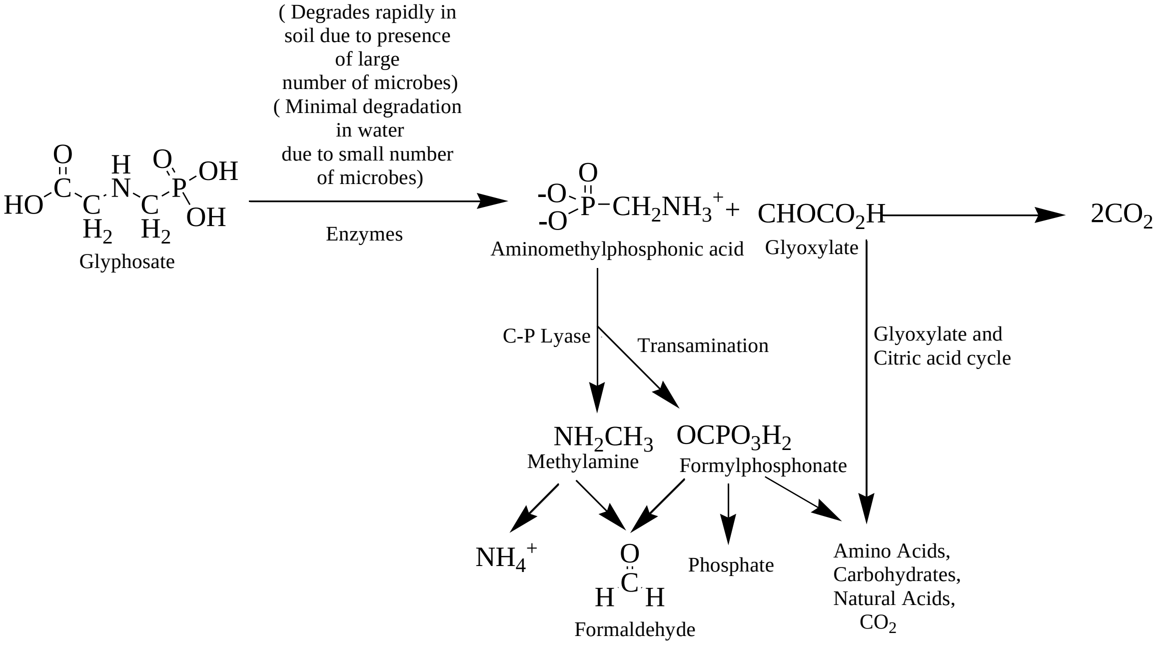


Figure 2.26 Degradation Pathway of Glyphosate

Several studies were conducted by different researchers to verify the degradation of glyphoste by the microbes present in soil and water.

Penaloza et al. studied the degradation behavior of thirty four bacterial strains of *Pseudomonas pseudomallia* extracted from soil. All the strains were grown on glyphosate and no additional aromatic amino acids were added to the media. Bacterial strain *Pseudomonas pseudomallia* 22 showed 50% degradation of glyphosate in 40 hours. The degradation capacity of *Pseudomonas pseudomallia* 22 to degrade glyphosate was checked by providing them mineral medium containing equimolar amounts of aminomethylphosphonic acid, glyphosate and phosphate. However no growth was noted in media containing glyphosate, AMPA and sarcosine as a C-source. Thus, in lieu of this glyphosate may endure stepwise degradation from the carboxyl group, with AMPAas main metabolite. Thus, these bacteria were capable to develop on glyphosate and AMPA as a P- source[108].

Pipke and Amrhein studied the degradation of glyphosate by using bacteria *Arthrobacter atrocyaneus* ATCC 13752. The bacteria were grown on increasing concentration of both phosphate and glyphosate (as the source of Phosphorus). Equal cellular yields were acquired from equimolar concentrations of both these compounds. This indicates that bacteria had utilized both the compounds to same extent. Thus, *Arthrobacter atrocyaneus* had degraded glyphosate to AMPA. AMPA was then completely transformed to CO_2[109].

Sviridov et al.2011 isolated the strains of bacteria that can decompose methylphosphonic acid and glyphosate from the soil contaminated with these organophosphonate herbicides. Bacterial strains extracted from methylphosphonic acid contaminated soils were able to grow only on it. Two different groups of bacterial strains were extracted from glyphosate contaminated soils. One of these groups was able to decompose only methylphosphonic acid while the other was able to degrade both glyphosate and methylphosphonic acid. Strains of *Achromobacter sp. MPS12 (VKM B-2694)* showed best degradative capability towards the degradation of methylphosphonic acid while the bacterial species *Ochrobactrum anthropi GPK 3* degrades glyphosate. In *Achromabacter sp.MPS12* the degradation of methylphosphonic acid was catalysed by enzyme C-P lyase which was incapable to degrade glyphosate. When the bacterial strain Achromobacter *sp. MPS12A* was grown on glyphosate, it gets adapted to it and hold its ability to degenerate methylphosphonic acid through C-P lyase I. It was also able to degrade glyphosate through the production of sarcosine. Degradation of methylphosphonic acid through *Ochrobactrum anthropi GPK 3* proceeds through C-P lyase and the degradation of glyphosate was commenced by glyphosate oxidoreductase enzyme through phosphonatase pathway[110].

In sod-podzol soil the biodegradation of glyphosate was studied by Shushkova et al. by using indigenous microflora and expatriate strain of *Ochrobactrum anthropi GPK 3*. All experiments were performed in columns that imitate the upright profile of soil. Results showed that up to 84% of glyphosate was absorbed by the soil during the first 24 hours of spray and the rest 16% remained in the soluble form in the soil containing the microbial communities. The experiment was performed for 21 days. Only a minute amount of glyphosate was left available in the soil at the end of the test . About 10-12% of glyphosate was degraded in the uppermost soil in the presence of native microorganisms. However in the presence of culture containing different bacteria *O.anthropi GPK 3* 42% of glyphosate was degraded. Results showed that the degradation of glyphosate occurred only in the top layer of soil containing viable *O.anthropi GPK 3* cells and indigenous microflora. However, results also suggested that the herbicide was not decomposed in the soil in the deficiency of these microbes[111].

Pipke et al. analyzed the metabolic process of glyphosate in bacterium *Arthrobacter sp.GLP-1* with the help of NMR technique and radiotracer analysis. Chief step in the decomposition of glyphosate include the transformation of glyphosate to glycine, a C-1 unit and phosphate moiety. Glycine produced as the degradation product of glyphosate was utilized in the synthesis of purines and proteins. Similar type of degradation pathway was also reported by *Pseudomonas sp.PG2982.* Degradation of glyphosate by *Arthrobacter sp. ATCC 13752* occurs through the breakage of C-P bond. This pathway of glyphosate degradation was quite different from the glyphosate’s degradation in soil via aminomethyl phosphonic acid formation[112].

Jacob et al. analysed the metabolic process of glyphosate using *Pseudomonas* sp. strain LBr (extracted from a glyphosate process waste stream) through solid state ^{13}C NMR and phosphonate content analysis of the growth medium. *Pseudomonas* sp. strain LBr showed two different pathways for glyphosate degradation. It degraded 20mM of glyphosate from the growth medium. The bacterium metabolized the high levels of glyphosate by converting it into aminomethyl phosphonoate. Only a small amount of aminomethyl phosphonic acid (about 0.5 t0 0.7 mM) was degraded by this bacterium. C-13 NMR analysis showed that 5% of glyphosate followed different degradation pathway and form glycine as a metabolite[113].

2.6. INSECTICIDAL AND TOXICOLOGICAL EVALUATION OF GLYPHOSATE ON *Drosophila melanogaster*:

Drosophila melanogaster is employed as a model organism in variety of studies involving ecology, behaviour, genetics and molecular biology. It is not categorized as a pest and is highly recommended for large-scale insecticide screening operations. It resembles with mosquitoes and other flies in its physiology, biochemistry and genetic makeup[114]. Apart from this there are many technical advantages of using *Drosophila* over other insect models; they are easy to culture and are economical to breed in the laboratory conditions. They have shorter life cycle. They produce large number of embryos that can be genetically modified[115].

Thus the insecticidal effects of glyphosate on *Drosophila melanogaster* may elaborate the toxicological effects and defense mechanisms of this herbicide on it. It may help to find out the harmful effects of various herbicides on different insects. Very less amount of work has been performed to ensure the insecticidal and toxicological effects of glyphosate on *Drosophila melanogaster*.

de Aguiar et al. checked the consequences of herbicide (involving glyphosate as the chief component) regarding antioxidant defence system, acetylcholinesterase activity and oxidative stress in *Drosophila melanogaster*. Flies (1 to 3 days old) were treated with fourr different concentrations (1mg/L, 2mg/L, 5mg/L and 10mg/L) of herbicide dose mixed in the diet for 24 hours and 96 hours. Herbicide exposure to the flies leads to the reduction in the reactive oxygen species (ROS) level in flies exposed to 96 hours. Antioxidant capacity against peroxyl radicals (ACAP) and gene expression of the antioxidant defence system show significant enlargement after 24 hours exposure. However lipid peroxidation level does not demonstrate any noteworthy change in both cases. Also the activity of enzyme the acetylcholinesterase was not affected by Roundup exposure. Thus it was concluded that exposure of flies to Roundup caused premature activation of the antioxidant defense system in the flies and stop the harm made by reactive oxygen species[116].

Kaya et al. analysed the genotoxicity effects of four different herbicides (maleic hydeazine, glyphosate, propanil and 2,4,5-tri chlorophenoxy acetic acid) in *Drosophila melanogaster* wing spot test . 3-day old larvae were chronically fed to these herbicides. Standard (ST) cross and high-bioactivation (HB) cross relating the flare-3 and multiple wing hair markers were used for the test. Glyphosate provoke a weak but momentous enlargement in the frequency of small single spots only in standard cross indicating that it is genotoxic in these strains. No response was noticed in high-bioactivation (HB) cross[117].

Another similar type of studies was conducted by Kale et al. They analysed the mutagenotoxic effects of nine herbicides and pesticides including glyphosate on *Drosophila melanogaster* using sex-linked recessive lethal mutation assay. *Drosophilla* larvae were fed on the media containing the test compounds. Glyphosate resulted in high frequency of mutants in spermatocytes and spermatogonia of the flies. Results showed that glyphosate induced mutagenotoxicity to the *Drosophila melanogaster*[118].

2.7 TOXICITY EVALUATION OF GLYPHOSATE ON EARTHWORMS:

Earthworms are the essential component of soil biota. They are helpful in maintaining the quality and ecosystem of soil[120]. Various acute and chronic toxicological tests were conducted by different researchers to evaluate the toxicological outcomes of glyphosate on these wigglers.

Correia et al. conducted laboratory tests on *Eisenia fetida* to investigate the toxicological effects produced by glyphosate on it. Five concentrations of glyphosate (1, 10, 100, 500 and 1000 mg) were taken as test concentrations. The experiment was carried out for 56 days. No mortality was observed in the soils treated with glyphosate at any of these concentrations. However, steady and considerable decrease in the mean body weight was found at all the test concentrations. Glyphosate revealed severe harmful effects on the fecundity and development of earthworms in the range of these concentrations. The soils treated with the herbicide does not contain any cocoon or juveniles Apart from this, significant anatomical changes were also observed after 30 days of the experiment. Morphological abnormalities like the elevation of body, curling and coiling were noticed in all the samples mixed with the highest amount of glyphosate in the soil [54]. Another research group checked the acute and chronic toxicological effects of AMPA on *Eisenia andrei* at field-relevant concentrations. No significant mortality was observed in both acute and chronic assays. In acute toxicity test, momentous loss in the biomass of earthworms was recorded in case of control as compared to the earthworms treated with aminomethylphosphonic acid. However, in chronic test, larger loss in the biomass of earthworms was recorded at the highest concentration of aminomethylphosphonic acid. Also there was an increase in the number of young earthworms and cocoons at the highest concentration of the herbicide. But the mean body weight of these juveniles was found to be decreased. These results confirmed that juveniles are more sensitive to AMPAthan the adults[120]. Ecotoxicological effects of glyphosate were evaluated on *Eisenia fetida* and *Eisenia andrei*. In the bioassays, earthworms were rendered to the soil samples collected fromsoya farms (treated with glyphosate), from Argentina. Both behavioral and biological changes were noticed in the test organisms of both species. It was observed that glyphosate decreased the cocoon viability, thereby decreasing the number of juveniles produced. Apart from this, they also avoided the soils treated with glyphosate and show reduction in their feeding activity[121].

Similar kind of studies was conducted by Yasmin and D’Souza on *Eisenia fetida* to check the toxicological effects of glyphosate and other pesticides on it. A regular diminution in the body weight of the test organisms was found, when they were exposed to glyphosate and mixture of glyphosate, carbendazim and dimethoate[122]. Hazardous effects of commonly used herbicide glyphosate on two annelid species *Eisenia fetida* and *Octolasion tyrtaeum* were studied by García-Torre et al. Both these test organisms were exposed to five different concentrations of glyphosate. Results revealed that earthworm species *Octolasion tyrtaeum* was more prone to the glyphosate (50,000 mg kg−1). 100% mortality was observed at this concentration after seventh day of treatment. However, in the case of *Eisenia fetida* no mortality was recorded, but a noticeable loss (40%) in the body weight was found. Unpleasant effects of the herbicide were also seen on the adult fertility and cocoon viability. The number of juveniles produced from the cocoons was also decreased[123]. Berghausen et al. also assessed the impact of glyphosatebased herbicides on two species of earthworms (*Lumbricus terrestris* and *Aporrectodea caliginosa*). The surface casting activity of *Lumbricus terrestris* was decreased after three weeks of herbicide spray. However, no change in this activity was recorded for other earthworm species (*Aporrectodea caliginosa*). Apart from this, reproduction rate in earthworms of both species was also reduced within 3 months after herbicide application[57]. Toxicity evaluation of two glyphosate-based herbicides was carried out by comparing their adverse effects on earthworm (*Eisenia andrei*). Glyphosate's commercial formulations, Roundup FG and Mon 8750, were used. Lethal concentration (LC-50) values reveal that Roundup FG was 4.5 times more toxic than Mon 8750. However, at sublethal concentrations noticeable weight loss was observed. Glyphosate acts as uncoupler of oxidative phosphorylation in the mitochondria of earthworms. Roundup FG showed venomous effects on the DNA of test organisms and caused lysosomal damage in them[56]. Harmful effects of herbicide on the population of earthworm species *Eisenia fetida* were carried out by Santadino et al. Two different concentrations of the herbicide were used. Glyphosate showed long- term effects on the test organisms with the decrease in the fertility of cocoons. This led to the local extinction of population of the earthworms in the soil[124]. Assessment of the effect of the pesticide to the nontarget organisms present in the soil was done by Santos et al. Three commercial insecticides (Chlorpyrifos, Endosulfan) and one herbicide (Glyphosate) were used. Treated soil was collected to verify the avoidance test and reproduction behavior of *Eisenia andrei*. These worms avoided the soil contaminated with Chlorpyrifos and Endosulfan. However, in the case of glyphosate, an equal number of worms were found on both sides indicating that glyphosate does not cause any harm to earthworms if used in recommended dose. Also it does not affect the reproduction activity of the worms[125]. Glyphosate molecule has many binding sites due to the presence of different functional groups present in it therefore it combines with metal ions and form metal complexes. Fan Zhou et al. found that Cu ions present in the soil form complex with glyphosate and reduce the acute toxicity on earthworm caused by Cu ions. This complexation declined the mortality rate in earthworms. Along with this glutathione (GSH) content , superoxide dismutase (SOD), and acetylcholinesterase activity were also reduced to the levels of control. These outcomes revealed that the complexation of glyphosate with metal ions present in soil could reduce the toxicity and accessibility of heavy metal ions present in the soil[126].

Another research group used glyphosate-based herbicide Groundclear (containing 5% of isopropylamine salt of glyphosate), to examine its acute toxicity on *Eisenia fetida*. Earthworms were treated with five concentrations of glyphosate ; however, the worms exposed to the recommended dose for 24–48 hours show very little mortality. But they show avoidance behavior against the herbicide. The presence of herbicide in the soil also affects the locomotor activity of the worms. Thus, the use of herbicide may not directly cause any harm to them, but it can cause severe long-term effects[127]. Zaller et al. studied the harmful outcomes of glyphosate-based herbicide on the correlation between earthworms (*Lumbricus terrestris*) and symbiotic mycorrhizal fungi. Herbicide application on the soil decreased the earthworm activity in the mesocosms containing arbuscular mycorrhizal fungi. It further declined the soil mycorrhizal fungi spore biomass, vesicles and reduced the root mycorrhization. This resulted in the poor interactions between the worms and the mycorrhizal fungi which pose a serious threat to the natural systems[128].

2.8 HERBICIDAL ACTIVITY OF GLYPHOSATE:

Glyphosate is a potent herbicide widely used by farmers all over the world. Before the advancement of glyphosate-resistant cropping systems, it was applied to the soils before sowing the plants and was used as a pre-emergent herbicide[6]. Now-a-days glyphosate is used after planting and is applied to the foliar part of the plant. To understand the herbicidal effects of this herbicide several researchers have conducted various experiments on different weeds commonly found in the agricultural fields.

Barivan et al. studied the activity, rain-fastness, translocation and absorption of glyphosate in absence and presence of surfactants on commonly found weed purple nutsedge. The weed reciprocates differently to different concentrations of the herbicide depending upon the growth stage of the plant. The growth of the weed was reduced significantly to 96%, when the herbicide was sprayed at different concentrations of 2.24kg ai/ha on a 17 day old plant and 4.48kg/ha on a10 week old plant. Glyphosate also reduced the resprouting of the tuber and diminishes the growth of the plant. The presence of organosilicone surfactant in the glyphosate doesn't affect the efficacy of the herbicide. Conversely, simulated rainfall of 2.5cm after 1 hour and 24 hour spray of glyphosate reduced its efficiency to one-half and one-third respectively. Apart from this ^{14}C-glyphosate absorption in the plant get increased from 2.8% at 1 hour after application to 21.4% at 168 hour after application and translocation increased from 0.43% to 5.18% after 1 hour to 168 hour respectively. Presence of organosilicone surfactant doesn't affect absorption and translocation in the plant[129].

Another similar type of activity was done by Claus et al. They studied the effect of glyphosate on the rhizome length and foliar height on rhizome bud of commonly found weed quackgrass (*Agropyron repens* (L) Beau). Translocation of glyphosate through the weed was also examined using ^{14}C-glyphosate. Foliar application of glyphosate at conc. 0.28kg/ha had significantly reduced the survival of quackgrass rhizome bud. However glyphosate treatment at conc. of 0.56, 0.84 and 1.12kg/ha had completely killed the bud of the weed. Glyphosate's effect was more prominent when the herbicide was applied to taller foliage. Glyphosate showed no effect on the rhizomes present near the mother shoot of the plant. Glyphosate's accumulation in the weed was found to be more in the nodes near the rhizome tip[130].

Akin et al. conducted the field studies to appraise the outcomes of glyphosate and other commonly used herbicides on purple nutsedge (*Cyperus rotundus*) and yellow nutsedge (*Cyperus esculentus*). Effects of different herbicides on the tuber density and viability was checked via growth chamber or triphenyl tetrazolium chloride test. Glyphosate containing herbicides were applied at conc. 0.84kg/ha followed by conc. of 0.56kg/ha, reduced the viability and total tuber density of purple nutsedge. Similar types of results were also obtained in case of yellow nutsedge[131].

Devlin et al.1986 evaluated the herbicidal outcomesof glyphosate in sandy soil. Both initial and residual herbicidal effects of this herbicide were studied on the bioassay of wheat (*Triticum vulgare* L.) . Glyphosate at conc. of 5kg/ha appreciably reduced the shoot length of the plant. This change was notified in the plants planted immediately and after 10 days of herbicidal application. Glyphosate at conc.2.5kg/ha and 5kg/ha also significantly reduced the root length of the plant even after 10 days of its application. Notable loss in the fresh and dry weight was noticed for both shoots and roots of the wheat plants. This shows that glyphosate can show its herbicidal effects in the sandy soil even after 10 days of its application[132].

Balah et al. studied the herbicidal outcomes of glyphosate in the presence and absence of different adjuvants on the weed *Cyperus rotundus*. Glyphosate reduced the fresh and dry weight of shoot and roots of the weed. Addition of adjuvant in the herbicide also manifested the decrease in the weight. Apart from this glyphosate also decreased the chlorophyll content of nutshell leaves after 3 weeks of the treatment. Presence of adjuvant also reduced the chlorophyll content in the leaves of the plant[133].

Herbicidal outcomes of glyphosate on Purple nutsedge (*Cyperus rotundus* L.) was studied by Zandstra et al. 4kg/ha of the herbicide was foliar applied to the plants grown in the greenhouse for 2 to 10 weeks, glyphosate hindered the germination of the tubers from the plant. However some of the tubers from plants of age 12-24 week were not affected by the glyphosate application. Translocation of ^{14}C-glyphosate in the leaves and other parts of the treated plant was found to be increased from 5% to 19% from day 1 to day 4. Presence of ^{14}C activity was more in the tubers of plants of age 2-6 week. In older purple nutsedge plants^{14}C translocation was found to be more in tubers and less in leaves[134].

Asad Shabir analyzed the effect of glyphosate on the common weed Parthenium hysterophorus in glass house and under field conditions. Different concentrations of glyphosate were used (One-half and three-fourth of the recommended doses). Under glass house conditions 100% mortality was observed in the weed plants 21 days after spray. However at half and three-fourth of the recommended doses about 60 and 90% of the mortality was observed in the plants. Under field conditions 100% and 80% mortality was noticed at these concentrations. This study concluded that glyphosate is very effective herbicide used to control *Parthenium hysterophorus*[135].

Norsworthy et al. analyzed the differential susceptibility of some common weeds (hemp sesbania, pitted morning glory, barnyardgrass and prickly sida) to glyphosate. It was measured by determining the assimilation of ^{14}C-glyphosate in the leaves and other parts of the plant, amount of epicuticular wax released and the wettability of leaf surfaces. Under green house conditions the use of commercial formulation of glyphosate (Roundup Ultra) reduced the biomass of weeds (barnyardgrass and Prickly sida) to 95%. However, other weeds like Hemp sesbania and pitted morning glory demonstrated more endurance towards this herbicide with only 66 and 51% average biomass reduction respectively. Under controlled conditions, the absorption of ^{14}C- glyphosate in various weeds doesn't follow any trend. Barnyardgrass showed absorption of 30%, Prickly sida absorbed only 18% , hemp sesbania with absorption of 52% and pitted morning glory absorbed only 6% of the ^{14}C- glyphosate. It was concluded that the high tolerance of Roundup Ultra by pitted morning glory may be due to its very low absorption. Addition of nonionic surfactants to the herbicide reduced its absorption in these weeds but had no effects on the herbicidal activity of glyphosate[136].

Irmaileh et al. conducted the study to analyse the outcomes of glyphosate on the chlorophyll content, carotenoid and catalse activity in the weed purple nutsedge (*Cyperus rotundas* L.). Glyphosate caused chlorosis in the leaves of the plants maintained under light conditions. This glyphosate induced chlorosis in the leaves of the weed was delayed by the presence of darkness but it reoccurred when the treated plants were placed in the light. Apart from this glyphosate caused appreciable reduction in the chlorophyll and carotenoid content of the plant. Also, the catalase specific activity was reduced to about 70% in the plants treated with glyphosate[137].

Another researcher Wang studied the outcomes of glyphosate on the amino acid metabolism in the sprouted weed, purple nutsedge tubers and shoots. Glyphosate at concentration of 33.5mM inhibited the bud enlargement and elevated the total free amino acid concentration. It also resulted in the rapid accretion of shikimic acid in the sprouted tubers of the plant. The percentage of tryptophan (aromatic amino acid) reduces rapidly to 22% after 3 days of treatment and remained low thereafter. Foliar application of glyphosate at concentration of 14.5mM on the plant shoots caused the rapid accumulation of the herbicide which was then converted into aminomethylphosphonic acid. The amount of free amino acids also increased in the leaves three days after the treatment. Apart from this, decrease in the soluble protein content and reduction in the acid protease activity was noticed in the plant. All these transformations were linked with fast accretion of glyphosate and large accretion of shikimic acid[138].

From the recent studies done on glyphosate, it has been found that due to the farming of transgenic glyphosate-persistant crops, its use in the fields and farmlands has increased many folds. This has led to the evolution of many new glyphosate- persistant plants, specially the weeds which create problems for the farmers. Also with the amplification in the growth of glyphosate -persistant weeds, the crop production has decreased which ultimately has resulted in declined food production.

From the literature reviewed, it has been found that derivatization of glyphosate is done earlier only to detect its presence in soil, water and living organisms. As glyphosate is highly polar and lacks fluorogenic and chromophoric groups, so it was derivatised with fluorogenic groups to detect its presence by various spectrophotometric techniques. Very few derivatives of glyphosate have been synthesized to overcome the problem of weeds.

So by taking into account all the considerations, further investigation is needed to delineate the derivatives of glyphosate molecule by its modifications which could show herbicidal properties so that the intricacy of weeds could be solved and the production of crops could be enhanced.

CHAPTER-3

HYPOTHESIS

Glyphosate is a broad spectrum, post emergent, systemic, foliar applied herbicide used widely in the whole world to control annual and perennial plants. Since from its discovery till date it is the commonly used herbicide. But the excessive and liberal application of glyphosate in the agricultural fields, gardens and public streets has resulted in the evolution of many glyphosate-tolerant weeds which have directly influenced the crop productivity. Apart from this glyphosate's metabolites have also been found in the soil and water systems which has caused severe toxicity to the non-target organisms present in the ecosystem.

To overcome the problems of weed resistivity and toxicity certain structural modifications in the glyphosate molecule are required, so that the structurally modified derivatives of glyphosate could behave as potent herbicides and could show minimal toxicity. On this assumption, different structurally modified derivatives of glyphosate were proposed to synthesize by doing different organic reactions on the glyphosate molecule. These modified derivatives of glyphosate were expected to show good herbicidal properties. Also they would be less polar as compared to the parent glyphosate molecule and can reduce the problem of their leaching into the groundwater. It is also expected that these structurally modified derivatives of glyphosate could be easily degraded by microbes present in the soil and would not penetrate in the soil.

CHAPTER-4

OBJECTIVES

1. To prepare the derivatives of glyphosate and their characterization by FTIR, NMR and Mass spectroscopy.
2. To study the effects of glyphosate derivatives on weeds and various types of grasses in the field.
3. To check the anti microbial and degradation activities of synthesized glyphosate derivatives and their comparison with metal complexes of various metal ions present in the soil.
4. To check the anti insecticidal activity and toxicity of synthesized glyphosate derivatives

CHAPTER-5

MATERIAL AND METHODS

5.1 EXTRACTION OF GLYPHOSATE FROM ITS COMMERCIALLY AVAILABLE FORMULATION:

Reagents:

Ammonium salt of glyphosate 71% SG (manufactured by Bharat insecticides limited) was purchased from market. Silica gel 60-120 mesh and Ethyl acetate were taken from Loba Chemie Pvt Ltd.

Procedure:

100g of Ammonium salt of glyphosate 71% SG was dissolved in 100mL of distilled water by continuous heating. On cooling, the solution was passed through silica column (60-120 mesh) and the solution was allowed to drain completely. Silica is slightly acidic in nature and retains basic compounds. So it was used to separate weakly polar impurities from the solution. To the clear solution of ammonium salt of glyphosate, conc. HCl was added drop wise till its pH became ~ 2-3. On adding HCl, ammonium chloride was formed which remains in the solution and white precipitate of glyphosate were settled down at the bottom. Formation of ammonium chloride was confirmed by detecting ammonium ions in the solution (To a small portion of solution, sodium hydroxide pellets were added, ammonia gas was liberated which turned damped red litmus paper to blue). Solid white precipitates of glyphosate were dried in vaccum dessicator containing calcium chloride and P_2O_5. Extraction of glyphosate from ammonium salt was confimed from its FTIR, ^{1}H NMR and ^{13}C NMR[139].

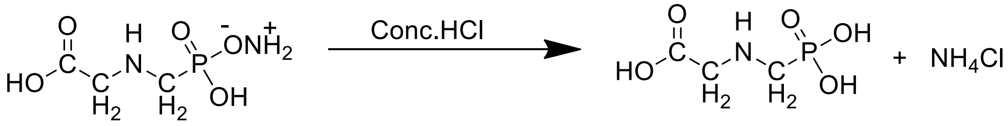


Figure 5.1 Extraction of glyphosate from ammonium salt of glyphosate using HCl

5.2 SYNTHESIS OF GLYPHOSATE DERIVATIVES:

Variety of derivatives of glyphosate were synthesized by solution phase methodology in which different functional group conversions were done. The progress of the reactions was checked by TLC and the synthesised compounds were purified by using column chromatography and further characterization was done by using various spectroscopic techniques (Fourier Transform Infrared (FTIR), Proton Nuclear Magnetic Resonance (^{1}H NMR) and Mass Spectroscopy (MS)

5.2.1 Synthesis of ester derivatives of glyphosate:

A series of ester derivatives of glyphosate were synthesized by using thionyl chloride and corresponding alcohol by the method developed by Hosangadi and Dave [140]. It is an effective and efficient method used for the synthesis of alkyl substituted ester derivatives of glyphosate.

Reagents:

Glyphosate (Technical Grade 95.10%), Thionyl chloride (AR)

Solvents; Methanol (AR), Ethanol (AR), Propan-2-ol (AR) and Butanol (AR)

Experimental:

At 0°C, a two- necked flask, attached with a stirrer, was loaded with 30 mL (6mmole) of alcohol (distilled). 3.45mL (17.7mmole) of thionyl chloride and 1g (5.9mmole) of glyphosate at ambient temperature. The contents of the flask were then refluxed for 3 hours. Rota evaporator was used to evaporate the solvent under reduced pressure and the product obtained was dried in vaccum dessicator using P_2O_5. The coarse product was cleansed using column containing the silica gel with MeOH: C_6H_{14} as solvent system to get the pure compound. The progress of the reaction was monitored by TLC on readymade silica gel plates (Merck, UV active, λ_{254} nm) using solvent system C_4H_9OH: CH_3COOH: H_2O in the ratio of 4:1:1.

Characterization was made using FTIR, ^{1}H NMR and MS. The IR spectra were recorded by Schimadzu spectrophotometer, ^{1}H NMR spectra were recorded in D_2O with Trimethyl silane as internal standard using Bruker Advance II instrument at 400MHz and Mass spectra were recorded on Waters Q-TOF Micro equipped with electronspray ionisation (ESI) and atomspheric-pressure chemical ionization (APcI) Source.

where R= CH_3, C_2H_5, $CH(CH_3)_2$, $CH_2CH_2CH_2CH_3$

Figure 5.2 Synthesis of alkyl ester derivatives of glyphosate

5.2.2 Synthesis of amide derivatives of glyphosate:

A series of amide derivatives of glyphosate were synthesized by condensation of glyphosate with required amine separately using mixed anhydride method of coupling with Isobutyl chloroformate as a coupling reagent in presence of inert solvent as described by Chen and Benoiton [141,142].

Reagents:

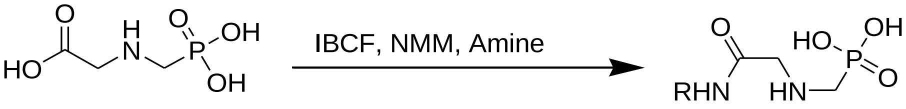
Glyphosate (Technical Grade 95.10%), N-Methylmorpholine (AR), Isobutyl chloroformate (AR), Methanamine, Propan-2-amine, Butan-1-amine,
Solvents; Dioxane (AR), Ethyl acetate (AR)

Experimental:

Glyphosate (10mmole,1.69g) was dissolved in 10mL of dioxane (minimum amount of NaOH pellets were dissolved in it) under ice-cold conditions (-5°C) at constant stirring. To that N-Methylmorpholine (13mmole,1.42mL) was added followed by addition of Isobutyl chloroformate (13mmole,1.68mL) as a coupling reagent. Then the corresponding amine [15 mmole ,0.46mL of methyl amine,15mmole ,1.28mL of isopropyl amine and 15mmole,1.48 mL of butyl amine] was mixed with the reaction mixture. The contents of the flask were stirred for 2 hours. The solvent (dioxane) was evaporated using rota-evaporator and the product obtained was extracted by using ethyl acetate followed by washings with cold 5% aqueous citric acid, brine, 5% aqueous $NaHCO_3$ and then again with brine. The organic layer (solvent layer containing ethyl acetate) was evaporated using rota evaporator and dehydrated over anhydrous Na_2SO_4 . The product was purged using silica gel column with ethyl acetate and hexane as mobile phase. The refined product was then dried in vaccum dessicator using P_2O_5. The progression of the reaction was regularly checked by TLC with readymade silica gel plates (Merck, UV active , λ_{254} nm) using solvent system CH_3OH: CH_3COOH: H_2O in the ratio of 4:1:1

Characterization was made using FTIR, ^1H NMR and MS. The IR spectra were recorded by Schimadzu spectrophotometer, ^1H NMR spectra were recorded in D_2O with Trimethyl silane as internal standard using Bruker Advance II instrument at 400MHz and Mass

spectra were recorded on Waters Q-TOF Micro equipped with electronspray ionisation (ESI) and atomspheric-pressure chemical ionization (APcI) Source.



Where R= CH_3, $CH(CH_3)_2$, $CH_2CH_2CH_2CH_3$

Figure 5.3 Synthesis of amide derivatives of glyphosate

5.2.3 Synthesis of Boc-protected glyphosate derivative:

Di-tert-butyl dicarbonate is used as a protecting group in the preparation of amino compounds. It is an acid anhydride and is known as Boc-anhydide. Addition of Boc-group to various amines and amino acids can be done under aqueous conditions in the presence of some base. Boc-Protection of glyphosate was done by doing slight modifications in the method described by Tarbell et al[143].

Reagents: Glyphosate (Technical Grade 95.10%), Sodium hydroxide (AR), Boc-anhydride (Di-tert-butyl dicarbonate) (AR),

Solvents: Dioxane (AR), Ethyl acetate (AR)

Experimental:

Glyphosate (10mmole, 1.69g) was dissolved in 10mL of water and sodium hydroxide (20mmole, 0.8g) was added to it with constant stirring. The contents of the reaction were then allowed to cool at 5°C and then they were mixed with (2.4mmole) Boc-anhydride dissolved in 10mL of dioxane. Contents of the flask were blended for 2 hours at 0°C. The pH of the reaction was maintained basic. The solvent (dioxane) was evaporated using rota-evaporator and the residue obtained was extorted by using ethyl acetate ($C_4H_8O_2$) followed by washings. The organic layer (solvent layer containing ethyl acetate) was evaporated using rota evaporator and dehydrated over anhydrous Na_2SO_4. The product was purged using silica gel column with ethyl ethanoate and hexane as mobile phase. The refined product was dried in vaccum dessicator using P_2O_5. The progression in the reaction was regularly checked by TLC with readymade silica gel plates (Merck, UV active , λ_{254} nm) using solvent system CH_3OH: CH_3COOH: H_2O in the ratio of 4:1:1.

Characterization was made using FTIR, ^{1}H NMR and MS. The IR spectra were recorded by Schimadzu spectrophotometer, ^{1}H NMR spectra were recorded in $CDCl_3$ with Trimethyl silane as internal standard using Bruker Advance II instrument at 400MHz and Mass spectra were recorded on Waters Q-TOF Micro equipped with electronspray ionisation (ESI) and atomspheric-pressure chemical ionization (APcI) Source.

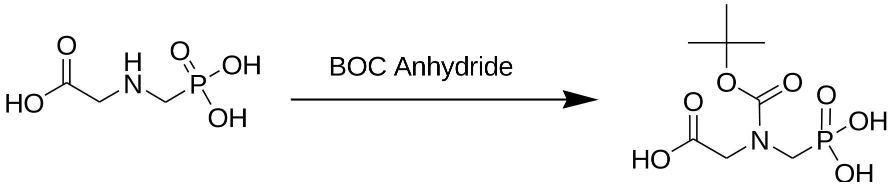


Figure 5.4 Synthesis of Boc-protected derivative of glyphosate

5.2.4 Synthesis of 2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid:

2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino)ethanoic acid was synthesized by treating glyphosate with guanidinylating reagent 1,3-Bis-(*tert*-butoxy carbonyl) -2-methyl-2-thiopseudourea in dioxane as a solvent by the method described by Solomon et al. [144].

Reagents: Glyphosate (Technical Grade 95.10%), Triethylamine, 1,3-Bis-(tert.-butoxy carbonyl) -2-methyl-2-thiopseudourea.

Solvent: Dioxane

Experimental:

Aqueous solution of glyphosate (2mmole, 0.338g) was taken in a round bottom flask. To that dioxane (10mL) and triethylamine (1.2mmole, 0.167mL) was added. After that 1,3-Bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudurea (3mmole, 0.87g) was mixed and the reaction mixture was heated at 60 ºC for 4 hour. The solvent (dioxane) was evaporated by using rota-evaporator and the residue obtained was extorted by using ethyl ethanoate followed by washings with 5% aqueous $NaHCO_3$ and NaCl. The organic layer (solvent layer containing ethyl ethanoate) was evaporated under reduced pressure using rota-evaporator and dried by using anhydrous Na_2SO_4. The coarse product was purified over silica gel column using ethyl acetate and hexane as solvent system and was dried in vaccum dessicator using P_2O_5. Progress of the reaction was regularly checked on readymade silica gel plates using Ethyl acetate and Hexane as a solvent system.

Characterization was made using FTIR, ^{1}H NMR and MS. The IR spectra were recorded by Schimadzu spectrophotometer, ^{1}H NMR spectra were recorded in D_2O with Trimethyl silane as internal standard using Bruker Advance II instrument at 400MHz and Mass spectra were recorded on Waters Q-TOF Micro equipped with electronspray ionisation (ESI) and atomspheric-pressure chemical ionization (APcI) Source.

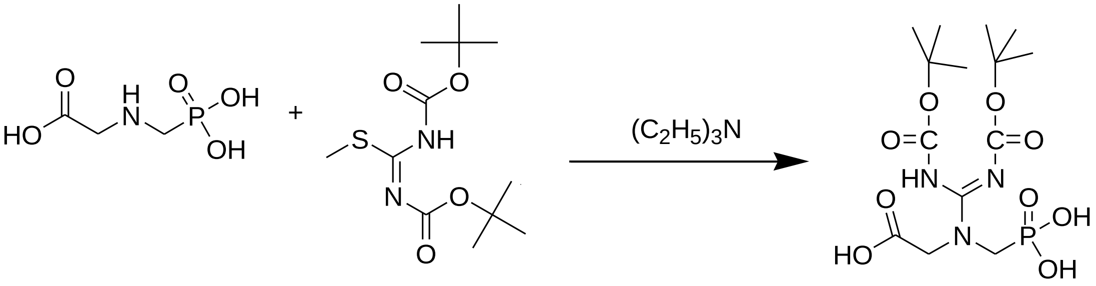


Figure 5.5 Synthesis of 2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid

5.2.5 Synthesis of 2-(1-phosphonomethyl) guanidino)ethanoic acid:

Guanidine derivative of glyphosate was obtained by acidolytic cleavage of Boc-groups using Trifluoroaceticacid (TFA) by the method as described by Lundt et al. [145].

Reagents: Trifluoroacetic acid

Solvent: Dichloromethane

Experimental: 2-(1-phosphonomethyl) guanidino)ethanoic acid (2mmole, 0.338g) was dissolved in 10mL of dichloromethane. To that trifluoroacetic acid (4mmole, 4mL) was added and the contents of the flask were blended for 1 hour at normal temperature. The progress of the reaction was monitored by TLC and the solvent was vapourised under reduced pressure. The residue obtained was purified in the column using acidic SiO_2 and was placed in dessicator containing P_2O_5. Progress of the reaction was regularly checked on readymade silica gel plates using Ethyl ethanoate and Hexane as a solvent system.

Characterization was made using FTIR, ^{1}H NMR and MS. The IR spectra were recorded by Schimadzu spectrophotometer, ^{1}H NMR spectra were recorded in D_2O with Trimethyl silane as internal standard using Bruker Advance II instrument at 400MHz and Mass

spectra were recorded on Waters Q-TOF Micro equipped with electronspray ionisation (ESI) and atomspheric-pressure chemical ionization (APcI) Source.

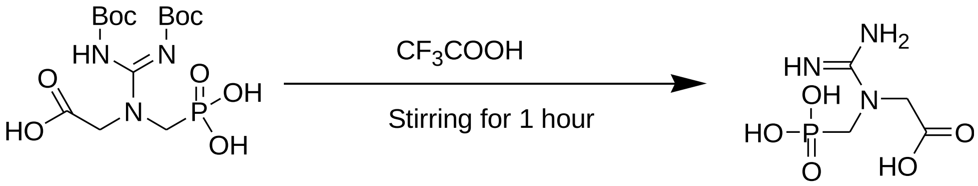


Figure 5.6 Synthesis of 2-(1-phosphonomethyl) guanidino)ethanoic acid

5.2.6 Synthesis of thioxylated ester derivatives of glyphosate: A series of thioxylated ester derivatives of glyphosate were prepared by refluxing the ester derivatives of glyphosate with phosphorus pentasulphide in dioxane as a solvent by the method given by Polshettiwar et al. [146,147]. In this method thioxo bond was introduced into the ester derivatives of glyphosate by using P_4S_{10}/Al_2O_3 as a reagent. Al_2O_3 act as a solid support as well as a catlayst in the reaction medium. It can be easily removed by filtration and do not involve any aqueous workup procedures.

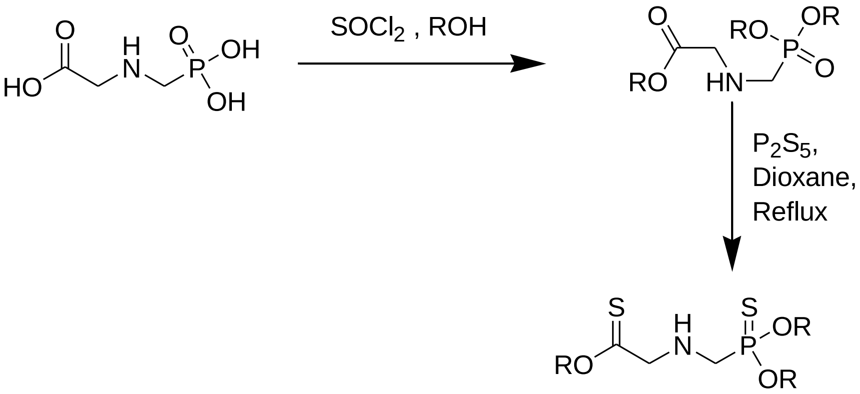
Reagents : Esters of glyphosate (Methyl, Ethyl, Isopropyl and Butyl), Phosphorus pentasulfide, Aluminium oxide

Solvent: Dioxane

Experimental: Ester derivatives of glyphosate (10mmol) were dissolved in minimum quantity of water. To that of Al_2O_3 and P_4S_{10} were added. 30mL of dioxane was mixed with the reaction mixture and the contents of the flask were refluxed for 4 hours. The reagent P_4S_{10}/Al_2O_3 was prepared by mixing P_4S_{10} (6g) with alumina Al_2O_3(basic, 10g) in a mortar and pestle and grinding it until a fine homogeneous powder was obtained. After finishing the reaction, the reagent (P_4S_{10}/Al_2O_3) was removed by filtration using G-3 crucible on suction pump. The solvent (filtrate) was vapourised by using rotaevaporator and coarse product was refined using silica gel column having Methanol: Chloroform solvent system.

Characterization was made using FTIR, ^{1}H NMR and MS. The IR spectra were recorded by Schimadzu spectrophotometer, ^{1}H NMR spectra were recorded in D_2O with Trimethyl silane as internal standard using Bruker Advance II instrument at 400MHz and Mass

spectra were recorded on Waters Q-TOF Micro equipped with electronspray ionisation (ESI) and atomspheric-pressure chemical ionization (APcI) Source.



Where R= CH_3, C_2H_5, CH $(CH_3)_2$, $CH_2CH_2CH_2CH_3$

Figure 5.7 Synthesis of thioxylated ester derivatives of glyphosate

5.2.7 Synthesis of Metal Complexes of Glyphosate:

5.2.7.1 Synthesis of Glyphosate-Metal(II) Complexes: Glyphosate-Metal (II) complexes were synthesized by dissolving glyphosate (0.338g, 3×10^{-4}mmole) and (0.1298g, 1.2×10^{-4} mmole of Co^{2+}salt, 0.219g,2.1×10^{-4}mmole of Zn^{2+} salt, 0.1996g,$.9\times10^{-4}$mmoel of Cu^{2+}salt and 0.262g,2.6×10^{-4}mmole of Ni^{2+} salt) in 50 mL of distilled water in a round bottom flask. 5mL of 0.05M NaOH solution was then added to it. The flask containing the reaction mixture was actuated on magnetic stirrer (at 100 rpm) for 6 h at normal temperature as described by Subramaniam et al[148]. The product obtained was filtered, washed (with water and methanol). Solid products were dehydrated at 45°C for 72 h and kept in desiccator before the spectral analysis.

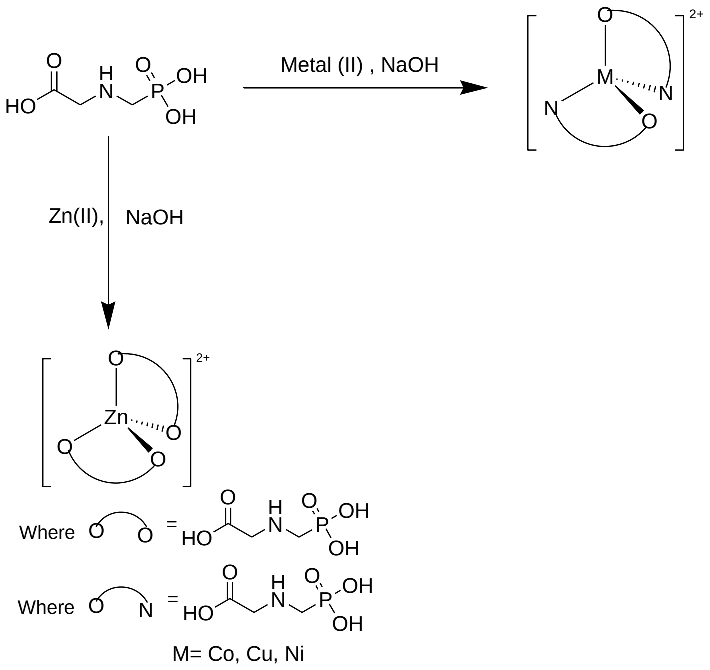


Figure 5.8 Synthesis of metal (II) complexes of glyphosate

5.2.7.2 Synthesis of Glyphosate-Fe(III) Complex: Glyphosate-Fe (III) complex was synthesized by dissolving glyphosate (1.97g, 1.97×10^{-3}mmole) and Fe salt (0.162g,1.62×10^{-4} mmole) in 50mL of double distilled water in a round bottom flask. 5mL of 0.05M NaOH solution was then added to it. The flask containing the reaction mixture was actuated on magnetic stirrer (at 100 rpm) for 6 h at normal temperature. The product obtained was filtered, washed (with water and methanol). Solid products were dehydrated at 45°C for 72 h and kept in desiccator before the spectral analysis.

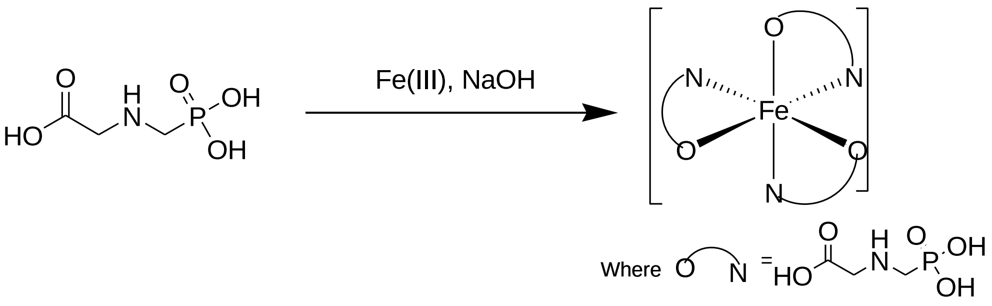
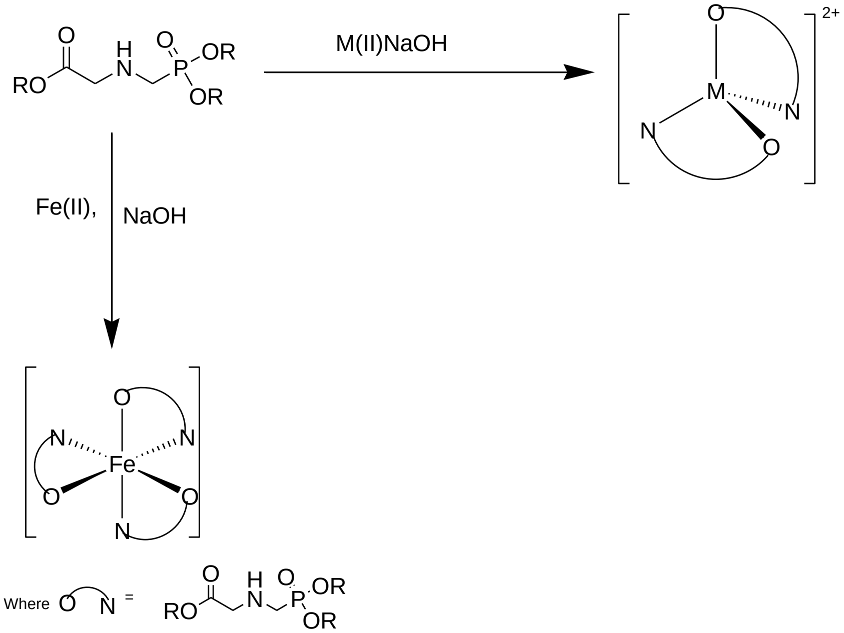


Figure 5.9 Synthesis of Fe(III) complexes of glyphosate

5.2.8 Synthesis of Metal Complexes of Glyphosate Esters: Similar procedure as above was used for the synthesis of different ester derivatives of glyphosate- metal complexes.



M= Zn, Co, Cu,Ni

R= CH_3, C_2H_5, $CH(CH_3)_2$,$CH_2CH_2CH_3$, $CH_3CH_2CH_2CH_2$

Figure 5.10 Synthesis of ester derivatives of glyphosate-Metal Complexes

5.3 HERBICIDAL ACTIVITY OF SYNTHESIZED GLYPHOSATE DERIVATIVES:

5.3.1 Test Plant: Herbicidal activity of the synthesized derivatives of glyphosate was checked on the commonly grown weeds, *Parthenium hysterophorus* and *Cyperus rotundus* and Wheat grass. Herbicidal activity was confirmed by using the method as given by Asad Shabbir[149].

5.3.2 Procedure:

Seeds of *Parthenium hysterophorus* and *Cyperus rotundus* were collected from wild population naturally grown around wild areas of Kapurthala, Punjab. The viability of seeds was checked in the laboratory, where the seeds were set to germinate in pre-sterilized Petri plates and exhibited 90 % germination. Poly bags were filled with 2 kg sandy loam soil. Ten seeds each of *Parthenium hysterophorus* and *Cyperus rotundus* were implanted in every pot and were watered regularly at field capacity. Two weeks after sowing, when maximum germination had been attained, the numbers of plants were reduced to have a uniform density. Three different concentrations of each derivative and the herbicide glyphosate (as negative control) were selected [1 X (recommended dose 441g/L per acre), 0.5 X (half of recommended dose) and 0.25 X (one-fourth of recommended dose)]. Each treatment was replicated three times. The required amount of each compound was applied to all bags except the control (Distilled water). A simple hand sprayer was used to apply the compounds on the plants. The plants foliage was sprayed sufficiently so that it is completely wet with the compounds. The experiment was carefully watched on daily basis for the development of any symptoms (necrosis, wilting and complete death of plant). Leaf samples were collected for the analysis of 'chlorophyll a' (Chl a), 'chlorophyll b' (Chl b)'and' total chlorophyll' (Chl a+b) content. Similar procedure was repeated to check the herbicidal activity of these synthesized derivatives on Wheat (*Triticum aestivum*).

5.3.3 Determination of Chlorophyll content:

Total chlorophyll content (Chl a+b) in the leaves of plants was measured by using the technique given by Lichtenthaler (1987) [150]. Chlorophyll content was measured from the leaves of treated plants. 'Chlorophyll a' and 'chlorophyll b' were measured from the extracts of treated plants at wavelength λ_{663} and λ_{645} nm using spectrophotometer. About 5 g of fresh, clean treated leaves were homogenized in 10 mL of extraction solution (10mL of 0.1N NH_4OH + 90 ml of acetone). The plant matter was carefully grounded (using pestle and mortar) in darkness and cold conditions to prepare fine slurry of tissue sample. The extracts were filtered and centrifuged at 5000 rpm for 20 minutes. After collecting supernatant, it was diluted with aqueous acetone to make final volume of 20 ml.

'Chl a', 'Chl b'and 'Chl a+b' content were determined by using formulae[150].

Chlorophyll a (µg/mL) = $12.70\cdot A_{663} - 2.69\cdot A_{645}$

Chorophyll b (µg/mL) = $22.90\cdot A_{645} - 4.68\cdot A_{663}$

Total Chlorophyll (µg/mL) = $20.21\cdot A_{645} + 8.02\cdot A_{663}$[151].

5.4 ANTIMICROBIAL ACTIVITY OF SYNTHESIZED GLYPHOSATE DERIVATIVES:

The antimicrobial activity of the synthesized glyphosate derivatives was checked using Kirby-Bauer disc diffusion method by measuring the diameter of zone of inhibition[152].

5.4.1 Microbial Cultures:

Bacterial Cultures:

Pseudomonas fulva (Gram negative) (Accession Number- MF 782684), *Pseudomonas putida* (Gram negative) (Accession Number- MF 782681)

After procuring the bacterial isolates from Biotechnology department of Lovely Professional University Phagwara (Punjab), the cultures were revived on Muller Hinton Agar Media (MHA). Further these cultures were inoculated into liquid media (Nutrient broth) and were used for in-vitro study.

Fungal Cultures:

Aspergillus fumigatus (NCIM- 902), *Candida albicans* (MTCC-183)

The fungal isolates were procured from the Biotechnology department of Lovely Professional University Phagwara (Punjab). These isolates were revived on Potato Dextrose Agar (PDA). Further these cultures were inoculated into liquid media (Potato Dextrose Broth) and were used for in- vitro study.

5.4.2. Reagents:

- Muller Hinton Agar Media (MHA) - To prepare the media, 33.9 g of Muller Hinton Agar (Hi Media) was dissolved in 1000mL of distilled water in a conical flask. The contents of the flask were sterilized by autoclaving them at 15 psi at 121°C for 15 minutes. After autoclaving, media was poured into petriplates (~ 25mL/plate).
- Potato Dextrose Agar (PDA)- 39 g of Potato Dextrose Agar (Hi media) was mixed in 1000mL of distilled water in a conical flask. The contents of the flask were sterilized by autoclaving them at 15 psi at 121°C for 15 minutes. After autoclaving the media, it was poured into petriplates (~ 25mL/plate).

5.4.3. Procedure:

Media agar plates (MHA for Bacterial strains and PDA for fungal strains) were prepared as discussed above. Microbial cultures were inoculated on the plates by the spread plate method. Paper discs (Whatman filter paper No.1) of diameter approximately 6 mm were soaked in synthesized derivatives of glyphosate (Different concentrations of 1000 ppm, 500 ppm and 250 ppm) and were placed in agar plates. Discs were placed in such a way that they should be in complete contact with the agar surface and should not be closer than 25 mm from each other. The plates containing bacterial isolates were then incubated at 37°C and fungal plates at 25-28°C in biological incubator. After incubation each plate was examined and the zone of inhibition was measured.

5.5 MICROBIAL DEGRADATION OF SYNTHESISED GLYPHOSATE DERIVATIVES :

Microbial degradation of synthesised derivatives of glyphosate was performed by minor modifications in the method given by Fan et al.[153]

5.5.1. Reagents:

Glyphosate (Technical Grade 95.10%), Minimal salt media (MSM), Synthesised derivatives of glyphosate (Ethyl ester of glyphosate, Thioxylated ethyl ester of glyphosate)

Solvent: Ethyl acetate (AR)

5.5.2 Microbial Cultures:

Actinomyces sp. (Gram positive) (Accession Number-KJ 854403.1)

After procuring the bacterial isolates from Biotechnology department of Lovely Professional University Phagwara (Punjab), the cultures were revived in Nutrient broth and were used for degradation studies.

5.5.3 Procedure:

In 250 mL of minimal salts media having 1000 ppm of glyphosate, 100µL of bacterial culture was inoculated. Growth parameters and degradation of glyphosate procedure was repeated for the degradation of other synthesized derivatives of glyphosate.

5.5.4 Sample preparation for ESI-MS analysis:

Spent media (about 100 mL) was centrifuged at 5000 rpm followed by its filtration through filter paper (Whatmann 1). The filtrate was then extracted with an equivalent amount of ethyl ethanoate for three times. The organic layer (Ethyl ethanoate layer) was then air dried and the filtrate left behind was dissolved in least volume of water (250 mL) and was analyzed in mass spectrophotometer.

5.6 INSECTICIDAL ACTIVITY OF SYNTHESIZED GLYPHOSATE DERIVATIVES:

Insecticidal activity of the synthesised derivatives of glyphosate were checked on *Drosophila melanogaster*.

5.6.1 Test organism:

Drosophila melanogaster (male, female) is well known as fruit fly or vinegar fly. It pertains to the family Drosophilidae. These flies are commonly used for conducting different experiments in research because they have high breeding rate, have only four pairs of chromosomes and eggs are produced in a huge amount. They are used as model insects to conduct various insecticidal and toxicological studies.

Drosophila melanogaster (Both male and female) flies were procured from the Zoology department of Lovely Professional University Phagwara (Punjab),

5.6.2 Media and Reagents:

Agar (2.73g), Dextrose (30.3g), Sucrose (15.2g), Brewer's yeast (9.1g), Corn meal (50.4g), Distilled water (500mL), Phosphoric acid (3µL), Propionic acid (3µL).

5.6.3 Procedure:

Parental *Drosophila melanogaster* flies were obtained from running cultures for producing assay flies. Flies coming ou from the eggs were gathered from the parental flies and were alienated after 4 hour interval. These flies were maintained in pre labeled holding vials containing standard corn media in BOD incubator (23±3°C) until the further use of glyphosate derivatives to check their activity. Ten flies of two populations (male and female) were transferred into each vial containing corn media mixed with three different concentrations (10ppm, 5ppm and 2.5ppm) of glyphosate and its synthesized derivatives[154].

5.7 TOXICOLOGICAL STUDIES OF SYNTHESISED GLYPHOSATE DERIVATIVES:

5.7.1 Test Organism:

Eisenia fetida (Earthworm), a lumbricoid was taken as the test species. The wobblers were procured from the vermiculture unit of Zoology department, Lovely Professional University, Phagwara.

5.7.2 Reagents:

The substrate used for experimentation was artificial soil (AS) which was made by mixing industrial quartz sand, kaolin clay and finely ground sphagnum peat in the ratio 7: 2:1 as described by OECD[155]. But sphagnum peat is not readily present in tropical countries and hence coco peat was used as a substitute.

5.7.3 Procedure:

The toxicological analysis was performed on the instructions given by the organization for economic co-operation and development (OECD)[155] which were there after adopted by international organization for standardization (ISO)[156] . Rectangular plastic trays of size 42×28 cm were filled with artificial soil (dry). The total weight of each tray was kept 1 kg. The synthesized glyphosate derivatives were taken as 10 mg/kg, 20 mg/kg and 40 mg/kg and mixed uniformly with artificial soil. 10 mature adult earthworms (300-500 mg net weight) having developed clitella were added in the artificial soil for toxicological tests. The moisture content was preserved to 60–70% all through the study period by regular spraying of adequate amount of water and was covered with jute mats. The test was carried out for a total of 14 days to check mortality. The experiment was carried out in triplicates.

CHAPTER-6

RESULTS AND DISCUSSIONS

6.1 EXTRACTION OF GLYPHOSATE FROM ITS COMMERCIALLY AVAILABLE FORMULATION:

FTIR (KBr cm^{-1})**:** The FTIR spectrum of the extracted glyphosate showed the following peak assignments.

Table 6.1 IR Absorption bands and their assignment for the extracted glyphosate

Absorption bands (cm-1)	**Band assignment for extracted glyphosate**
3016	NH^{2+} stretching
2827	CH_2 groups
2536	COOH
2409	NH^{2+} H-bonded
1732	COOH H-bonded
1558	NH^{2+} deformation
1483	NH^{2+} deformation
1425	CO, OH group
1334	CH_2 deformation
1269	PO_3H- group
1244	CH_2 group
1091	P-O-
1030	CCNC skeletal Vibration
979	P-OH
916	CCNC skeletal Vibration, CH_2 deformation
862	COH deformation
831	P-OH deformation
794	PO_3H- group
779	C-C skeletal vibration
646	COO- deformation

^{1}H NMR (400 MHz, D_2O): 3.15-3.19 (2H, d, J= 16Hz, HOOC$\underline{CH_2}$NH), 3.93 (2H, s, HN$\underline{CH_2}$PO$(OH)_2$) (Figure 6.1)

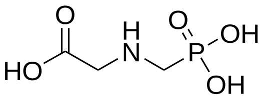


Figure 6.1 Glyphosate molecule

^{13}CNMR (400 MHz D_2O)**:** 43.08, 44.46, 48.69, 48.79, 169.05

6.2 SYNTHESIS OF GLYPHOSATE DERIVATIVES:

6.2.1 Synthesis of Ester derivatives of glyphosate:

6.2.1.1 Methyl 2- {[(dimethoxyphosphoryl)methyl]amino}acetate hydrochloride :

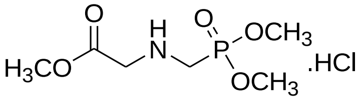


Figure 6.2 Structure of Methyl 2- {[(dimethoxyphosphoryl)methyl]amino}acetate hydrochloride

Molecular formula: $C_6H_{14}NO_5P$, Yield: 57.03%, Brown oily liquid

FTIR (KBr cm^{-1})**:** 3394 (-NH stretching), 2962 (CH stretching), 1745 (C=O stretching), 1627 (-NH bending), 1437 ($-CH_2$ bending), 1377 ($-CH_3$ bending), 1242 (C-O-C stretching), 1193 ($P-O-CH_3$ stretching), 1055(C-N stretching), 1010 (P-O-C stretching), 949 (P-O-C rocking), 773 (P-O-C stretching), 719 (P-C stretching)

^{1}H NMR (400 MHz, D_2O): 3.16-3.20 (6H, d, J=12.84Hz, $OP(O\underline{CH}_3)_2$), 3.69 (2H, s,$OC\underline{CH}_2NH$), 3.94 (2H, s, $HN\underline{CH}_2PO$), 3.98 (3H, s, $\underline{CH}_3OCO$)

^{31}P NMR (500 MHz, D_2O): 9.1

ESI-MS: 243 (M+1)

6.2.1.2 Ethyl 2-{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride:

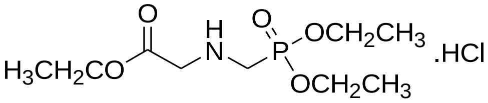


Figure 6.3 Structure of Ethyl 2- {[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride

Molecular formula: $C_9H_{20}NO_5P.HCl$, yield: 59.03%, Brown oily liquid,

FTIR (KBr cm^{-1}): 3412 (-NH stretching), 2987 (-CH stretching), 1743.71(C=O stretching), 1627 (-NH bending), 1429 ($-CH_2$ and $-CH_3$ deformations), 1381 (CH_2 bending) 1356($-CH_3$ bending), 1300 (P=O stretching), 1238 (C-O-C stretching), 1203 (C-N stretching), 1018 (P-O-C stretching), 952 (P-O-C rocking), 856 (P-O-C stretching)

^{1}H NMR (400 MHz, D_2O): 0.99-1.03(6H, t, J=7.12Hz, $OPO(CH_2)_2(\underline{CH}_3)_2$), 1.12-1.15 (3H, t, J= 7.08Hz, $\underline{CH}_3CH_2O$), 3.18-3.21(2H, d, J=12.96Hz, $CO\underline{CH}_2NH$), 3.46-3.51 (4H, q, J=7.08Hz,$OPO(\underline{CH}_2)_2(CH_3)_2$), 3.94-3.96 (2H, d, J=7.84Hz, $HN\underline{CH}_2OPO$), 4.13-4.18 (2H, q, J=7.12Hz, $CH_3\underline{CH}_2O$)

^{31}P NMR (500 MHz, D_2O): 9.3

ESI-MS: 254 (M+1)

6.2.1.3 Isopropyl 2- ({[diisopropoxyphosphoryl] methyl}amino) acetate hydro-chloride:

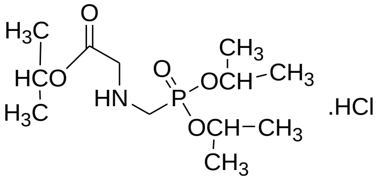


Figure 6.4 Structure of Isopropyl 2- ({[diisopropoxyphosphoryl] methyl}amino) acetate hydrochloride

Molecular formula: $C_{12}H_{26}NO_5P$, yield: 49.40%, Brown oily liquid

FTIR (KBr cm^{-1})**:** (-NH stretching), 2965 (-CH stretching), 2945 (-CH stretching), 2848 (CH_2 symmetric stretching), 2823 (C-H stretching), 2353 (C-H stretching), 1737 (C=O stretching), 1627 (N-H bending), 1460(-CH_2 bending), 1425 (P-CH_2 deformation), 1383 (CH deformation (Gem dimethyl)), 1242 (C=O stretching), 1188 (P-O-C rocking), 1101 (P=O stretching), 1053 (P-O-C stretching), 1024 (C-O-C stretching) 952 (-P-O-C stretching), 813 (P-O-C stretching), 771 (P-C stretching)

^{1}H NMR (400 MHz, D_2O): 0.99-1.01 (12H, d, J= 6.12Hz,OPO(CH_2)($\underline{CH_3}$)$_4$), 1.13-1.14 (16H, d, J=6.2Hz, OCH($\underline{CH_3}$)$_2$), 3.18-3.23 (2H, distorted dd, J= 13.12 and 3.20Hz, CO$\underline{CH_2}$NH), 3.83-3.86 (2H, m, OPO($\underline{CH_2}$)(CH_3)$_4$), 3.92-3.94 (1H, s, O$\underline{CH}$(CH_3)$_2$), 4.93-4.99(2H,m, HN$\underline{CH_2}$PO)

^{31}P NMR (500 MHz, D_2O): 10.3

ESI-MS: 297 (M+2)

6.2.1.4 Butyl 2-{[(dibutoxyphosphoryl)methyl]amino}acetate hydrochloride:

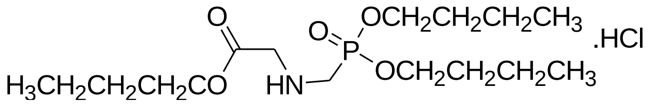


Figure 6.5 Structure of Butyl 2-{[(dibutoxyphosphoryl)methyl]amino}acetate

Molecular formula: $C_{15}H_{32}NO_5P$, yield: 46.53%, Brown oily liquid

FTIR (KBr cm^{-1}): (-NH stretching), 2962 (-CH stretching), 2937 (-CH stretching), 2874,2845 (-CH stretching), 1745 (C=O stretching), 1464 (CH_2 bending), 1423 (P-CH_2 deformation), 1240 (-C-O-C stretching), 1224 (P=O stretching), 1060 (P-O-C stretching), 1016 (C-O stretching), 950 (P-O-C stretching), 719 (P-C stretching)

^{1}H NMR (400 MHz, D_2O): 0.71-0.77(9H, q, J=7.2 Hz, $\mathrm{P(OCH_2CH_2CH_2\underline{C\mathit{H}}_3)_2}$, $\mathrm{COCH_2CH_2CH_2\underline{C\mathit{H}}_3}$), 1.14-1.26 (6H, m, $\mathrm{P(OCH_2CH_2\underline{C\mathit{H}}_2CH_3)_2}$, $\mathrm{COCH_2CH_2\underline{C\mathit{H}}_2CH_3}$)), 1.32-1.37(4H, m, $\mathrm{P(OCH_2\underline{C\mathit{H}}_2CH_2CH_3)_2}$), 1.47-1.54(2H, m, $\mathrm{COCH_2\underline{C\mathit{H}}_2CH_2CH_3}$), 3.19-3.22(2H, d, J=12Hz, $\mathrm{CO\underline{C\mathit{H}}_2NH}$), 3.42-3.45(4H, t, J=6.64Hz, $\mathrm{P(O\underline{C\mathit{H}}_2CH_2CH_2CH_3)_2}$), 3.94-3.96(2H ,d, J=11.08Hz, $\mathrm{NH\underline{C\mathit{H}}_2OP}$), 4.10-4.13(2H, t, J=6.64, $\mathrm{CO\underline{C\mathit{H}}_2CH_2CH_2CH_3}$)

^{31}P NMR (500 MHz, D_2O): 9.1

ESI-MS: 339 (M+2)

6.2.2 Synthesis of amide derivatives of glyphosate:

6.2.2.1 ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid :

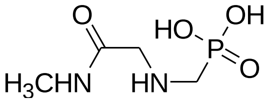


Figure 6.6 Structure of ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid

Molecular formula: $C_4H_{11}N_2O_4P$, yield: 23%, Brown gummy solid

FTIR (KBr cm^{-1}): 3620 (-NH stretching), 2966 (-CH asym stretching), 2871(-CH sym stretching), 2362, 1736 (O=P-OH), 1655 (C=O stretching), 1514 (-NH bending), 1462

(C-H asym bending), 1281 (P=O stretching), 1127 (-CN stretching), 1073 (P-O stretching), 742 (P-C stretching).

^{1}H NMR (400 MHz, D_2O): 0.556 (2H, s, HN$\underline{C\mathit{H}}_2$PO), 2.54 (3H, s, $\underline{C\mathit{H}}_3$NHCO), 3.69 (2H, s, CO$\underline{C\mathit{H}}_2$NH)

6.2.2.2 ({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid:

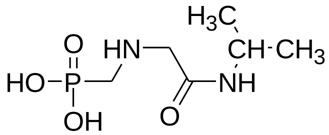


Figure 6.7 Structure of ({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid

Molecular formula: $C_6H_{15}N_2O_4P$, yield: 33%, Colourless liquid

FTIR (KBr cm^{-1}) : 3327 (-OH stretching), 3056 (-NH stretching), 2969 (-CH asym stretching), 2875(-CH sym stretching), 2362 (O=P-OH), 1695 (C=O stretching), 1536 (-NH bending), 1469 (C-H asym bending), 1385 [CH_2 deformation (gem-dimethyl)], 1250 (P=O stretching), 1187 (-CN stretching), 1086 (P-O stretching), 779 (P-C stretching).

^{1}H NMR (400 MHz, D_2O): 0.82-0.86 (6H, dd, J=1Hz, NHCH($\underline{C\mathit{H}}_3$)$_2$), 1.06 (2H, s, OP$\underline{C\mathit{H}}_2$NH), 1.81 (2H, s, NH$\underline{C\mathit{H}}_2$CO), 3.83-3.86 (1H, m,NH$\underline{C\mathit{H}}$($CH_3$)$_2$)

6.2.2.3 ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid:

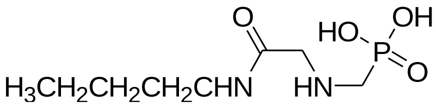


Figure 6.8 Structure of ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid

Molecular formula: $C_7H_{17}N_2O_4P$, yield: 48%, Colourless liquid

FTIR (KBr cm^{-1}): 3334 (-OH stretching), 3068 (-NH stretching), 2959 (-CH asym stretching), 2874(-CH sym stretching), 2340 (O=P-OH), 1698 (C=O stretching), 1537(-NH bending), 1468 (C-H asym bending), 1250 (P=O stretching), 1141 (-CN stretching)1054,966 (P-O stretching),739 (P-C stretching).

^{1}H NMR (400 MHz, D_2O): 0.78- 0.86 (3H, m, $NHCH_2CH_2CH_2\underline{C\mathit{H}_3}$), 1.16-1.28 (2H, m, $NHCH_2CH_2\underline{C\mathit{H}_2}CH_3$), 1.32-1.41 (2H, m, $NHCH_2\underline{C\mathit{H}_2}CH_2CH_3$), 2.53 (2H, s, $NH\underline{C\mathit{H}_2}PO$), 2.98- 3.04 (2H, m, $NH\underline{C\mathit{H}_2}CH_2CH_2CH_3$), 3.54-3.56 (2H,d, J=7.2 Hz, $CO\underline{C\mathit{H}_2}NH$)

ESI-MS: 225 (M+1)

6.2.3 Synthesis of (2-((tert-butoxycarbonyl) (phosphonomethyl)amino)ethanoic acid (Boc-protected glyphosate derivative):

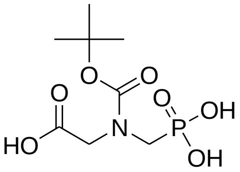


Figure 6.9 Structure of 2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid

Molecular Formula: $C_8H_{16}NO_7P$, yield: 28%, Colourless gummy solid

FTIR (KBr cm^{-1}): 3444 (-OH stretching), 2926 (-CH asym stretching), 2854 (-CH sym stretching), 2623 (O=P-OH), 1730 (C=O stretching), 1440 (C-H asym bending, 1377 [-CH stretching (t-Butyl group)] 1218 (P=O stretching), 1124 (C-O stretching), 1075 (-CN stretching) 741 (P-C stretching).

^{1}H NMR (400 MHz, $CDCl_3$): 1.46 (9H, s,$OC(\underline{C\mathit{H}_3})_3$), 1.96 (2H, s, $OP(\underline{O\mathit{H}})_2$), 3.16- 3.19 (2H, d, J=12.8Hz, $N\underline{C\mathit{H}_2}PO(OH)_2$), 3.90 (2H, s, $N\underline{C\mathit{H}_2}COOH$), 7.61-7.64 (1H, m, $NCH_2CO\underline{O\mathit{H}}$)

ESI-MS: 271 (M+2)

6.2.4 Synthesis of 2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid (Boc-protected guanidine derivative of glyphosate):

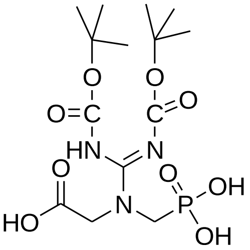


Figure 6.10 Structure of 2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid

FTIR (KBr cm^{-1}): 3308 (-OH stretching), 3118 (-NH stretching), 2927 (-CH asym stretching), 2855 (-CH sym stretching), 1668 (C=O stretching), 1437 (C-H asym bending), 1144 (C-O stretching), 1285 (P=O stretching), 726 (P-C stretching).

^{1}H NMR (400 MHz, D_2O): 1.50-1.76 (18H, m, (OCOC(<u>C*H*</u>$_3$)$_3$)$_2$), 2.54(2H, s, N<u>C*H*</u>$_2$PO), 3.60- 3.69 (2H, dd, J=14.5Hz, N<u>C*H*</u>$_2$COOH)

ESI-MS: 413 (M+2)

6.2.5 Synthesis of 2-(1-(Phosphonomethyl)guanidino) ethanoic acid (Guanidine derivative of glyphosate):

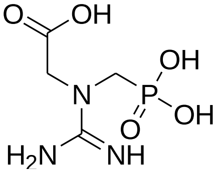


Figure 6.11 Structure of 2-(1-(Phosphonomethyl)guanidino) ethanoic acid

Molecular formula: $C_4H_{10}N_3O_5P$, yield: 14% ,Gummy solid

FTIR (KBr cm^{-1}): 3417 (-NH stretching), 2926 (-CH asym stretching), 2854 (-CH sym stretching), 1665 (C=O stretching), 1437 (C-H asym bending), 1261 (P=O stretching), 725, 604 (P-C stretching).

^{1}H NMR (400 MHz, D_2O): (Racemic mixture) 2.04 (3H, s, <u>N*H*</u>$_2$C, OP(<u>O*H*</u>)$_2$), 2.89 - 2.95(2H, distorted t, J=8.04Hz, N<u>C*H*</u>$_2$PO), 3.90 (2H, s, COOH<u>C*H*</u>$_2$N), 7.61-7.64 (1H, m, NC<u>N*H*</u>), 7.69-7.72(1H, m,CO<u>O*H*</u>)

ESI-MS: 213 (M+2)

6.2.6 Synthesis of thioxylated ester derivatives of glyphosate:

6.2.6.1 *O*-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate:

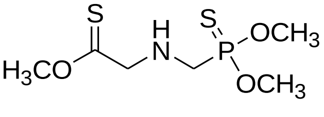


Figure 6.12 Structure of *O*-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate

Molecular formula: $C_6H_{14}NO_3PS_2$, yield: 14%, Brown gummy solid

FTIR (KBr cm^{-1}): 3440(-NH stretching), 2926 (-CH asym stretching), 2855 (-CH sym stretching), 1737 (-CO stretching), 1648 (-NH bending), 1451, 1374 ($-CH_2$ deformation), 1258 (C=S stretching), 1024 (P-O stretching), 801,604 (-PS stretching).

^{1}H NMR (400 MHz, D_2O): 3.16-3.20 (6H, d, J=12.84Hz, $OP(O\underline{C\mathit{H}}_3)_2$), 3.69 (2H, s,$OC\underline{C\mathit{H}}_2NH$), 3.94 (2H, s, $HN\underline{C\mathit{H}}_2PO$), 3.98 (3H, s, $\underline{C\mathit{H}}_3OCO$)

ESI-MS: 243 ($M+H^+$)

6.2.6.2 *O*-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate:

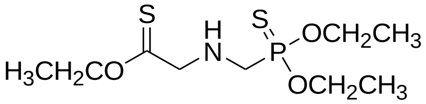


Figure 6.13 Structure of *O*-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate molecule

Molecular formula: $C_9H_{20}NO_3PS_2$, yield: 19%, Brown gummy solid

FTIR (KBr cm^{-1}): 3443(-NH stretching), 2960 (-CH asym stretching), 1730 (-CO stretching), 1640 (-NH bending), 1459 ($-CH_2$ deformation), 1261 (C=S stretching), 1014 (P-O stretching), 813,662 (-PS stretching).

^{1}H NMR (400 MHz, D_2O): 0.99-1.03(6H, t, J=7.12Hz, $OPO(CH_2)_2(\underline{C\mathit{H}}_3)_2$), 1.12-1.15 (3H, t, J= 7.08Hz, $\underline{C\mathit{H}}_3CH_2O$), 3.18-3.21(2H, d, J=12.96Hz, $CO\underline{C\mathit{H}}_2NH$), 3.46-3.51 (4H, q, J=7.08Hz,$OPO(\underline{C\mathit{H}}_2)_2(CH_3)_2$), 3.94-3.96 (2H, d, J=7.84Hz, $HN\underline{C\mathit{H}}_2OPO$), 4.13-4.18 (2H, q, J=7.12Hz, $CH_3\underline{C\mathit{H}}_2O$)

ESI-MS: 285($M+H^+$)

6.2.6.3 *O*-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino) ethanethioate:

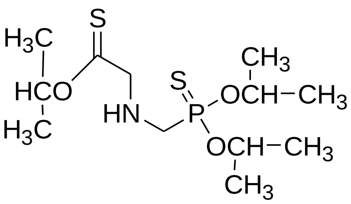


Figure 6.14 Structure of *O*-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino)

Molecular formula: $C_{12}H_{26}NO_3PS_2$, yield: 22%, Brown gummy solid

FTIR (KBr cm^{-1})-8400S: 3442(-NH stretching), 2924 (-CH asym stretching), 2854 (-CH sym stretching), 1729 (-CO stretching), 1648 (-NH bending), 1461 (-CH_2 deformation), 1374 (CH deformation (Gem dimethyl)), 1257 (C=S stretching), 1167 (P=O stretching), 802,663 (-PS stretching).

^{1}**H NMR** (400 MHz, D_2O): 0.99-1.01 (12H, d, J= 6.12Hz,$OPO(CH_2)(\underline{C\mathit{H}}_3)_4$), 1.13-1.14 (16H, d, J=6.2Hz, $OCH(\underline{C\mathit{H}}_3)_2$), 3.18-3.23 (2H, distorted dd, J= 13.12 and 3.20Hz, $CO\underline{C\mathit{H}}_2NH$), 3.83-3.86 (2H, m, $OPO(\underline{C\mathit{H}}_2)(CH_3)_4$), 3.92-3.94 (1H, s, $O\underline{C\mathit{H}}(CH_3)_2$), 4.93-4.99(2H,m, $HN\underline{C\mathit{H}}_2PO$)

ESI-MS: 329(M+2)

6.2.6.4 *O*-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate:

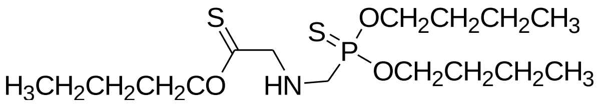


Figure 6.15 Structure of *O*-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate

Molecular formula: $C_{15}H_{32}NO_3PS_2$, yield: 10%, Brown gummy solid

FTIR (KBr cm^{-1})-8400S: 3444(-NH stretching), 2958 (-CH asym stretching), 2924 (-CH sym stretching), 2856, 2364 (-CH stretching), 1727 (-CO stretching), 1646 (-NH bending), 1455 (-CH_2 deformation), 1250 (C=S stretching), 1117 (P-O stretching), 802,671 (-PS stretching).

^{1}**H NMR** (400 MHz, D_2O): 0.71-0.77(9H, q, J=7.2 Hz, $P(OCH_2CH_2CH_2\underline{C\mathit{H}}_3)_2$, $COCH_2CH_2CH_2\underline{C\mathit{H}}_3$), 1.14-1.26 (6H, m, $P(OCH_2CH_2\underline{C\mathit{H}}_2CH_3)_2$, $COCH_2CH_2\underline{C\mathit{H}}_2CH_3$)), 1.32-1.37(4H, m, $P(OCH_2\underline{C\mathit{H}}_2CH_2CH_3)_2$), 1.47-1.54(2H, m, $COCH_2\underline{C\mathit{H}}_2CH_2CH_3$), 3.19-3.22(2H, d, J=12Hz, $CO\underline{C\mathit{H}}_2NH$), 3.42-3.45(4H, t, J=6.64Hz, $P(O\underline{C\mathit{H}}_2CH_2CH_2CH_3)_2$), 3.94-3.96(2H ,d, J=11.08Hz, $NH\underline{C\mathit{H}}_2OP$), 4.10-4.13(2H, t, J=6.64, $CO\underline{C\mathit{H}}_2CH_2CH_2CH_3$)

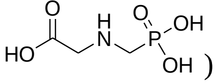
ESI-MS: 369($M+H^+$).

6.2.7 Synthesis of metal complexes of glyphosate:

FTIR (KBr cm^{-1}); The FTIR spectrum of the glyphosate-Metal complexes showed the following peak assignments

Table .6.2 IR peak values for Glyphosate-Metal complexes

Group Vibrations	Band Assignments (cm^{-1})[157]	**HL**	**$[Zn(L)_2]^{2+}$**	**$[Co(L)_2]^{2+}$**	**$[Cu(L)_2]^{2+}$**	**$[Ni(L)_2]^{2+}$**	**$[Fe(L)_3]Cl_3$**
***v* (C=O)$_{COOH}$**	1690-1760	1732	------	1733	1733	1716	1742
***v*(C=O)$_{COO^-}$ asym**	1550-1610	1558	1561	1566	1605	1569	-------
***v*(C=O)$_{COO^-}$ sym**	1300-1420	1334	1334	1333	1420	1333	-------
***v*(N-H)$_{NH_3^+}$**	3030-3300	3016	3075	------	------	-------	3023
***v*(P=O)**	~1150	1159	1161	1165	1156	--------	-------
***v*(O-H)**	3200-3600	------	-------	------	------	--------	3455
***v*(P-OH)**	910-1040	1030	1008	1030	917	980	------

(Where HL= )

The comparative FTIR analysis of glyphosate and its metal complexes have shown variations in the wave number and intensity of stretching bands of the (N-H), (C=O)$_{COOH,}$ (C=O)$_{COO^-}$asym and (C=O)$_{COO^-}$sym (Table.6.2).

The spectrum of $[Zn(L)_2]^{2+}$ complex has shown the absence of (C=O)$_{COOH}$ stretching bands and presence of (C=O)$_{COO^-}$ stretching bands indicating that Zn^{2+} binds the glyphosate molecule through partial double bond between O atoms of carbonyl group of glyphosate molecules. The complex is expected to adopt the tetrahedral geometry.

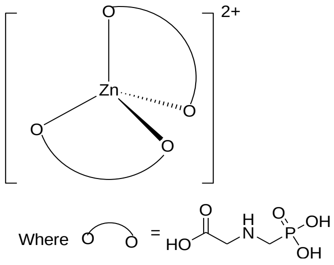


Figure 6.16 Structure of Zn-Glyphosate complex

FTIR spectrums of $[Co(L)_2]^{2+}$, $[Cu(L)_2]^{2+}$ and $[Ni(L)_2]^{2+}$ have shown the presence of *v* (C=O)$_{COOH}$ band and absence of the *v*(N-H) band. Apart from this there is variation in the absorbance peaks of *v*(C=O) $_{COO^-}$asym and *v*(C=O) $_{COO^-}$sym. From this it is expected that the metal ions co-ordinates the glyphosate molecules through O atom of carbonyl group and N atom (by deprotonation of H atom) from the amino group of glyphosate molecule. The expected structure of the complexes is as follows:

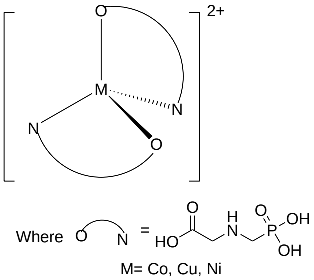


Figure 6.17 Structure of Co-Glyphosate, Cu-Glyphosate and Ni-Glyphosate complexes

FTIR spectra of $[Fe(L)_3]\ Cl_3$ has shown the absence of *v*(C=O) $_{COO^-}$asym and *v*(C=O) $_{COO^-}$ sym absorbance bands. While $(C=O)_{COOH}$ band and ν(N-H) band are present. It is expected that Fe^{3+} co-ordinates the glyphosate molecule through O atom of carbonyl group and lone pair of electrons from N atom of amino group of glyphosate molecule.

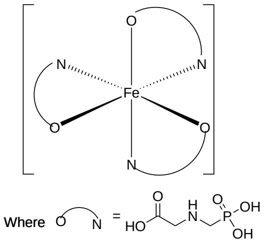


Figure 6.18 Structure of Fe-Glyphosate complex

6.2.8 Synthesis of Metal Complexes of Ester derivatives of glyphosate:

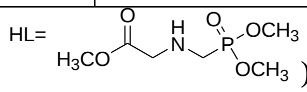
6.2.8.1 Methyl Ester of glyphosate - Metal Complexes:

FTIR (KBr cm^{-1}); The FTIR spectrum of the Methyl Ester of glyphosate-Metal complexes showed the following peak assignments

Table.6.3 IR peak values for Methyl Ester of glyphosate-Metal complexes

Group Vibrations	Band Assignments (cm^{-1}) [158]	**HL**	**$[Zn(L)_2]^{2+}$**	**$[Co(L)_2]^{2+}$**	**$[Cu(L)_2]^{2+}$**	**$[Ni(L)_2]Cl_2$**	**$[Fe(L)_3]\ Cl_3$**
***v* (N-H)**	3300-3500	3394	------	-------	-------	3321	3008
***v* $(C=O)_{COOR}$**	1730- 1750	1745	1735	1734	1733	1733	1743
***v*$(P-O-C)_{POR}$ (outphase)**	920-1088	1055	1088	1092	1092	1092	-------
***v*$(P-O-C)_{POR}$ (inphase)**	725-845	773	778	777	796	829	786

***v*(P=O)**	1230-1260	1242	1245	1245	-------	1245	-------

(Where 
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The spectrum of $[Zn(L)_2]^{2+}$,$[Co(L)_2]^{2+}$ and$[Cu(L)_2]^{2+}$ complexes have shown the absence of *v* (N-H) band indicating that Zn^{2+} binds the ligand molecules through the O atom of carbonyl group of ester moiety and N atom of amino group of glyphosate molecule. The complexes are expected to adopt the tetrahedral geometry.

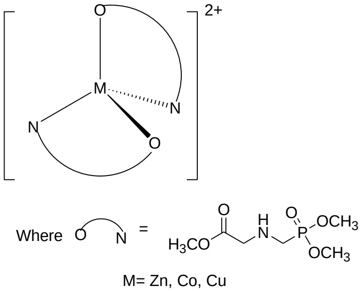


Figure 6.19 Structure of Zn-Methyl ester of glyphosate, Co- Methyl ester of glyphosate and Cu- Methyl ester of glyphosate complexes

The FTIR spectrum of $[Ni(L)_2]Cl_2$ complex have shown the presence of both N-H band and $(C=O)_{COOR}$ band. It is expected that Ni co-ordinates the ligand molecules through O atom of carbonyl and N atom of amino group of the ester derivative of glyphosate molecules. The expected geometry of the complex is as shown below:

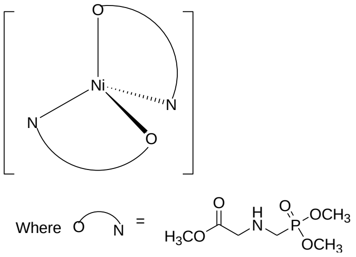


Figure 6.20 Structure of Ni-Methyl ester of glyphosate complex

For $[Fe(L)_3]$ Cl_3 complex, both *v* (N-H) band and *v* $(C=O)_{COOR}$ absorption bands are present. It is expected that the complex formed is neutral and the co-ordination of the Fe^{3+} ion to the ligand occurs through the O atom of carboxyl and N atom of amino group of the ligand and adopted the octahedral structure.

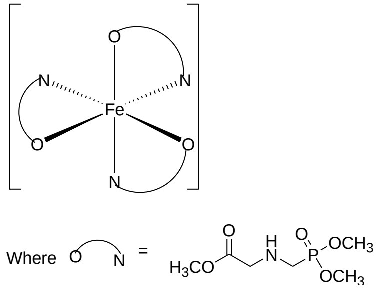


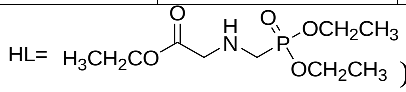
Figure 6.21 Structure of Fe-Methyl ester of glyphosate complex

6.2.8.2 Ethyl Ester of glyphosate-Metal Complexes:

FTIR (KBr cm^{-1}); The FTIR spectrum of the Ethyl Ester of glyphosate-Metal complexes showed the following peak assignments.

Table 6.4 IR peak values for Ethyl Ester of glyphosate-Metal complexes

Group Vibrations	Band Assignments (cm^{-1})[158]	HL	[Zn(L)$_2$]	[Co(L)$_2$]	[Cu(L)$_2$]	[Ni(L)$_2$]	[Fe(L)$_3$]$^{3+}$
***v* (N-H)**	3300-3500	3412	3555	3399	3481	3285	-----
***v* (C=O)$_{\mathbf{COOR}}$**	1730- 1750	1743	1716	1728	1728	1733	1734
***v*(P-O-C)$_{\mathbf{POR\ (outphase)}}$**	920-1088	1018	1030	1030	1030	1030	-------
***v*(P-O-C)$_{\mathbf{POR\ (inphase)}}$**	725-845	856	830	830	830	829	775
***v*(P=O)**	1230-1260	1238	1245	1246	1245	1245	-------

(Where )

The FTIR spectrum of complexes of Ethyl ester derivative of glyphosate with metal ions (Zn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+}) have shown the presence of both N-H band and (C=O)$_{COOR}$ band. It is expected that these complexes are neutral in which the metal ions co-ordinates the ligand molecules through O atom of carbonyl and N atom of amino group of the ligand. The expected geometry of the complexes is as shown below:

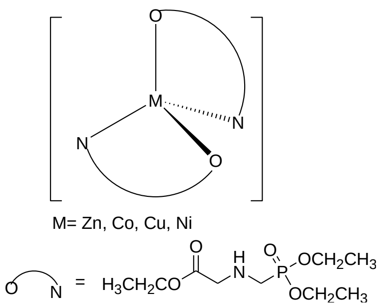


Figure 6.22 Structure of Zn-Ethyl ester of glyphosate, Co-Ethyl ester of glyphosate, Cu-Ethyl ester of glyphosate and Ni-Ethyl ester of glyphosate complexes

From the FTIR spectra of $[Fe(L)_3]^{3+}$ complex, it has been shown that the C=O stretching band is present while NH stretching absorption band is absent. In this complex it is expected that the Fe co-ordinates the ligand molecules through N atom (by deprotonation of H atom) from the amino group and O- atom of carbonyl group and adopted octahedral structure for the anionic complex.

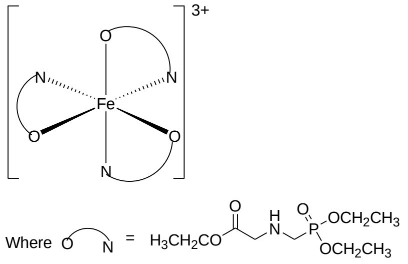


Figure 6.23 Structure of Fe-Ethyl ester of glyphosate complex

6.2.8.3 Isopropyl Ester of glyphosate-Metal Complexes:

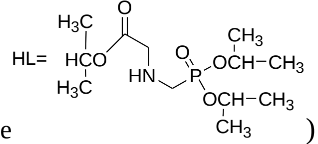
FTIR (KBr cm^{-1}): The FTIR spectrum of the Isopropyl Ester of glyphosate-Metal complexes showed the following peak assignments.

Table 6.5 IR peak values for Isopropyl Ester of glyphosate-Metal complexes

Group Vibrations	Band Assignments $(cm^{-1})^{158}$	**HL**	**$[Zn(L)_2]$**	**$[Co(L)_2]$**	**$[Cu(L)_2]$**	**$[Ni(L)_2]$**	**$[Fe(L)_3]^{3+}$**
ν (N-H)	3300-3500	3400	------	------	------	--------	3012
ν $(C=O)_{COOR}$	1730- 1750	1737	1733	1736	1731	1733	1735
ν(P-O-C)$_{POR\ (outphase)}$	920-1088	1053	1030	1030	1030	1030	1031
ν(P-O-C)$_{POR\ (inphase)}$	725-845	813	830	829	830	829	776

ν(P=O)	1230-1260	1242	------	1244	1245	1245	1244

(Where



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From the FTIR spectras of the complexes of isopropyl ester derivative of glyphosate and metal ions, two types of geometries are expected.

In the complexes with metal ions (Zn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} ions) FTIR spectra have shown the absence of NH absorption bands, which indicates that there occurs deprotonation at the amino group present in the ligand molecule. So the metal ion co-ordinates the ligand molecules through the N- atom of amino group and O-atom of carbonyl group. Expected geometry for these complexes is as follows.

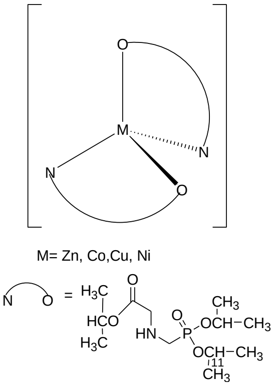


Figure 6.24 Structure of Zn-Isopropyl ester of glyphosate, Co-Isopropyl ester of glyphosate, Cu-Isopropyl ester of glyphosate and Ni-Isopropyl ester of glyphosate complexes

Fe^{3+} ion, co-ordinates the ligand molecules through the lone pairs of N atom of amino group and O-atom of carbonyl group of the ligand. It is expected that the complex adopts the octahedral structure.

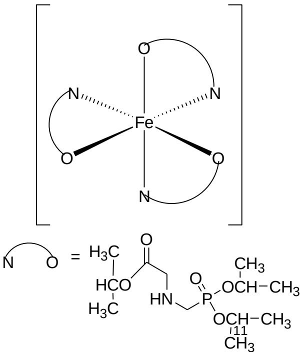


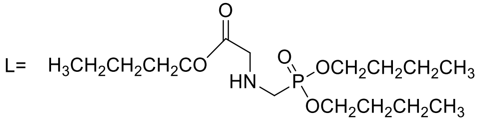
Figure 6.25 Structure of Fe-Isopropyl ester of glyphosate complex

6.2.8.4 Butyl Ester of glyphosate-Metal Complexes:

FTIR (KBr cm^{-1}): The FTIR spectrum of the Butyl Ester of glyphosate-Metal complexes showed the following peak assignments.

Table 6.6 IR peak values for Butyl Ester of glyphosate-Metal complexes

Group Vibrations	Band Assignments (cm^{-1}) [158]	**HL**	**[Cu(L)$_2$]**	**[Ni(L)$_2$]**
***v* (N-H)**	3300-3500	3417	---------------	---------------
***v* (C=O)$_{\mathrm{COOR}}$**	1730- 1750	1745	1713	1729
***v*(P-O-C)$_{\mathrm{POR\ (outphase)}}$**	920-1088	1060	1030	1030
***v*(P-O-C)$_{\mathrm{POR\ (inphase)}}$**	725-845	------	830	830
***v*(P=O)**	1230-1260	1240	-------------	1269

(Where )

In the complexes with metal ions (Cu^{2+} and Ni^{2+} ions) FTIR spectra have shown the absence of NH absorption bands, which indicates that there occurs deprotonation at the amino group present in the ligand molecule. So the metal ion co-ordinates the ligand molecules through the N- atom of amino group and O-atom of carbonyl group. Expected geometry for these complexes is as follows.

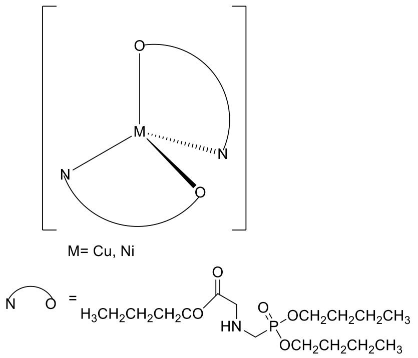


Figure 6.26 Structure of Cu-Butyl ester of glyphosate and Ni-Butyl ester of glyphosate complex

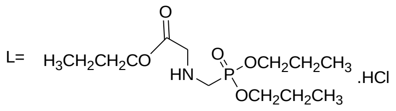
6.2.8.5 Propyl Ester of glyphosate-Metal Complexes:

FTIR (KBr cm^{-1}): The FTIR spectrum of the Propyl Ester of glyphosate-Metal complexes showed the following peak assignments.

Table 6.7 IR peak values for Propyl Ester of glyphosate-Metal complexes

Group Vibrations	Band Assignments (cm^{-1}) [159]	**HL**	**[Cu(L)$_2$]**	**[Ni(L)$_2$]**
***v* (N-H)**	3180-3030	3016	---------------	---------------
***v* (C=O)$_{COOR}$**	1730- 1750	1720	1727	1711
***v*(P-O-C)$_{POR\ (outphase)}$**	920-1088	1030	1031	916
***v*(P-O-C)$_{POR\ (inphase)}$**	725-845	831	830	830
***v*(P=O)**	1230-1260	---------	1241	--------

Where



In the complexes with metal ions (Cu^{2+} and Ni^{2+} ions) FTIR spectra have shown the absence of NH absorption bands, which indicates that there occurs deprotonation at the amino group present in the ligand molecule. So the metal ion co-ordinates the ligand molecules through the N- atom of amino group and O-atom of carbonyl group. Expected geometry for these complexes is as follows.

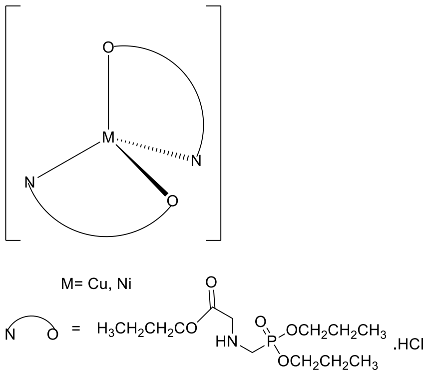


Figure 6.27 Structure of Cu-Propyl ester of glyphosate and Ni-Propyl ester of glyphosate

6.3 HERBICIDAL ACTIVITY OF SYNTHESIZED GLYPHOSATE DERIVATIVES: As glyphosate is a post-emergence herbicide, accordingly the post-emergent herbicidal effects of synthesized derivatives of glyphosate were checked against two the common weeds (*Parthenium hysterophorus*, *Cyperus rotundus*) and common wheat (*Triticum aestivum*). Determination of the Chlorophyll content (Chlorophyll a, chlorophyll b and total chlorophyll) and mortality of plants were used as important parameter to evaluate the detrimental and herbicidal effects of these synthesized derivatives on it. Three different concentrations i.e. [1X (recommended dose 441g/L per acre), 0.5X (half of recommended dose) and 0.25X (one-fourth of recommended dose)] of glyphosate and the synthesized derivatives were applied on the plants. A significant decrease in the chlorophyll a, chlorophyll b and total chlorophyll was observed in all the test concentrations after the application of these synthesized derivatives. A comparison was done on the basis of reduction in the chlorophyll content between the Control (only water), glyphosate and the derivatives. Mortality of the plants was also analysed on the basis of the necrosis and wilting in the plants. Synthesized derivatives had shown moderate to good herbicidal properties at all the test concentrations in comparison to glyphosate.

6.3.1 Herbicidal Activity Of Synthesized glyphosate derivatives on *Parthenium hysterophorus*:

6.3.1.1 Ester Derivatives of glyphosate:

Ethyl 2-{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride (**Ethyl ester of glyphosate**) have shown enhanced herbicidal activity than the glyphosate even on one fourth of the recommended dose after first day of its application. However, the compounds Methyl 2- {[(dimethoxyphosphoryl)methyl]amino}acetate hydrochloride (**Methyl ester of glyphosate**), Isopropyl 2- ({[diisopropoxyphosphoryl] methyl}amino) acetate hydrochloride (**Isopropyl ester of glyphosate**) and Butyl 2-{[(dibutoxyphosphoryl)methyl]amino}acetate hydrochloride (**Butyl ester of glyphosate**) manifested herbicidal activity only on the recommended doses after fifth day of their application. Significant reduction of chlorophyll content in the leaves of *Parthenium hysterophorus* was noticed in all the cases after 15 days.. Use of these ester derivatives of glyphosate on the *Parthenium hysterophorus* had a linear effect in decreasing the content of photosynthetic pigments. In comparison to control (water treatment) and glyphosate, ethyl ester of glyphosate at all the three test concentrations 0.25X. 0.5 X and 1X had shown much enhanced effect in decreasing the chlorophyll content (**Table 6.8**). After 15 days of the treatment, the 'chlorophyll a', was found to be 0.0056 µg/g FW at 0.25X, 0.0023 µg/g FW at 0.5X and 0.005µg/g FW at 1X **(Figure 6.28(a-c))** concentration of ethyl ester of glyphosate in comparison to glyphosate (0.166 µg/g FW at 0.25X, 0.159 µg/g FW at 0.5X and 0.132 µg/g FW at 1X), Similar results were also obtained in 'chlorophyll b' and 'total chlorophyll' as shown in **Table 6.8**. The chlorophyll b in *Parthenium hysterophorus* was also reduced at three different concentrations of ethyl ester of glyphosate, it was found to be 0.106 µg/g FW at 0.25X , 0.086 µg/g FW at 0.5X and 0.007 µg/g FW at 1X **(Figure 6.29(a-c)).** Similar reduction was also observed in 'total chlorophyl'l content [0.203 µg/g FW at 0.25X, 0.196 µg/g FW at 0.5X and 0.130 µg/g FW at 1X **(Figure 6.30(a-c)**]. However other ester derivatives of glyphosate have also reduced the chlorophyll content in comparison to control but in comparison to glyphosate their effect is less pronounced as shown in **Table 6.8**.

Apart from chlorophyll content analysis in the leaves of *Parthenium hysterophorus*, herbicidal activity was also measured on the basis of % of plants dead after the exposure to the ester derivatives of glyphosate. **Figure 6.31** shows the number of plants that become dead after the employment of different concentrations of the synthesized ester derivatives of glyphosate. At 0.25X in comparison to glyphosate, ethyl ester of glyphosate has resulted in the death of maximum number of plants (2 after 5 days, 4 after 10 days and 6 after 15 days after the exposure to derivatives). However with increase in the concentration (0.5X), the number of dead plants has increased (4 after 5days, 6 after 10 days and 8 after 15 days). In comparison to glyphosate at recommended dose (1X) ethyl ester of glyphosate has shown enhanced herbicidal activity, it has killed all the plants after 15 days exposure. With the analysis of above data, it could be concluded that, the rate of killing of plants is more in case of ethyl ester of glyphosate (6 after 5days, 9 after 10 days and 10 after 15 days).

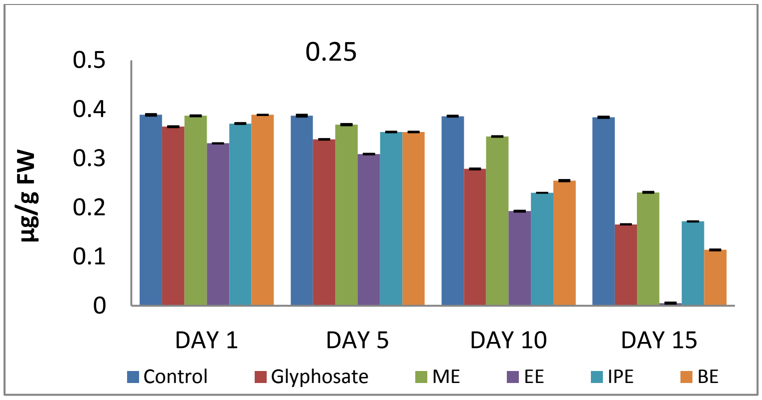


Figure (a)

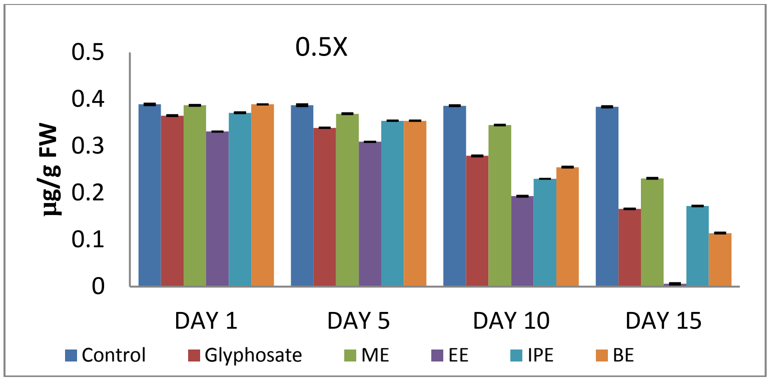


Figure (b)

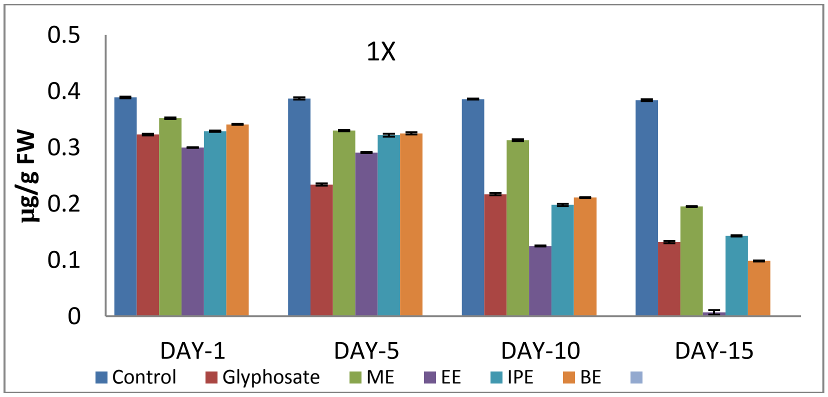


Figure (c)

Figure 6.28 (a)-(c) Effect of glyphosate and synthesized ester derivatives of glyphosate on'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

ME-Methyl ester of glyphosate; EE- Ethyl ester of glyphosate; IPE-Isopropyl ester of glyphosate; BE- Butyl ester of glyphosate

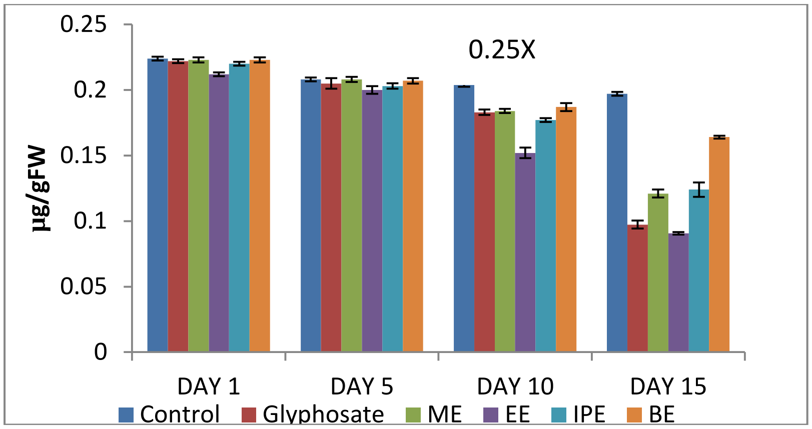


Figure (a)

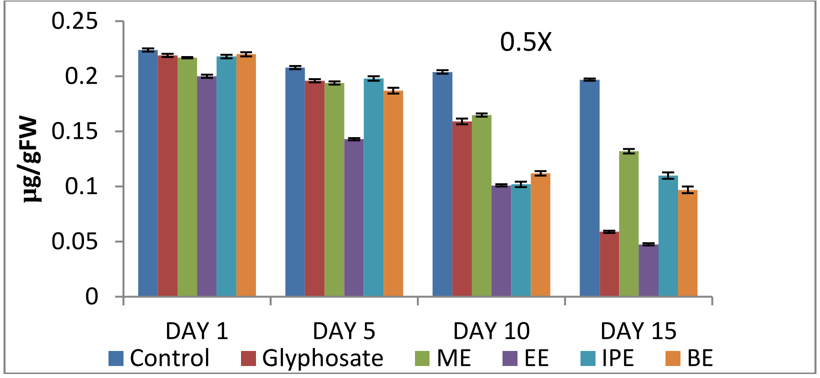


Figure (b)

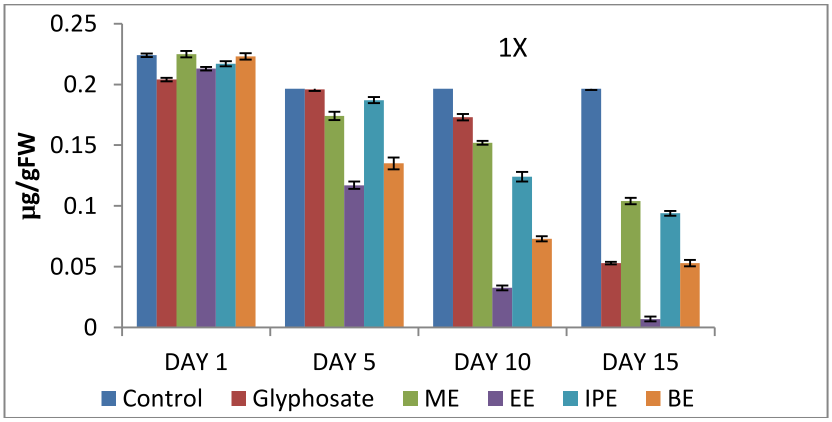


Figure (c)

Figure 6.29(a)-(c) Effect of glyphosate and synthesized ester derivatives of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
ME-Methyl ester of glyphosate; EE- Ethyl ester of glyphosate; IPE-Isopropyl ester of glyphosate; BE- Butyl ester of glyphosate

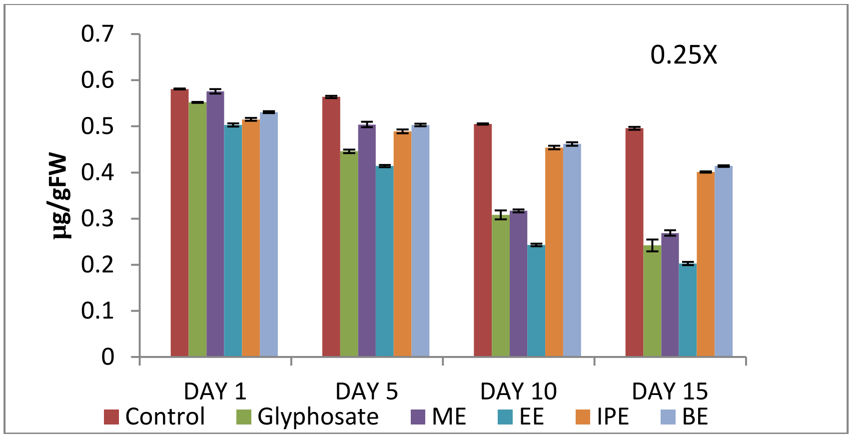


Figure (a)

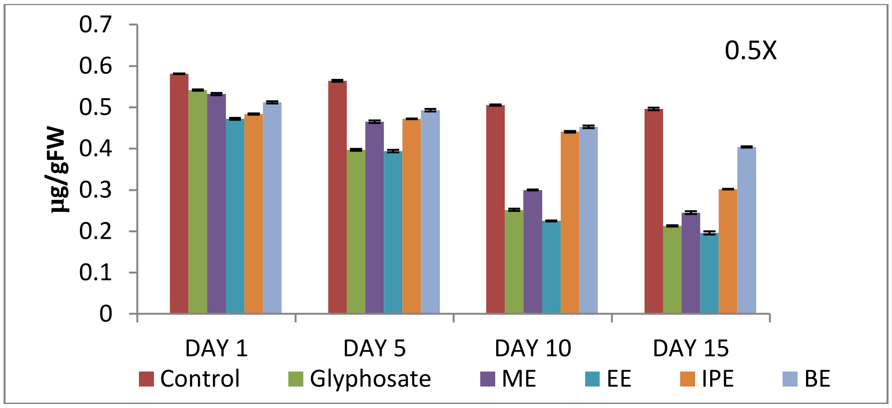


Figure (b)

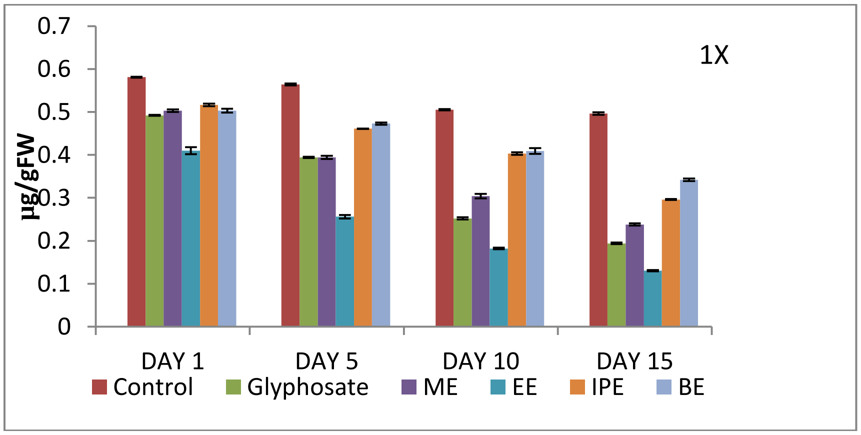


Figure (c)

Figure 6.30 (a)-(c) Effect of glyphosate and synthesized ester derivatives of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

ME-Methyl ester of glyphosate; EE- Ethyl ester of glyphosate; IPE-Isopropyl ester of glyphosate; BE- Butyl ester of glyphosate

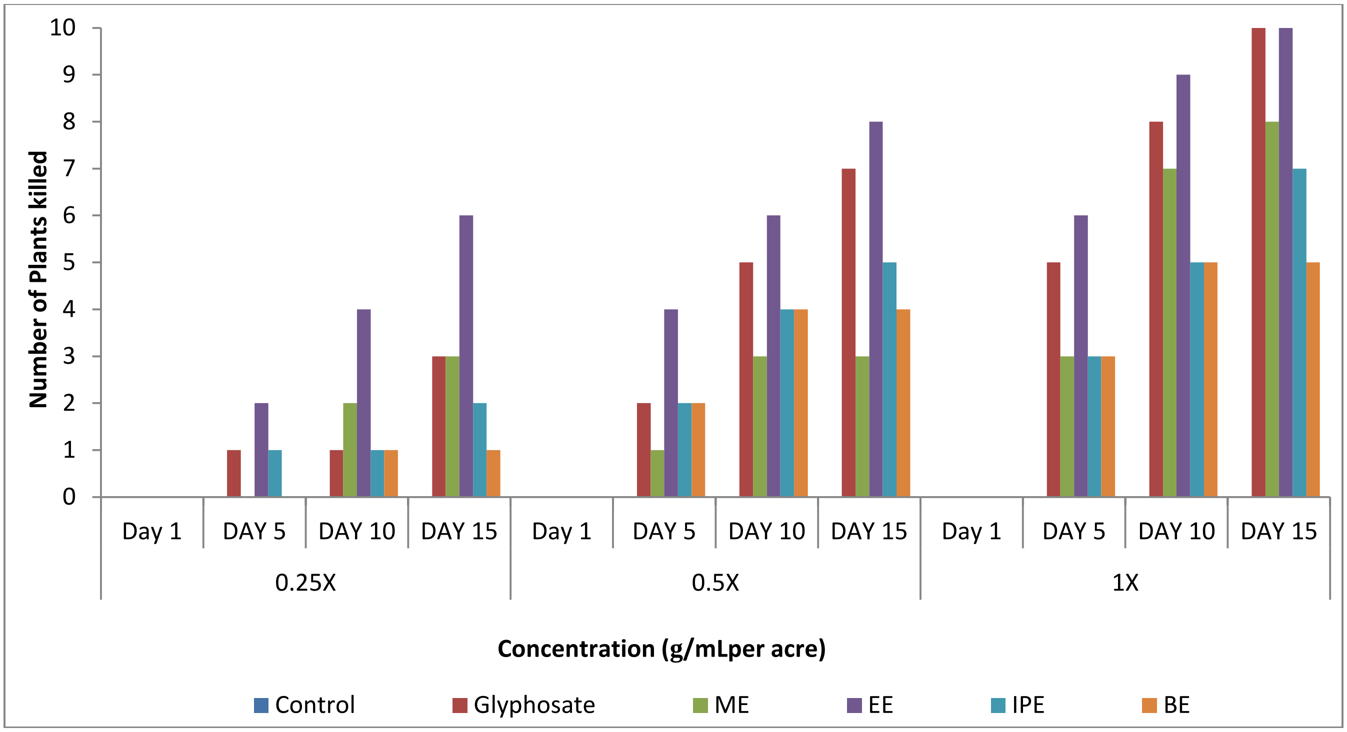


Figure 6.31 Lethal effect of exposure of Ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days Values are mean of 3 (n=3)

(Where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). ME-Methyl ester of glyphosate; EE- Ethyl ester of glyphosate; IPE-Isopropyl ester of glyphosate; BE- Butyl ester of glyphosate

Table 6.8. Effect of synthesized ester derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyl'l content (µg/gFW) in the leaves of *Parthenium hysterophorus*.

Days →		1			5			10			15		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
Chlorophyll a	**Compound**												
	Control	0.389± 0.0015^{a}	0.389± 0.0015 ^{a}	0.389± 0.0015^{a}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}
	Glyphosate	0.365± 0.001^{c}	0.334± 0.002^{e}	0.323± 0.0015^{e}	0.339± 0.00058^{e}	0.242± 0.002^{h}	0.234± 0.0020^{h}	0.279± 0.001^{h}	0.226± 0.0035^{h}	0.217± 0.0020^{i}	0.166± 0.00058^{m}	0.159± 0.001^{j}	0.132± 0.0020^{m}
	Methyl(((dimethylphosphoryl)methyl)amino)acetate hydrochloride	0.387± 0.0010^{b}	0.370± 0.0015^{c}	0.352± 0.0015^{c}	0.369± 0.0011^{c}	0.351± 0.0026^{d}	0.330± 0.0010^{d}	0.345± 0.0005^{e}	0.328± 0.0015^{e}	0.313± 0.0015^{e}	0.231± 0.0010^{j}	0.206± 0.0035^{i}	0.195± 0.001^{l}
	Ethyl 2-(((diethoxyphosphoryl) methyl)amino)acetate hydrochloride	0.331± 0.0005^{f}	0.318± 0.00153^{f}	0.300± 0.0005^{f}	0.309± 0.0005^{g}	0.303± 0.00265^{g}	0.291± 0.001^{g}	0.193± 0.001^{l}	0.153± 0.00153^{l}	0.125± 0.001^{m}	0.0056± 0.00153^{o}	0.0023± 0.0005^{n}	0.007± 0.0041^{o}
	Isopropyl 2-(((diisopropoxyphosphoryl) methyl)amino)acetate hydrochloride	0.371± 0.001^{c}	0.349^{a}± 0.0016^{d}	0.329± 0.001^{d}	0.354± 0.0005^{d}	0.336± 0.001^{e}	0.322± 0.0025^{e}	0.230± 0.000^{j}	0.208± 0.002^{i}	0.198± 0.002^{l}	0.172± 0.0005^{m}	0.163± 0.001^{j}	0.143± 0.001^{m}
	Butyl 2-(((dibutoxyphosphoryl) methyl)amino)acetate hydrochloride	0.389± 0.0004^{a}	0.352± 0.0015^{d}	0.341± 0.001^{d}	0.354± 0.0004^{d}	0.341± 0.001^{e}	0.325± 0.002^{e}	0.255± 0.001^{i}	0.237± 0.0025^{h}	0.211± 0.001^{j}	0.114± 0.001^{n}	0.109± 0.001^{m}	0.0983± 0.001^{n}
Chlorophyll b	**Control**	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.208± 0.0015^{b}	0.208± 0.0015^{c}	0.208± 0.0015^{c}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.197± 0.0015^{d}	0.197± 0.0015^{d}	0.197± 0.0015^{d}
	Glyphosate	0.222± 0.0015^{a}	0.219± 0.0015^{b}	0.204± 0.0015^{c}	0.205± 0.0040^{c}	0.196± 0.0015^{d}	0.196± 0.0015^{d}	0.183± 0.002^{e}	0.159± 0.0026^{g}	0.173± 0.0026^{f}	0.0973± 0.003^{l}	0.059± 0.001^{m}	0.053± 0.001^{n}
	Methyl(((dimethylphosphoryl)methyl)amino)acetate hydrochloride	0.223± 0.002^{a}	0.217± 0.0005^{b}	0.225± 0.0026^{a}	0.208± 0.002^{b}	0.194± 0.0015^{d}	0.174± 0.0034^{f}	0.184± 0.0015^{e}	0.165± 0.0015^{f}	0.152± 0.0015^{g}	0.146± 0.003^{i}	0.132± 0.0020^{i}	0.104± 0.0026^{j}
	Ethyl 2-(((diethoxyphosphoryl) methyl)amino)acetate hydrochloride	0.212± 0.0015^{b}	0.215± 0.0015^{b}	0.213± 0.0015^{b}	0.203± 0.003^{c}	0.143± 0.001^{h}	0.117± 0.0030^{i}	0.177± 0.004^{f}	0.101± 0.001^{j}	0.0326± 0.002^{o}	0.121± 0.001^{j}	0.086± 0.001^{l}	0.007± 0.002^{p}
	Isopropyl 2-(((diisopropoxyphosphoryl) methyl)amino)acetate hydrochloride	0.371± 0.001^{c}	0.349^{a}± 0.0016^{d}	0.329± 0.001^{d}	0.354± 0.0005^{d}	0.336± 0.001^{e}	0.322± 0.0025^{e}	0.230± 0.000^{j}	0.208± 0.002^{i}	0.198± 0.002^{l}	0.172± 0.0005^{m}	0.163± 0.001^{j}	0.143± 0.001^{m}

	Butyl 2-(((dibutoxyphosphoryl) methyl)amino)acetate hydrochloride	0.389 ± 0.0004^{a}	0.352 ± 0.0015^{d}	0.341 ± 0.001^{d}	0.354 ± 0.0004^{d}	0.341 ± 0.001^{e}	0.325 ± 0.002^{e}	0.255 ± 0.001^{i}	0.237 ± 0.0025^{h}	0.211 ± 0.001^{j}	0.114 ± 0.001^{n}	0.109 ± 0.001^{m}	0.0983 ± 0.001^{n}
Total Chlorophyll	**Control**	0.581 ± 0.0010^{a}	0.581 ± 0.0010^{a}	0.581 ± 0.0010^{a}	0.564 ± 0.0025^{b}	0.581 ± 0.0010^{b}	0.564 ± 0.0025^{b}	0.505 ± 0.0015^{d}	0.505 ± 0.0015^{e}	0.505 ± 0.0015^{c}	0.496 ± 0.0030^{d}	0.496 ± 0.0030^{f}	0.496 ± 0.0030^{c}
	Glyphosate	0.552 ± 0.0010^{b}	0.542 ± 0.0015^{c}	0.492 ± 0.0011^{c}	0.446 ± 0.0040^{f}	0.397 ± 0.0020^{j}	0.394 ± 0.0015^{g}	0.308 ± 0.0096^{j}	0.283 ± 0.0025^{n}	0.252 ± 0.0026^{j}	0.242 ± 0.0130^{m}	0.213 ± 0.0015^{p}	0.194 ± 0.0020^{m}
	Methyl(((dimethylphosphoryl)methyl)amino)acetate hydrochloride	0.576 ± 0.0049^{a}	0.532 ± 0.0025^{d}	0.503 ± 0.0030^{c}	0.504 ± 0.0060^{d}	0.465 ± 0.0015^{h}	0.394 ± 0.0035^{a}	0.317 ± 0.0030^{i}	0.416 ± 0.0015^{i}	0.304 ± 0.0051^{i}	0.269 ± 0.0060^{l}	0.396 ± 0.0015^{j}	0.238 ± 0.0025^{l}
	Ethyl 2-(((diethoxyphosphoryl) methyl)amino)acetate hydrochloride	0.503 ± 0.0036^{c}	0.472 ± 0.0025^{g}	0.410 ± 0.0081^{e}	0.414 ± 0.0026^{d}	0.394 ± 0.0030^{j}	0.256 ± 0.0043^{j}	0.243 ± 0.0026^{m}	0.311 ± 0.0010^{m}	0.182 ± 0.0017^{n}	0.203 ± 0.0035^{n}	0.255 ± 0.0037^{o}	0.130 ± 0.0015^{o}
	Isopropyl 2-(((diisopropoxyphosphoryl) methyl)amino)acetate hydrochloride	0.515 ± 0.0032^{c}	0.484 ± 0.0015^{a}	0.516 ± 0.0030^{c}	0.489 ± 0.0043^{d}	0.492 ± 0.0010^{f}	0.461 ± 0.0005^{d}	0.454 ± 0.0037^{e}	0.461 ± 0.0020^{h}	0.403 ± 0.0030^{f}	0.401 ± 0.0015^{h}	0.342 ± 0.0010^{l}	0.326 ± 0.0010^{h}
	Butyl 2-(((dibutoxyphosphoryl) methyl)amino)acetate hydrochloride	0.531 ± 0.0020^{c}	0.512 ± 0.0026^{d}	0.503 ± 0.0047^{c}	0.503 ± 0.0030^{d}	0.493 ± 0.0030^{e}	0.473 ± 0.0025^{d}	0.462 ± 0.0037^{e}	0.463 ± 0.0032^{h}	0.409 ± 0.0070^{c}	0.414 ± 0.0015^{g}	0.414 ± 0.0015^{i}	0.342 ± 0.0030^{h}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).

The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.1.2 Amide Derivatives of glyphosate:

Amide derivatives of glyphosate have also shown the herbicidal effects on the weed *Parthenium hysterophorus*. Significant decrease in the photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) have been observed in all cases with respect to time and concentration as compared to normal control (**Table 6.9**). But this decrease was not comparable to glyphosate. Out of the three different synthesized amide derivatives, **Isopropyl amide derivative of glyphosate** [({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid] is more effective in decreasing the 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' content in the plant at all the three concentrations. At 0.25X concentration, an effective decrease was observed in 'chlorophyll a' (0.292µg/gFW) (**Figure 6.32 (a-c)**), 'chlorophyll b' (0.181µg/gFW) (**Figure 6.33 (a-c**) and 'total chlorophyll' (0.441 µg/gFW) (**Figure 6.34 (a-c)**). However with increase in concentration of isopropyl amide derivative i.e. at 1X concentration, further noticeable drop off in the chlorophyll pigments have been noticed ['chlorophyll a' (0.242 µg/gFW); 'chlorophyll b' (0.145 µg/gFW); 'total chlorophyll' (0.407 µg/gFW)].

Other two amide derivatives also follow the same trend of reduction in chlorophyll content with increase in concentration of the derivative with respect to time. After 15 days of application, a momentous change in the quantity of photosynthetic pigments have been observed in comparison to control. In case of **methyl amide of glyphosate** [({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid] and **butyl amide of glyphosate** [({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid] at 0.25X concentration 'chlorophyll a' found was 0.331µg/gFW and 0.311µg/gFW respectively. While at 1X, 'chlorophyll a' get reduced to 0.274 µg/gFW and 0.260 µg/gFW respectively. Similarly amount of 'chlorophyll b' left at 0.25X (after 15 days) was 0.152 µg/gFW in case of **methyl amide of glyphosate** and 0.173 µg/gFW in case of **butyl amide of glyphosate**. This reduction is more manifested at higher concentration of 1X (**Table 6.9**).

Along with chlorophyll content analysis, a noteworthy enlargement in the number of dead plants was observed with exposure of *Parthenium hysterophorus* to the synthesized amide derivatives of glyphosate. This increase was manifested with increase in the concentration of the derivative and time. At 0.25X, in case **of methyl amide of glyphosate** only 1 plant was dead even after 15 days. At 0.5X, not much change was noticed even after 15 days of treatment. But at 1X, half of the plant population (5 out of 10) was dead. **Isopropyl amide of glyphosate** also followed the similar trend at all the three test concentrations (**Figure 6.35**). However **butyl amide of glyphosate** killed the minimum number of plants (only 2 after 15 days at 1X) and does not show much herbicidal activity.

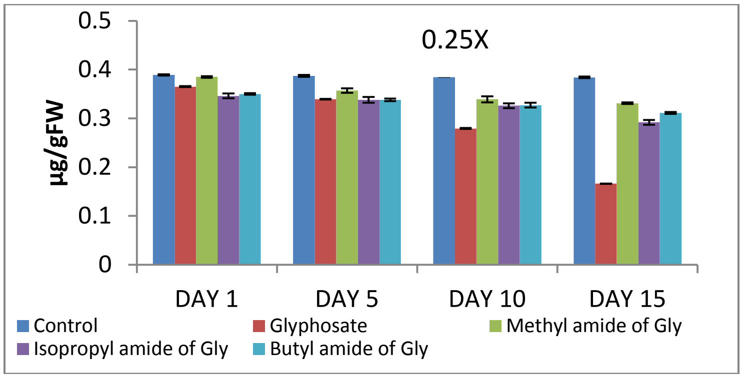


Figure (a)

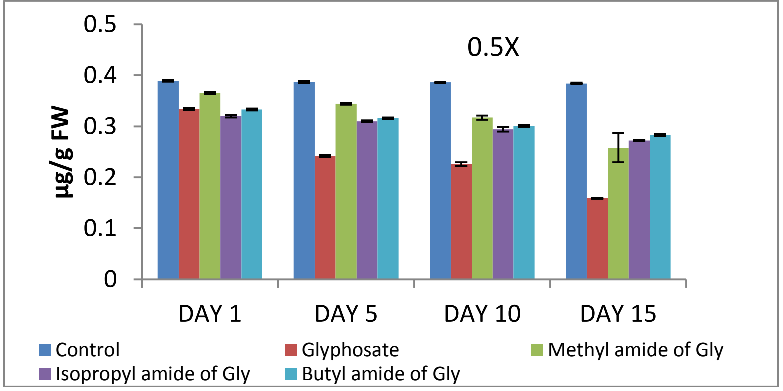


Figure (b)

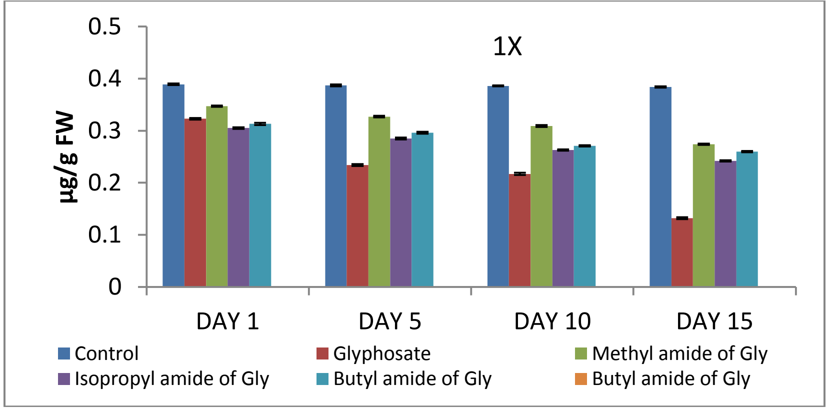


Figure 6.32 (a)-(c) Effect of glyphosate and synthesized amide derivatives of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Methyl amide of gly = ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid; Isopropyl amide of gly = ({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid ;Butyl amide of gly = ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid

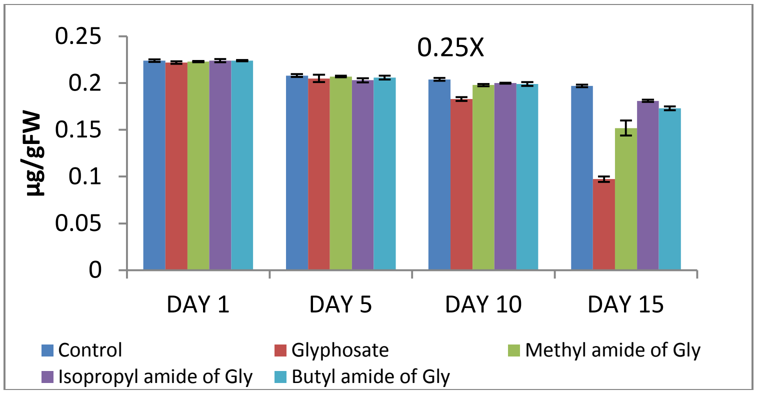


Figure (a)

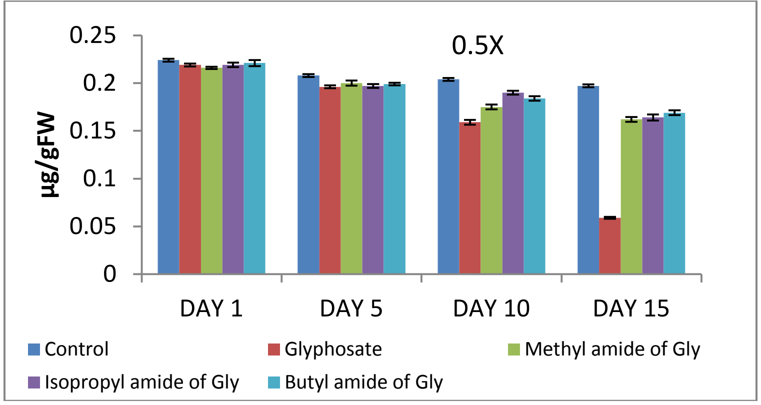


Figure (b)

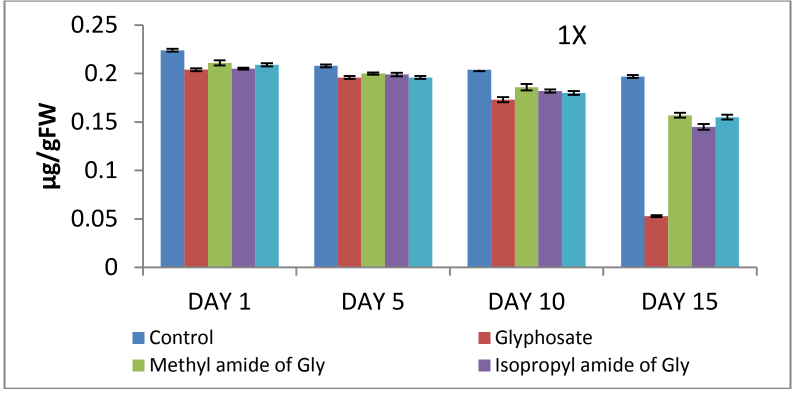


Figure (c)

Figure6.33 (a)-(c) Effect of glyphosate and synthesized amide derivatives of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Methyl amide of gly = ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid; Isopropyl amide of gly = ({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid ;Butyl amide of gly = ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid

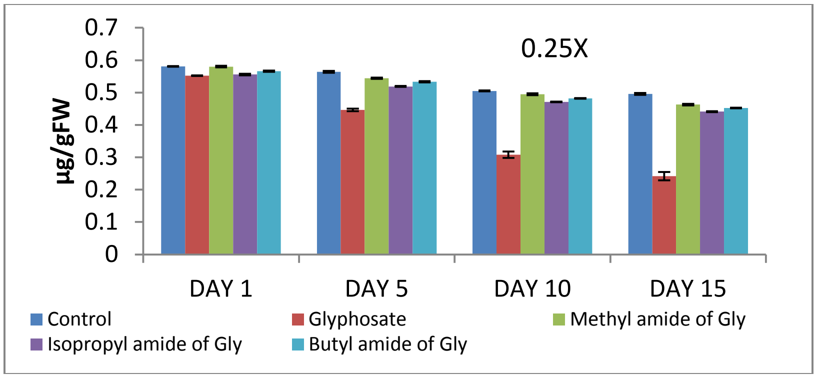


Figure (a)

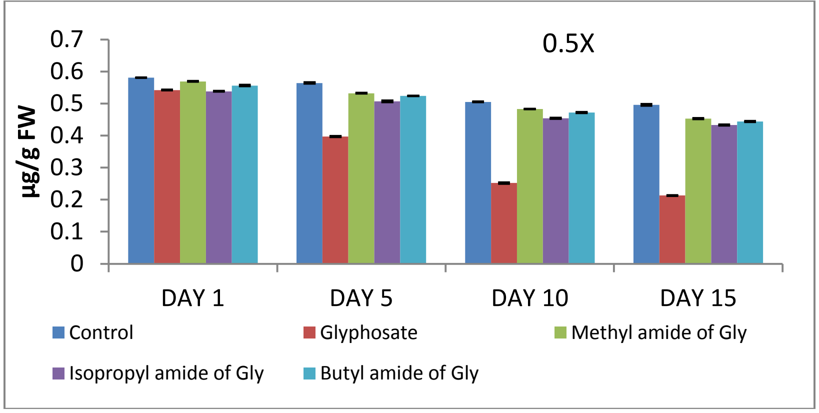


Figure (b)

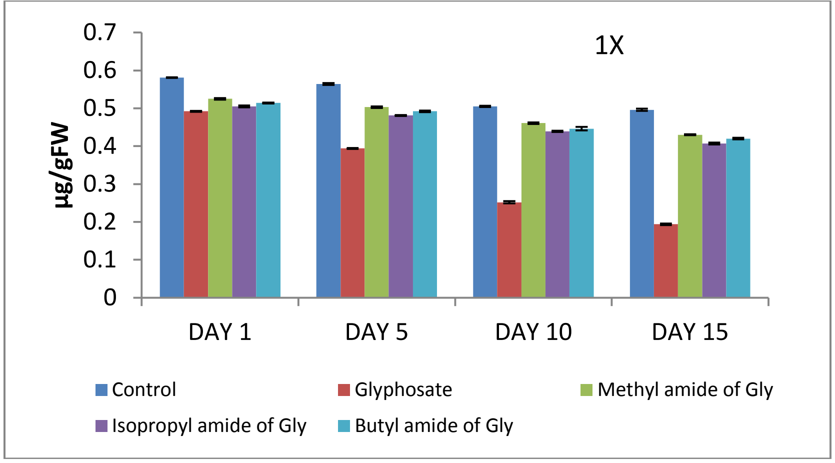


Figure (c)

Figure 6.34 (a)-(c) Effect of glyphosate and synthesized amide derivatives of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Methyl amide of gly = ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid; Isopropyl amide of gly = ({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid ;Butyl amide of gly = ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid.

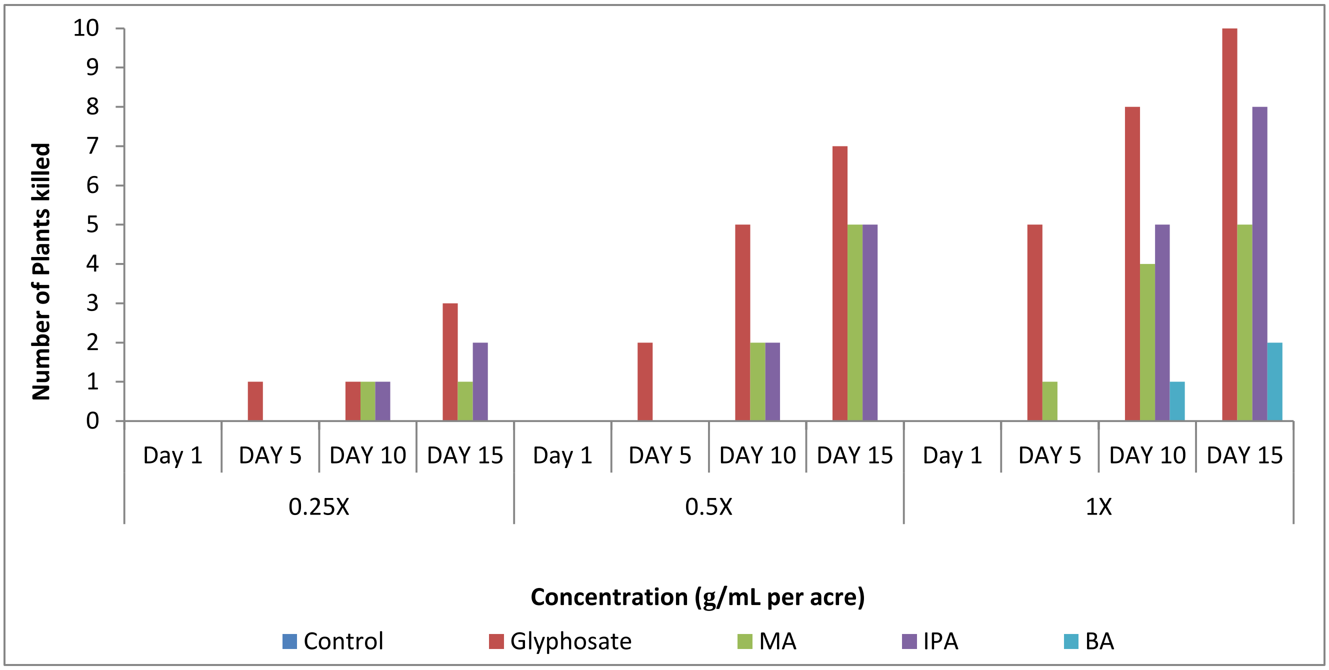


Figure 6.35 Lethal effect of exposure of Amide derivatives of glyphosate on *Parthenium hysterophorus* after 15 days Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

MA = ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid; IPA = ({[2-oxo-2-(propan-2-ylamino)ethyl] amino}methyl)phosphonic acid; BA = ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid.

Table 6.9 Effect of synthesized amide derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll' content (µg/gFW) in the leaves of *Parthenium hysterophorus*.

	Days →	**1**			**5**			**10**			**15**		
	Concentration →	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.389± 0.0015^{a}	0.389± 0.0015^{a}	0.389± 0.0015^{a}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}
	Glyphosate	0.365± 0.001^{c}	0.334± 0.002^{e}	0.323± 0.0015^{e}	0.339± 0.00058^{e}	0.242± 0.002^{h}	0.234± 0.0020^{h}	0.279± 0.001^{h}	0.226± 0.0035^{h}	0.217± 0.0020^{i}	0.166± 0.00058^{m}	0.159± 0.001^{j}	0.132± 0.0020^{m}
	({[2-(methylamino)-2-oxoethyl] amino} methyl) phosphonic acid	0.385± 0.0016^{b}	0.367± 0.0021^{c}	0.347± 0.0012^{e}	0.357± 0.0045^{d}	0.344± 0.0016^{e}	0.327± 0.0016^{e}	0.339± 0.0063^{d}	0.317± 0.0038^{f}	0.309± 0.00163^{g}	0.331± 0.00163^{d}	0.258± 0.0283^{h}	0.274± 0.0012^{h}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino} methyl)phosphonic acid	0.346± 0.0048^{e}	0.320± 0.0024^{f}	0.305± 0.0016^{g}	0.338± 0.0057^{d}	0.310± 0.0016^{f}	0.285± 0.0021^{g}	0.326± 0.0050^{e}	0.294± 0.0044^{g}	0.263± 0.0012^{h}	0.292± 0.0049^{g}	0.272± 0.0012^{h}	0.242± 0.0012^{h}
	({[2-(butylamino)-2-oxoethyl]amino}methyl) phosphonic acid	0.350± 0.0016^{c}	0.333± 0.0020^{e}	0.313± 0.0020^{f}	0.348± 0.0028^{c}	0.316± 0.0016^{f}	0.296± 0.0020^{g}	0.327± 0.0050^{e}	0.310± 0.002^{f}	0.271± 0.0012^{h}	0.311± 0.0020^{f}	0.283± 0.0020^{g}	0.260± 0.0012^{h}
Chlorophyll b	**Control**	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.208± 0.0015^{b}	0.208± 0.0015^{b}	0.208± 0.0015^{b}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.197± 0.0015^{d}	0.197± 0.0015^{d}	0.197± 0.0015^{d}
	Glyphosate	0.222± 0.0015^{a}	0.219± 0.0015^{b}	0.204± 0.0015^{c}	0.205± 0.0040^{c}	0.196± 0.0015^{d}	0.196± 0.0015^{d}	0.183± 0.002^{e}	0.159± 0.0026^{g}	0.173± 0.0026^{f}	0.0973± 0.003^{e}	0.059± 0.001^{m}	0.053± 0.001^{n}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.223± 0.0008^{a}	0.216± 0.0012^{b}	0.211± 0.0026^{b}	0.207± 0.0009^{c}	0.200± 0.0026^{c}	0.200± 0.0012^{c}	0.198± 0.0012^{d}	0.175± 0.0026^{f}	0.186± 0.0032^{d}	0.152± 0.008^{g}	0.162± 0.0024^{f}	0.157± 0.0024^{g}
	({[2-oxo-2-(propan-2ylamino)ethyl]amino} methyl) phosphonic acid	0.185± 0.0016^{d}	0.219± 0.0024^{b}	0.205± 0.0009^{c}	0.153± 0.0021^{g}	0.197± 0.0020^{d}	0.199± 0.0016^{d}	0.130± 0.0004^{i}	0.190± 0.0020^{d}	0.182± 0.0016^{d}	0.218± 0.0012^{b}	0.164± 0.0032^{f}	0.145± 0.0028^{h}
	({[2-(butylamino)-2-oxoethyl]amino}methyl) phosphonic acid	0.224± 0.0008^{a}	0.221± 0.0032^{a}	0.209± 0.0016^{c}	0.206± 0.0021^{c}	0.199± 0.0012^{d}	0.196± 0.0016^{d}	0.199± 0.0020^{d}	0.184± 0.0024^{e}	0.180± 0.0021^{e}	0.173± 0.0020^{f}	0.169± 0.0024^{j}	0.155± 0.0024^{g}
Total Chlorophyll	**Control**	0.581± 0.0010^{a}	0.581± 0.0010^{a}	0.581± 0.0010^{a}	0.564± 0.0025^{b}	0.581± 0.001^{a}	0.564± 0.0025^{b}	0.505± 0.0015^{d}	0.505± 0.0015^{d}	0.505± 0.0015^{d}	0.496± 0.0030^{d}	0.496± 0.0030^{d}	0.496± 0.0030^{d}
	Glyphosate	0.552± 0.0010^{b}	0.542± 0.0015^{c}	0.492± 0.0011^{c}	0.446± 0.0040^{f}	0.397± 0.0020^{j}	0.394± 0.0015^{j}	0.308± 0.0096^{j}	0.283± 0.0025^{n}	0.252± 0.0026^{j}	0.242± 0.0130^{m}	0.213± 0.0015^{p}	0.194± 0.0020^{m}
	({[2-(methylamino)-2-oxoethyl] amino} methyl) phosphonic acid	0.580± 0.0024^{a}	0.569± 0.0016^{b}	0.525± 0.0020^{c}	0.544± 0.0021^{c}	0.532± 0.0016^{c}	0.503± 0.0020^{d}	0.495± 0.0028^{f}	0.483± 0.0012^{d}	0.461± 0.0021^{d}	0.463± 0.00249^{d}	0.453± 0.0024^{e}	0.430± 0.0012^{f}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino} methyl) phosphonic acid	0.556± 0.0024^{c}	0.538± 0.0016^{c}	0.505± 0.0024^{d}	0.519± 0.0012^{c}	0.507± 0.0026^{d}	0.481± 0.0012^{d}	0.471± 0.0008^{d}	0.454± 0.0021^{e}	0.439± 0.00169^{f}	0.441± 0.0016^{e}	0.433± 0.0020^{f}	0.407± 0.0024^{f}

	({[2-(butylamino)-2-oxoethyl]amino}methyl) phosphonic acid	0.566± 0.0024^{b}	0.556± 0.0024^{b}	0.514± 0.0012^{c}	0.533± 0.0020^{b}	0.524± 0.0008^{b}	0.492± 0.0024^{d}	0.482± 0.0012^{d}	0.472± 0.0024^{d}	0.446± 0.0047^{e}	0.452± 0.0008^{h}	0.444± 0.0021^{e}	0.420± 0.002^{g}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.1.3 Boc-protected glyphosate derivative:

Boc-Protected glyphosate derivative also showed similar kind of results of herbicidal activity as shown by amide derivatives of glyphosate. As compared to control (water treatment), the plants treated with Boc-protected glyphosate have shown momentous reduction in photosynthetic pigments. A considerable decrease in the amount of 'Chl a', 'Chl b' and 'Chl a+b' has been observed at all test concentrations with respect to time (**Table 6.10**). After 15 days, the 'chlorophyll a' content present in the control plant was found to be 0.384 µg/gFW which get decreased to 0.316 µg/gFW at 0.25X , 0.294 µg/gFW at 0.5X and 0.261 µg/gFW at 1X concentration of the Boc-protected glyphosate (**Figure 6.36 (a-c)**). Analogous trend was also scrutinized in case of 'chlorophyll b' and 'total chlorophyll' content of the *Parthenium hysterophorus*. 'Chlorophyll b' content present in control plant after 15 days was found to be 0.197 µg/gFW , however after the application of the Boc-protected glyphosate on the weed plant the 'chlorophyll b' was reduced to 0.181 µg/gFW at 0.25 X, 0.170 µg/gFW at 0.5X and 0.166 µg/gFW at 1X concentration (**Figure 6.37 (a-c)**). Comparable results were observed with 'total chlorophyll' content (**Figure 6.38 (a-c)**).

Mortality rate of *Parthenium hysterophorus* with respect to concentration of Boc- protected glyphosate and time was perceived by using 10 plants in each pot. It was observed that Boc-protected glyphosate did not show any significant effect on the mortality of the plants. This compound has shown its herbicidal activity only at higher concentration (0.5X and 1X) . Foliar spray of Boc-protected glyphosate at 0.5X has resulted in death of 3 plants after 15 days, whereas glyphosate has shown significant reduction in the number of plants even after 10th day (5 plants were dead out of 10). At 1X concentration of Boc-protected glyphosate, half of the plants (5 out of 10) has become dead (In case of glyphosate at 1X concentration, 8 out of 10 plants were dead) (**Figure 6.39**). This data illustrates that application of Boc-protected glyphosate on the weed significantly reduced the content of its photosynthetic pigments at all the three test concentrations, but it killed the plant completely only at higher concentrations (1X).

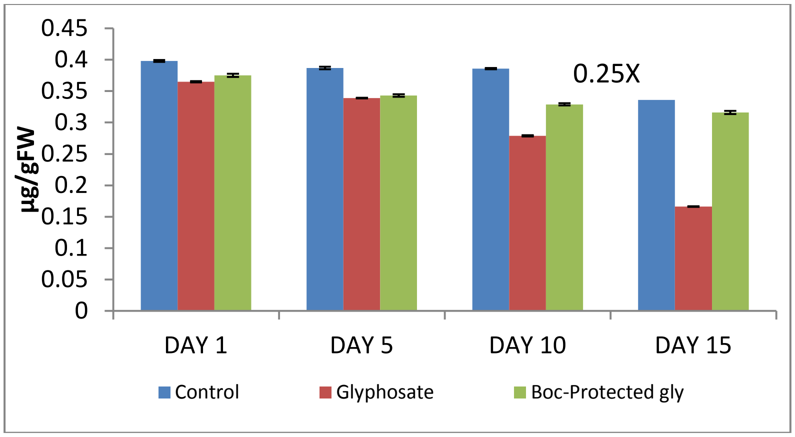
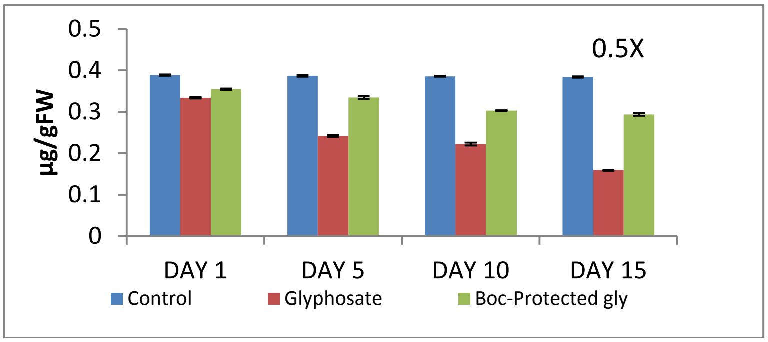


Figure (a)



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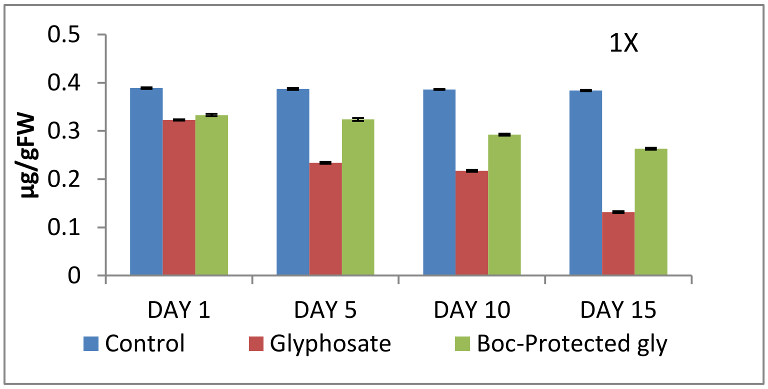


Figure (c)

Figure 6.36(a)-(c) Effect of glyphosate and Boc-protected glyphosate derivative on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-Protected Gly= (2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid

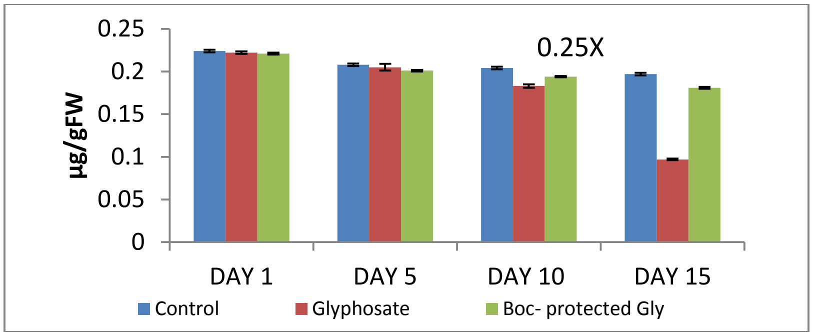


Figure (a)

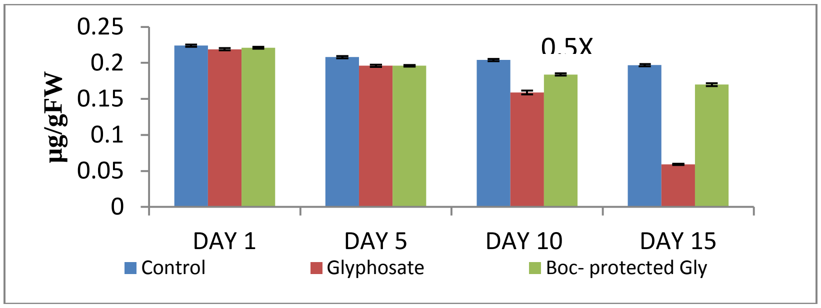


Figure (b)

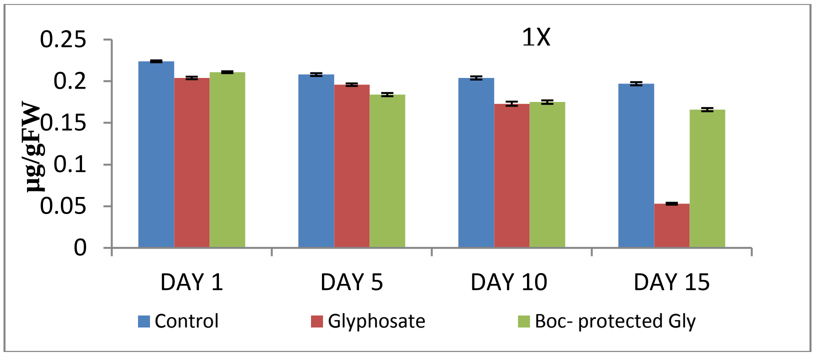


Figure (c)

Figure 6.37 (a)-(c) Effect of glyphosate and Boc-protected glyphosate derivative on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-Protected Gly= (2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid

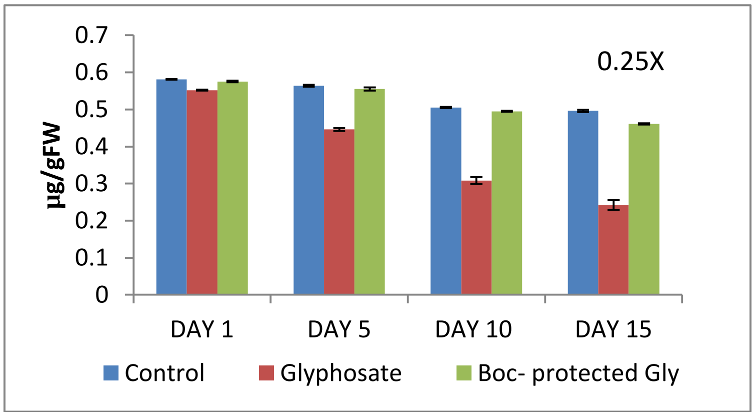


Figure (a)

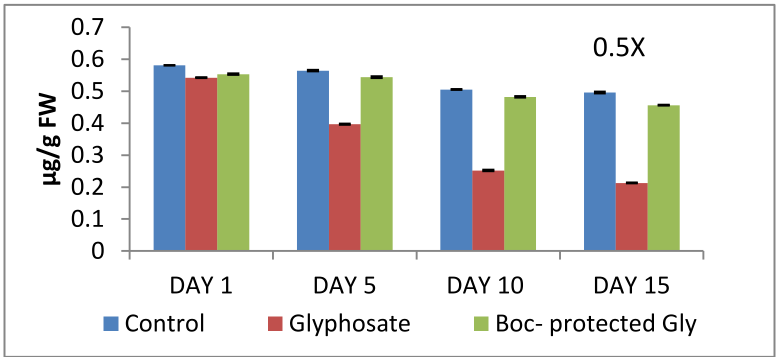


Figure (b)

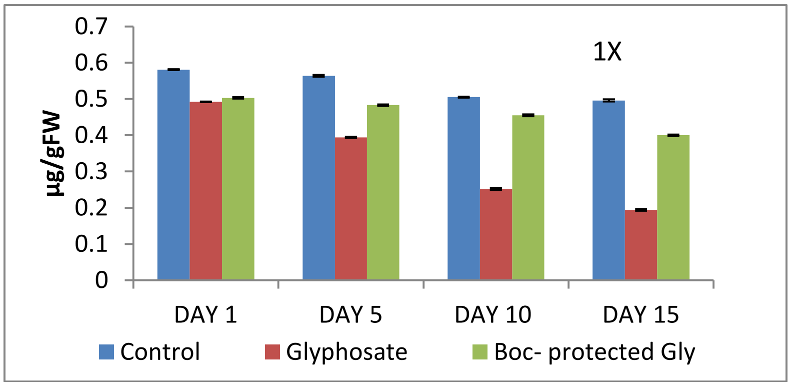


Figure (c)

Figure 6.38(a)-(c) Effect of glyphosate and Boc-protected glyphosate derivative on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Boc-Protected Gly= (2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid

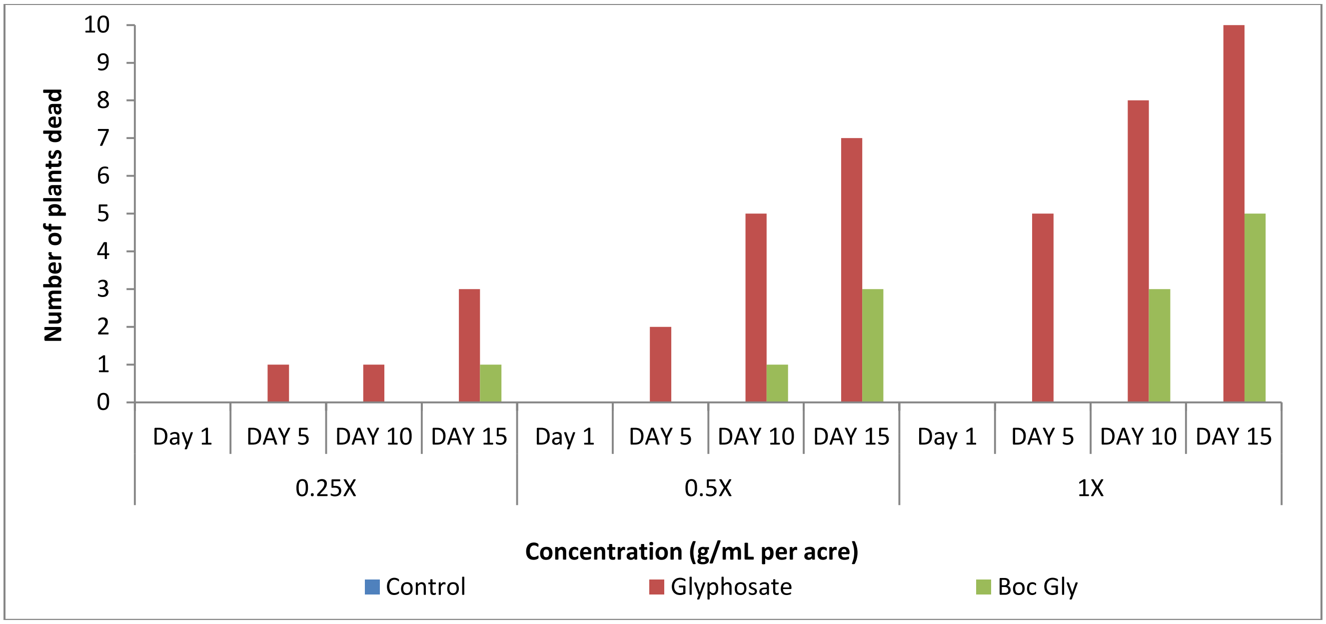


Figure 6.39 Lethal effect of exposure of Boc- Protected glyphosate on *Parthenium hysterophorus* after 15 days Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-Protected Gly= (2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid

Table 6.10 Effect of Boc-protected glyphosate derivative on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll' content (µg/gFW) in the leaves of *Parthenium hysterophorus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.389± 0.0015^{a}	0.389± 0.0015^{a}	0.389± 0.0015^{a}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}
	Glyphosate	0.365± 0.001^{c}	0.334± 0.002^{e}	0.323± 0.0015^{e}	0.339± 0.00058^{e}	0.242± 0.002^{h}	0.234± 0.0020^{h}	0.279± 0.001^{h}	0.226± 0.0035^{h}	0.217± 0.0020^{i}	0.166± 0.00058^{m}	0.159± 0.001^{j}	0.132± 0.0020^{m}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.375± 0.0026^{c}	0.355± 0.0016^{f}	0.333± 0.0025^{d}	0.343± 0.0020^{f}	0.335± 0.0036^{d}	0.324± 0.003^{d}	0.329± 0.0017^{d}	0.303± 0.00081^{e}	0.292± 0.002^{f}	0.316± 0.00251^{d}	0.294± 0.0037^{f}	0.263± 0.0020^{e}
Chlorophyll b	**Control**	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.208± 0.0015^{b}	0.208± 0.0015^{b}	0.208± 0.0015^{b}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.197± 0.0015^{d}	0.197± 0.0015^{d}	0.197± 0.0015^{d}
	Glyphosate	0.222± 0.0015^{a}	0.219± 0.0015^{b}	0.204± 0.0015^{c}	0.205± 0.0040^{c}	0.196± 0.0015^{d}	0.196± 0.0015^{d}	0.183± 0.002^{e}	0.159± 0.0026^{g}	0.173± 0.0026^{f}	0.0973± 0.003^{j}	0.059± 0.001^{k}	0.053± 0.001^{k}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.221± 0.0012^{a}	0.221± 0.0012^{a}	0.211± 0.0012^{b}	0.201± 0.001^{c}	0.196± 0.0012^{d}	0.184± 0.0018^{e}	0.194± 0.0008^{d}	0.184± 0.0016^{e}	0.175± 0.002^{f}	0.181± 0.0012^{e}	0.170± 0.002^{h}	0.163± 0.0021^{i}
Totall Chlorophyll	**Control**	0.581± 0.0010^{a}	0.581± 0.0010^{a}	0.581± 0.0010^{a}	0.564± 0.0025^{b}	0.581± 0.0010^{b}	0.564± 0.0025^{b}	0.505± 0.0015^{d}	0.505± 0.0015^{d}	0.505± 0.0015^{d}	0.496± 0.0030^{d}	0.496± 0.0030^{d}	0.496± 0.0030^{d}
	Glyphosate	0.552± 0.0010^{b}	0.542± 0.0015^{c}	0.492± 0.0011^{c}	0.446± 0.0040^{f}	0.397± 0.0020^{j}	0.394± 0.0015^{j}	0.308± 0.0096^{i}	0.283± 0.0025^{k}	0.252± 0.0026^{l}	0.242± 0.0130^{m}	0.213± 0.0015^{n}	0.194± 0.0020^{o}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.575± 0.0021^{h}	0.533± 0.002^{h}	0.503± 0.0021^{d}	0.555± 0.0043^{b}	0.544± 0.0032^{c}	0.483± 0.002^{f}	0.495± 0.0016^{f}	0.482± 0.0024^{d}	0.455± 0.0029^{f}	0.461± 0.0016^{g}	0.456± 0.0016^{f}	0.400± 0.00205^{e}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

6.3.1.4 Boc-protected guanidine derivative of glyphosate and its de-protected analogue:

Boc-protected guanidine derivative of glyphosate and its de-protected analogue (guanidine derivative of glyphosate) have also shown herbicidal properties on the common weed *Parthenium hysterophorus*. A regular decrease in the chlorophyll content was found in both the cases at all the test concentrations. In comparison to control (water treatment) both of these newly synthesized derivatives are effective in controlling the weed population.

In case of **Boc-protected guanidine derivative of glyphosate**, at 0.25X after 15 days, 'chlorophyll a' has been reduced to 0.274 µg/gFW in comparison to control (0.384 µg/gFW). However at 0.5X, reduction in' chlorophyll a' was 0.262 µg/gFW and at 1X, 'chlorophyll a' left behind was found to be 0.252 µg/gFW (**Figure 6.40 (a-c)**). But in case of 'chlorophyll b', at 0.25X no significant change was observed after 15 days treatment. 'Chlorophyll b' was negligibly reduced from 0.197 µg/gFW (control) to 0.196 µg/gFW. However the reduction in 'chlorophyll b' content became significant at 0.5X [chlorophyll b(treated plant=0.165 µg/gFW] and at 1X [chlorophyll b(treated plant=0.154 µg/gFW] (**Figure 6.41 (a-c)**). Considerable dimiution in 'Chl a+b' content of plant was also observed at all test concentrations (**Figure 6.42 (a-c)**)

The **guanidine derivative of glyphosate** (de-protected guanidine derivative of glyphosate) is more effective in showing its herbicidal effects in comparison to the Boc-protected guanidine derivative of glyphosate. It has shown greater herbicidal effects by decreasing the content of photosynthetic pigments in the plant. At all the test concentrations a marked reduction in the 'Chl a,'Chl b' and 'Chl a+b' has been observed. At 0.25X 'chlorophyll a' left in the plant was 0.261µg/gFW which get further reduced 0.254 µg/gFW and 0.243 µg/gFW at 0.5X and 1X respectively. Similarly a considerable decrease in 'chlorophyll b' and 'total chlorophyll' (**Table 6.11**) was also examined in the weed plant at all the test concentrations (0.25X, 0.5X and 1X).

Herbicidal activity of the synthesized Boc-protected guanidine derivative of glyphosate and guanidine derivative of glyphosate was also established by means of the total number of plants dead after their exposure to these derivatives. It was found that in comparison to glyphosate no considerable decrease in the number of plants was found in both these derivatives. However at higher concentration (1X) guanidine derivative of glyphosate was effective in killing the plants (4 after 10 days and 6 after 15 days). But these results are not noteworthy with respect to glyphosate. Use of Boc-protected guanidine glyphosate and guanidine derivative of glyphosate on the weed (*Parthenium hysterophorus*), at all the three test concentrations has effectively decreased the content of photosynthetic pigments in the plant but the number of deaths caused in the plant was less (Boc-protected guanidine glyphosate killed 3 plants at 1X after 10 and 15 days and guanidine derivative of glyphosate killed 4 plants after 10 and 6 plants after 15 days) and was not crucial (**Figure 6.43**).

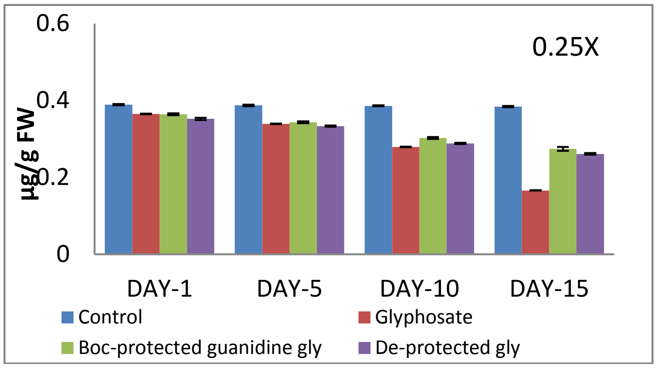


Figure (a)

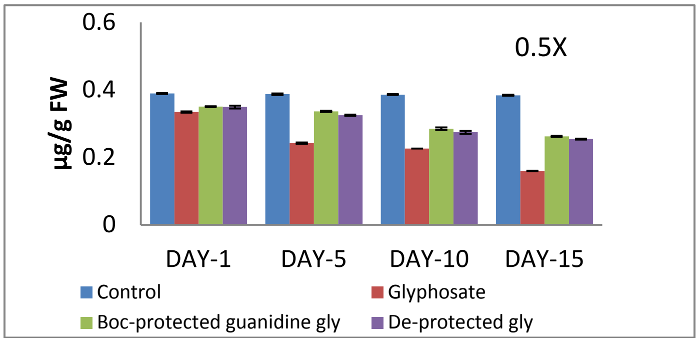
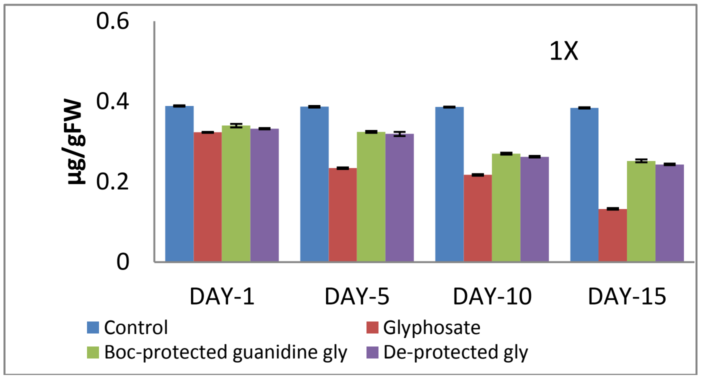


Figure (b)



Figure(c)

Figure 6.40 (a)-(c) Effect of Boc-protected guanidine glyphosate and guanidine derivative of glyphosate on 'chlorophyll 'a content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-protected guanidine gly = 2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid

De- protected gly =2-(1-(Phosphonomethyl)guanidino) ethanoic acid

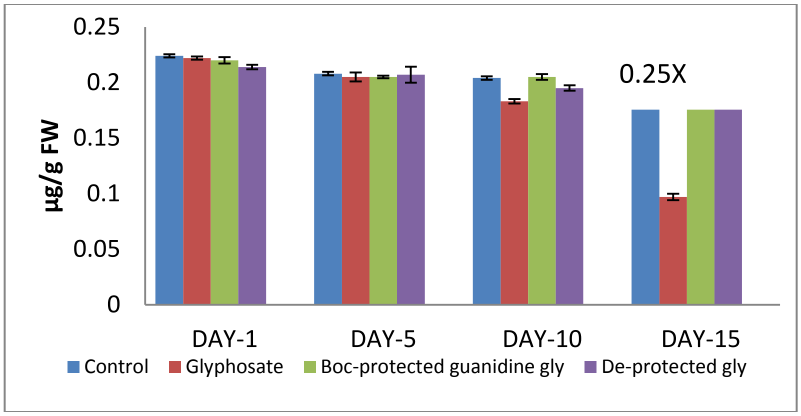


Figure (a)

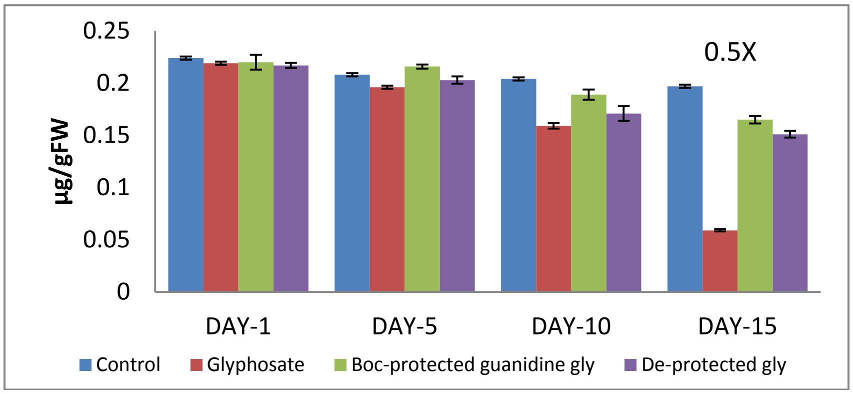


Figure (b)

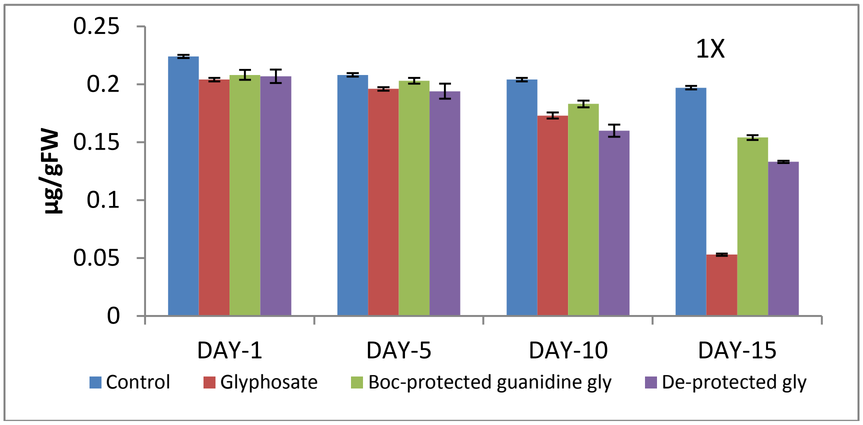


Figure (c)

Figure 6.41 (a)-(c) Effect of Boc-protected guanidine glyphosate and guanidine derivative of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-protected guanidine gly = 2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid

De- protected gly = 2-(1-(Phosphonomethyl)guanidino) ethanoic acid

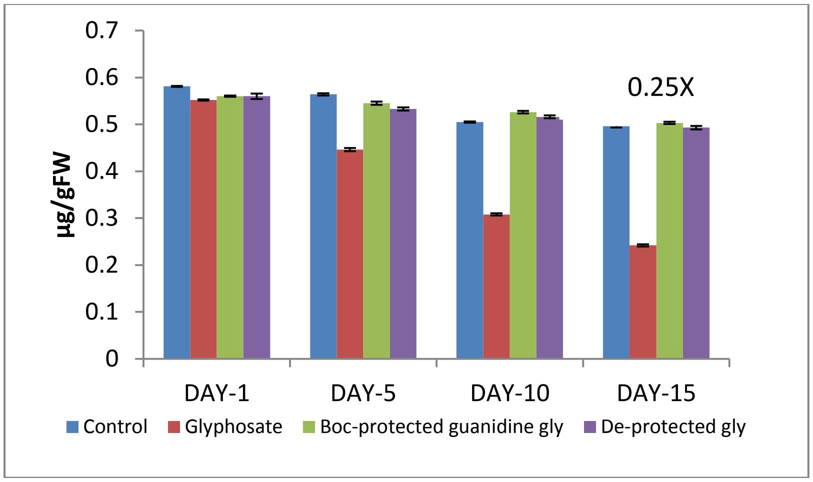


Figure (a)

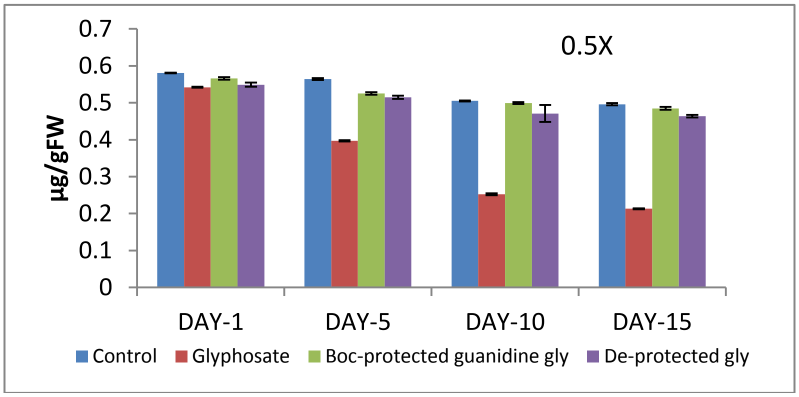


Figure (b)

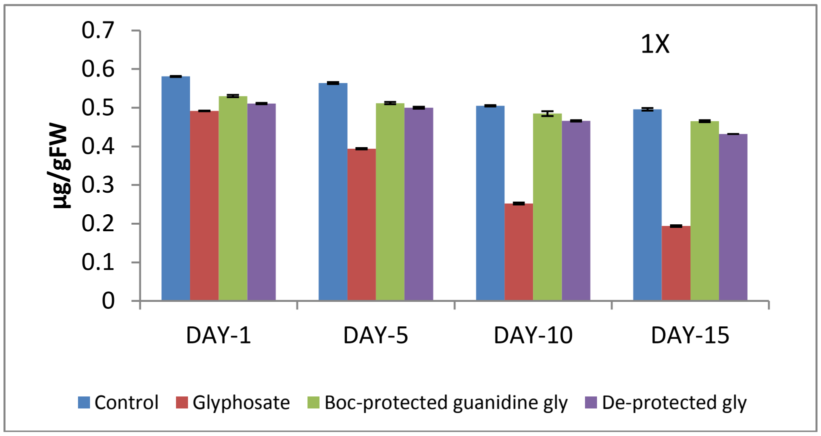


Figure (c)

Figure 6.42 (a)-(c) Effect of Boc-protected guanidine glyphosate and guanidine derivative of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-protected guanidine gly = 2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid

De- protected gly = 2-(1-(Phosphonomethyl)guanidino) ethanoic acid

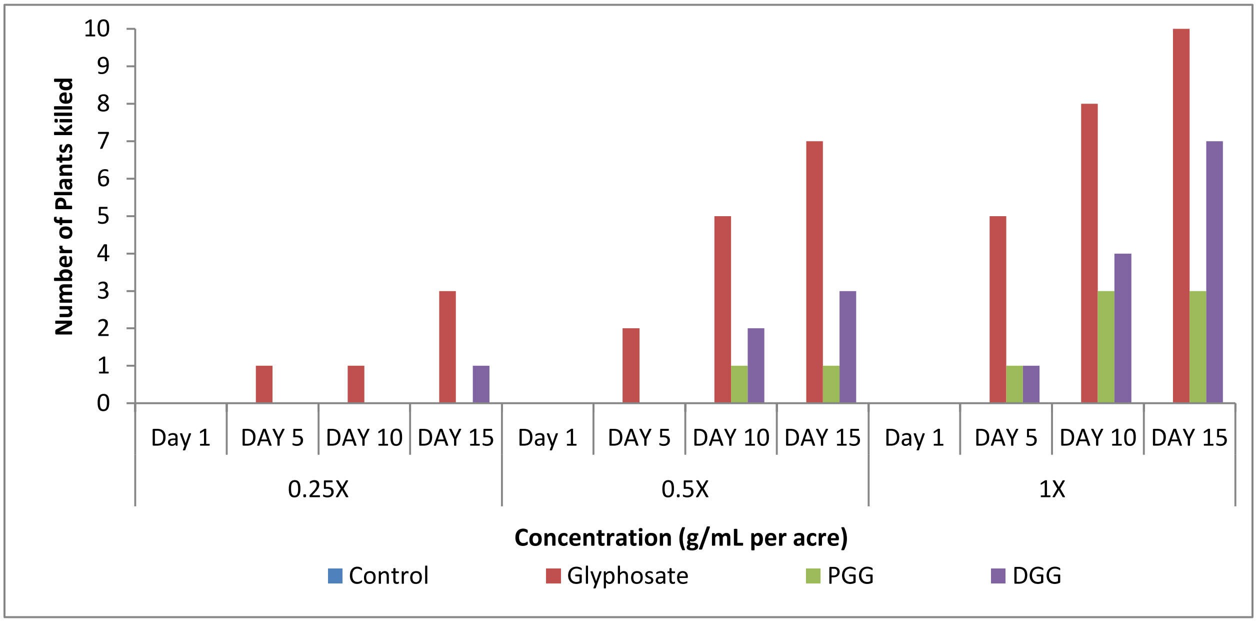


Figure 6.43 Lethal effect of exposure of of Boc-protected guanidine glyphosate and De-protected guanidine glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

PGG (Boc-protected guanidine gly) = 2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid ;DGG (De- protected guanidine gly) = 2-(1-(Phosphonomethyl)guanidino) ethanoic acid

Table 6.11 Effect Boc-protected guanidine derivative of glyphosate and its deprotected analogue (guanidine derivative of glyphosate) on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b' (µg/gFW) and ' total chlorophyll content' (µg/gFW) in the leaves of *Parthenium hysterophorus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.389±0.0015^{a}	0.389±0.0015^{a}	0.389±0.0015^{a}	0.387±0.002^{b}	0.387±0.002^{b}	0.387±0.002^{b}	0.386±0.001^{b}	0.386±0.001^{b}	0.386±0.001^{b}	0.384±0.0015^{b}	0.384±0.0015^{b}	0.384±0.0015^{b}
	Glyphosate	0.365±0.001^{c}	0.334±0.002^{d}	0.323±0.0015^{d}	0.339±0.00058^{d}	0.242±0.002^{e}	0.234±0.0020^{e}	0.279±0.001^{f}	0.226±0.0035^{e}	0.217±0.0020^{e}	0.166±0.00058^{f}	0.159±0.001^{k}	0.132±0.0020^{l}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino)ethanoic acid	0.364±0.0021^{c}	0.350±0.0015^{d}	0.340±0.0045^{e}	0.333±0.0015^{d}	0.336±0.002^{d}	0.324±0.0026^{d}	0.302±0.0025^{g}	0.285±0.0036^{h}	0.270±0.0025^{h}	0.261±0.0020^{h}	0.262±0.0015^{h}	0.252±0.0032^{i}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.352±0.0032^{d}	0.325±0.002^{d}	0.332±0.0017^{e}	0.333±0.0015^{e}	0.325±0.002^{d}	0.319±0.005^{d}	0.288±0.00152^{h}	0.274±0.0041^{h}	0.262±0.002^{h}	0.261±0.002^{h}	0.254±0.00015^{i}	0.243±0.002^{e}
Chlorophyll b	**Control**	0.224±0.0015^{a}	0.224±0.0015^{a}	0.224±0.0015^{a}	0.208±0.0015^{b}	0.208±0.0015^{b}	0.208±0.0015^{b}	0.204±0.0015^{c}	0.204±0.0015^{c}	0.204±0.0015^{c}	0.197±0.0015^{d}	0.197±0.0015^{d}	0.197±0.0015^{d}
	Glyphosate	0.222±0.0015^{a}	0.219±0.0015^{b}	0.204±0.0015^{c}	0.205±0.0040^{c}	0.196±0.0015^{d}	0.196±0.0015^{d}	0.183±0.002^{g}	0.159±0.0026^{h}	0.173±0.0026^{g}	0.0973±0.003^{k}	0.059±0.001^{l}	0.053±0.001^{l}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	0.244±0.003^{e}	0.226±0.007^{a}	0.208±0.0043^{e}	0.225±0.0011^{a}	0.216±0.002^{e}	0.203±0.0025^{e}	0.205±0.0025^{e}	0.189±0.005^{g}	0.183±0.0028^{g}	0.196±0.0036^{j}	0.165±0.0035^{i}	0.154±0.002^{h}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.234±0.002^{f}	0.217±0.0025^{b}	0.207±0.0058^{e}	0.214±0.0072^{b}	0.203±0.0030^{e}	0.194±0.0064^{j}	0.195±0.0025^{j}	0.171±0.007^{i}	0.160±0.0052^{i}	0.185±0.0035^{g}	0.151±0.0032^{h}	0.133±0.001^{h}
Total Chlorophyll	**Control**	0.581±0.0010^{a}	0.581±0.0010^{a}	0.581±0.0010^{a}	0.564±0.0025^{b}	0.581±0.0010^{b}	0.564±0.0025^{b}	0.505±0.0015^{d}	0.505±0.0015^{d}	0.505±0.0015^{d}	0.496±0.0030^{g}	0.496±0.0030^{g}	0.496±0.0030^{g}
	Glyphosate	0.552±0.0010^{b}	0.542±0.0015^{c}	0.492±0.0011^{f}	0.446±0.0040^{f}	0.397±0.0020^{i}	0.394±0.0015^{i}	0.308±0.0096^{j}	0.283±0.0025^{k}	0.252±0.0026^{l}	0.242±0.0130^{l}	0.213±0.0015^{l}	0.194±0.0020^{m}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	0.560±0.0015^{b}	0.566±0.0036^{b}	0.530±0.003^{e}	0.545±0.0035^{c}	0.525±0.0003^{e}	0.512±0.0032^{c}	0.526±0.0025^{e}	0.499±0.0025^{g}	0.485±0.00624^{g}	0.503±0.0025^{d}	0.485±0.0036^{g}	0.465±0.0026^{f}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.560±0.0058^{b}	0.549±0.005^{e}	0.511±0.001^{c}	0.533±0.0035^{f}	0.515±0.004^{c}	0.500±0.0028^{d}	0.516±0.003^{c}	0.471±0.0235^{f}	0.466±0.002^{f}	0.493±0.004^{g}	0.464±0.0036^{f}	0.432±0.00020^{h}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.1.5 Thioxylated ester derivatives of glyphosate:

A series of four new thioxylated ester derivatives of glyphosate were synthesized and their herbicidal activity was compared with control (water treatment) and glyphosate. These newly synthesized derivatives have shown excellent herbicidal effects on the weed *Parthenium hysterophorus* after 15 days treatment on three test concentrations (0.25X, 0.5X and 1X). Among these thioxylated esters of glyphosate, **thioxylated ethyl ester of glyphosate** (*O*-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate) have shown best herbicidal effects. It has significantly decreased the chlorophyll content ('Chl a', 'Chl b'and 'Chl a+b') and killed the plant. At 0.25X after 15 days, a considerable decline in 'chlorophyll a' (0.00466µg/gFW) , 'chlorophyll b'(0.0937 µg/gFW, 'total chlorophyll' (0.222 µg/gFW) **[Figure 6.44 (a), 6.45 (a) and 6.46 (a)]** was observed in the treated plant as compared to control ['chlorophyll a' (0.389 µg/gFW), 'chlorophyll b'(0.224 µg/gFW), 'total chlorophyll' (0.581 µg/gFW] and glyphosate ['chlorophyll a' (0.166 µg/gFW), 'chlorophyll b' (0.0973 µg/gFW), 'total chlorophyll' (0.242 µg/gFW)]. Similar significant reduction was observed at 0.5X and a remarkable decline in chlorophyll content was observed at 1X (**Table 6.12**). Thioxylated ethyl ester of glyphosate behave as a potent herbicide and is extremely effective in decreasing the content of photosynthetic pigments of plant in comparison to glyphosate. Plants treated with thioxylated ethyl ester derivative of glyphosate at 1X have shown tremendous decrease in 'chlorophyll a' (0.0016 µg/gFW), 'chlorophyll b' (0.0201 µg/gFW) and 'total chlorophyll' (0.136 µg/gFW) **[Figure 6.44 (c), 6.45 (c) and 6.46 (c)]** in contrast to glyphosate. Other thioxylated ester derivatives of glyphosate are also effectual in killing the weed. They have also reduced the content of photosynthetic pigments in the plant at all the test concentrations. Major diminution in 'chlorophyll a' (0.156 µg/gFW) , 'chlorophyll b' (0.0956 µg/gFW) and 'total chlorophyll' (0.237 µg/gFW) was found in the plants treated with 0.25X of **thioxylated isopropyl ester of glyphosate** (O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl] methyl}amino) ethanethioate). However this decrease became much pronounced with increase in the concentration of the thioxylated isopropyl ester of glyphosate. At 1X, chlorophyll content was greatly reduced ['chlorophyll a' (0.126 µg/gFW), 'chlorophyll b' (0.0426 µg/gFW) and 'total chlorophyll' (0.190 µg/gFW) in the plant and it exterminate the plant completely.

Transience of plant is the main criterion for determining the herbicidal effects of any pesticide. Glyphosate is a well known herbicide that effectually killed the plant. Synthesized Thioxylated ester derivatives of glyphosate also act as potent herbicides and competently killed the plant. Thioxylated ethyl ester of glyphosate is adequate in killing the plant in contrast to glyphosate. Even at the lowest concentration(0.25X) half of the plants were exterminated by this compound after 10 days of the treatment. The number of plants killed by the thioxylated ethyl ester of glyphosate was augmented with increase in the concentration of the compound. At 0.5X, 6 plants were killed after 5 days and 9 plants were killed after 15 days. The rate at which the plants become dead after their exposure to this compound was greatly increased with increase in concentration and time. However at 1X all the plants (10) were dead even after 10 days exposure. Whereas, glyphosate killed 8 out of 10 plants even after 10 days exposure. Similar type of results were achieved in case of Thioxylated isopropyl ester of glyphosate. It has also shown its herbicidal effects even at the lowest concentration of 0.25X. It effectively killed 7 out of 10 plants after 15 days **(Figure 6.47)**. At 1X more than half of the plants were killed by this compound after 10 days exposure. All these findings showed that thioxylated ester derivatives of glyphosate are potent herbicides and effectively killed the plant.

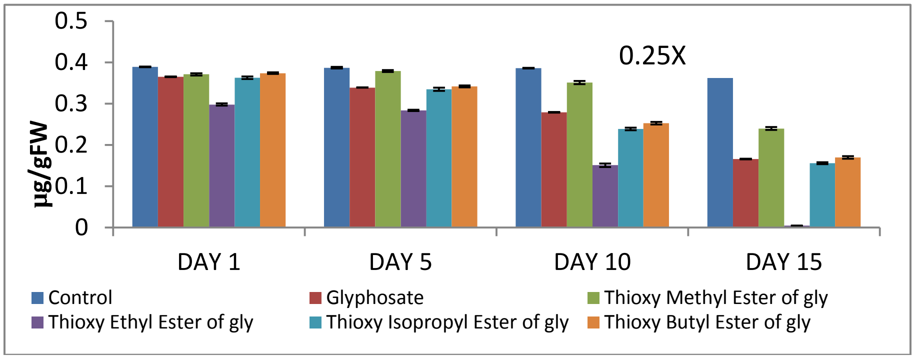


Figure (a)

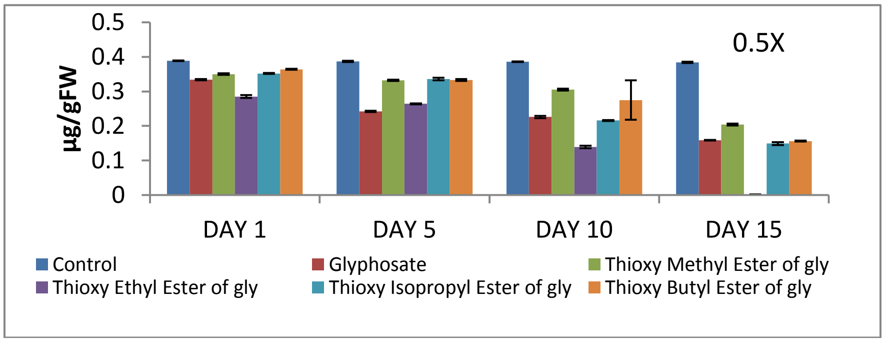


Figure (b)

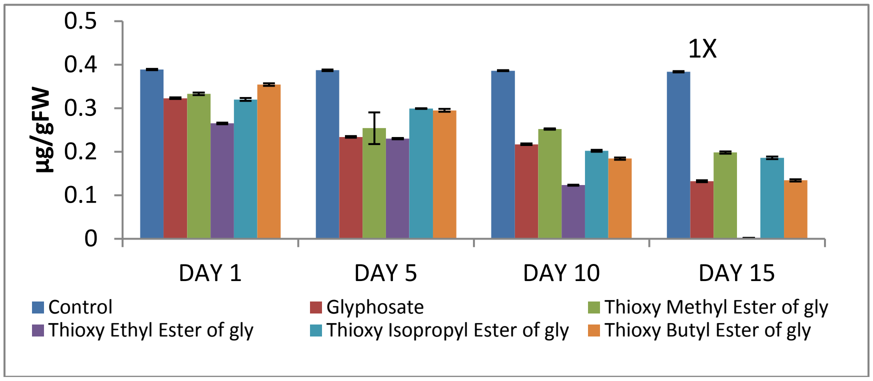


Figure (c)

Figure 6.44(a)-(c) Effect of Thioxylated ester derivatives of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Where, Thioxy Methyl Ester of gly= O-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thioxy Ethyl Ester of gly= O-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thioxy Isopropyl Ester of gly= O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino)ethanethioate
Thioxy Butyl Ester of gly= O-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate

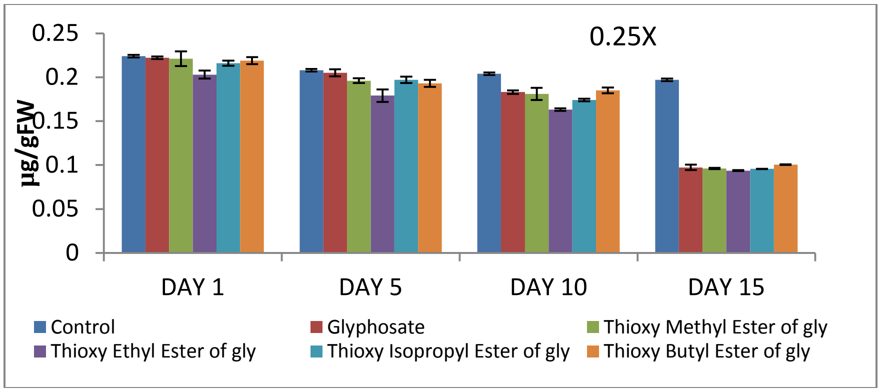


Figure (a)

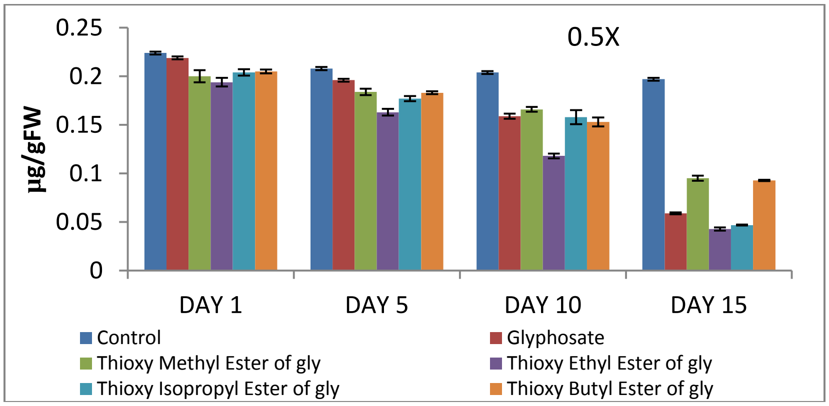


Figure (b)

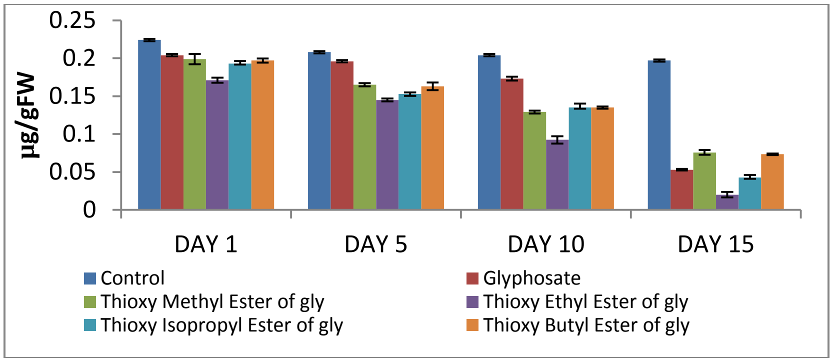


Figure (c)

Figure 6.45(a)-(c) Effect of Thioxylated ester derivatives of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Where, Thioxy Methyl Ester of gly= O-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thioxy Ethyl Ester of gly= O-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thioxy Isopropyl Ester of gly= O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino)ethanethioate
Thioxy Butyl Ester of gly= O-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate

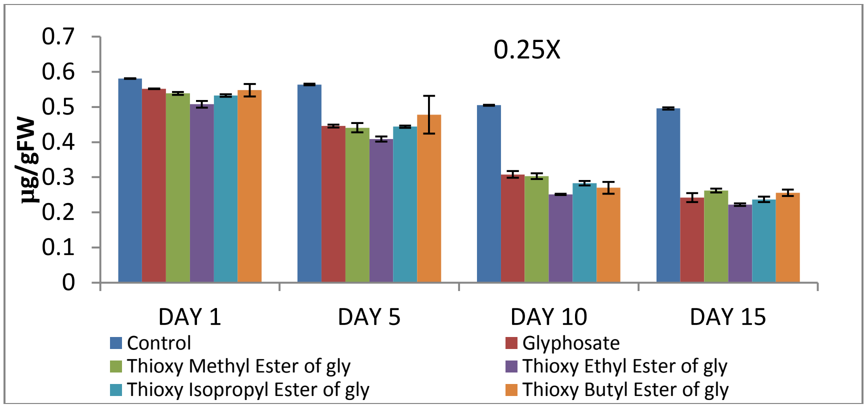


Figure (a)

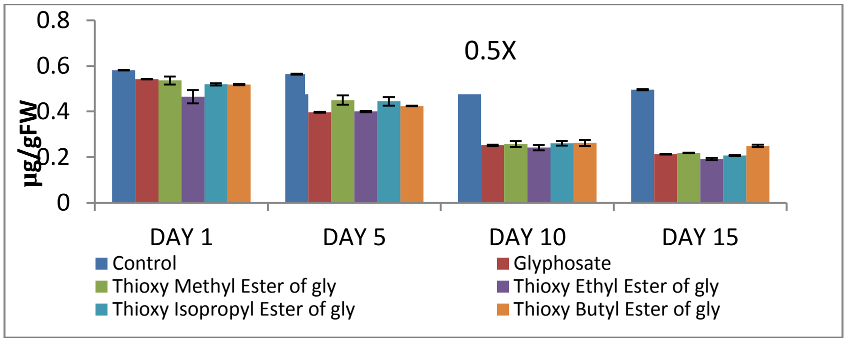


Figure (b)

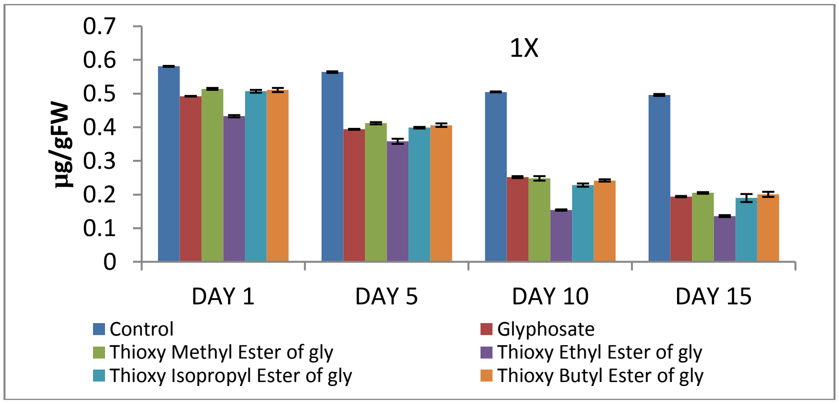


Figure (c)

Figure 6.46(a)-(c) Effect of Thioxylated ester derivatives of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Where, Thioxy Methyl Ester of gly= O-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thioxy Ethyl Ester of gly= O-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thioxy Isopropyl Ester of gly= O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino)ethanethioate
Thioxy Butyl Ester of gly= O-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate

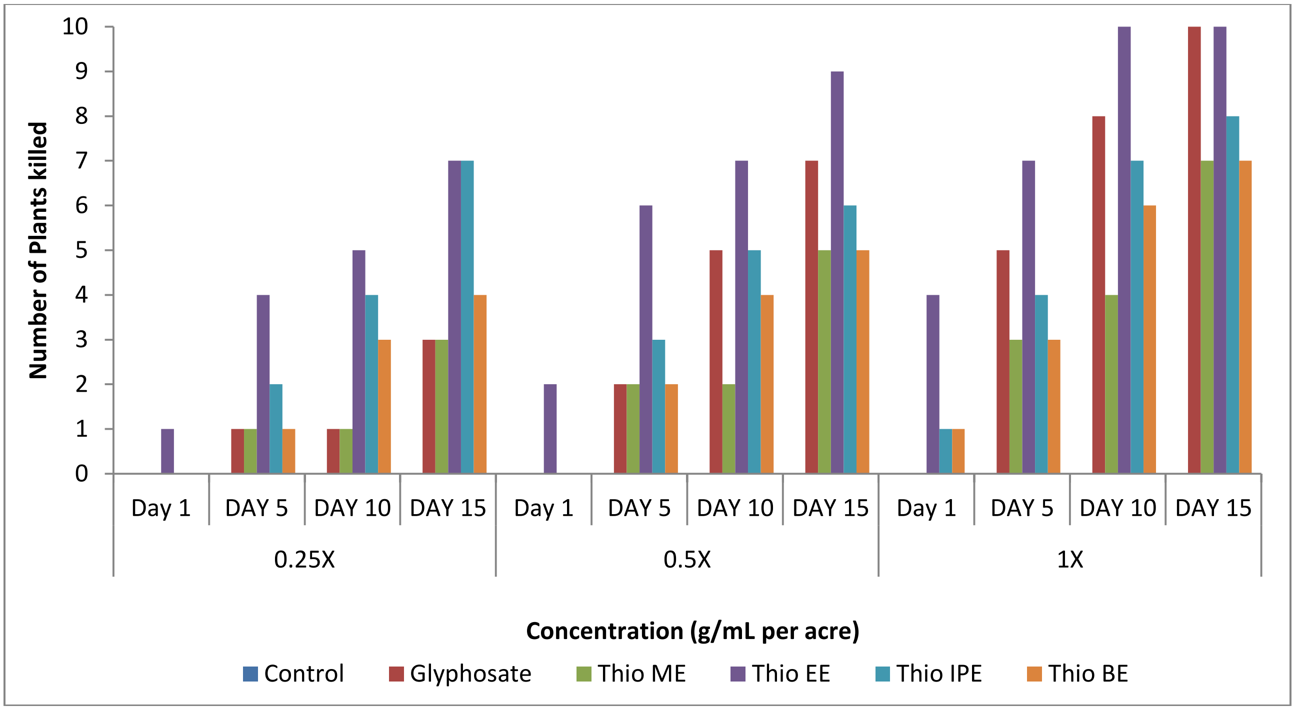


Figure 6.47 Lethal effect of exposure of Thioxylated ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3)
where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Where, Thio ME = O-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thio EE = O-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate,
ThioIPE = O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino)ethanethioate
Thio BE = O-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate

Table 6.12 Effect Thioxylated Ester derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Parthenium hysterophorus*.

Days →		1			5			10			15		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
Chlorophyll a	**Compound**												
	Control	0.389± 0.0015^{a}	0.389± 0.0015^{a}	0.389± 0.0015^{a}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.387± 0.002^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.386± 0.001^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}	0.384± 0.0015^{b}
	Glyphosate	0.365± 0.001^{c}	0.334± 0.002^{d}	0.323± 0.0015^{d}	0.339± 0.00058^{d}	0.242± 0.002^{f}	0.234± 0.0020^{g}	0.279± 0.001^{f}	0.226± 0.0035^{h}	0.217± 0.0020^{h}	0.166± 0.00058^{i}	0.159± 0.001^{i}	0.132± 0.0020^{i}
	O-methyl {[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.371± 0.0025^{c}	0.360± 0.0020^{c}	0.339± 0.003^{d}	0.381± 0.0020^{b}	0.332± 0.0015^{d}	0.254±0. 036^{g}	0.351± 0.0040^{c}	0,305± 0.0028^{b}	0.252± 0.0015^{g}	0.241± 0.0032^{g}	0.204± 0.0025^{h}	0.198± 0.0026^{i}
	O-ethyl{[(diethoxyphosphorothioy) methyl]amino}ethanethioate	0.298± 0.0025^{f}	0.285± 0.0041^{f}	0.265± 0.002^{f}	0.284± 0.0015^{f}	0.264± 0.0011^{f}	0.230± 0.0015^{g}	0.151± 0.0040^{i}	0.139± 0.0035^{i}	0.123± 0.0012^{i}	0.0040± 0.00050j	0.0020± 0.0025^{j}	0.0016± 0.00016^{j}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl] methyl} amino)ethanethioate	0.363± 0.0032^{c}	0.352± 0.0015^{e}	0.320± 0.0035^{d}	0.335± 0.0041^{d}	0.326± 0.0036^{d}	0.299± 0.001^{f}	0.239± 0.0030^{g}	0.216± 0.0015^{h}	0.202± 0.002^{h}	0.156± 0.0025^{i}	0.149± 0.004^{i}	0.126± 0.003^{i}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.364± 0.0020^{c}	0.354± 0.0015^{e}	0.334± 0.0003^{d}	0.342± 0.0020^{e}	0.333± 0.0025^{d}	0.295± 0.0036^{f}	0.253± 0.0030^{g}	0.245± 0.057^{g}	0.184± 0.0025^{i}	0.170± 0.0030^{i}	0.156± 0.0015^{i}	0.134± 0.0025^{i}
Chlorophyll b	**Control**	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.224± 0.0015^{a}	0.208± 0.0015^{b}	0.208± 0.0015^{b}	0.208± 0.0015^{b}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.204± 0.0015^{c}	0.197± 0.0015^{d}	0.197± 0.0015^{d}	0.197± 0.0015^{d}
	Glyphosate	0.222± 0.0015	0.219± 0.0015	0.204± 0.0015	0.205± 0.0040	0.196± 0.0015	0.196± 0.0015	0.183± 0.002	0.159± 0.0026	0.173± 0.0026	0.0973± 0.003	0.059± 0.001	0.053± 0.001
	O-methyl{[(dimethoxyphosphorothioyl) methyl] amino} ethanethioate	0.221± 0.0083^{a}	0.200± 0.00624^{c}	0.199± 0.0068^{d}	0.196± 0.0026^{d}	0.184± 0.0032^{e}	0.165± 0.002^{f}	0.181± 0.0068^{e}	0.166± 0.0025^{f}	0.129± 0.002^{i}	0.0962± 0.00073^{j}	0.095± 0.0026^{m}	0.0758± 0.00326^{l}
	O-ethyl{[(diethoxyphosphorothioyl)methyl] amino} ethanethioate	0.203± 0.0047^{c}	0.194± 0.0045^{d}	0.171± 0.0035^{e}	0.179± 0.007^{e}	0.163± 0.0035^{f}	0.145± 0.002^{h}	0.163± 0.0015^{f}	0.118± 0.0025^{i}	0.0923± 0.0050^{j}	0.0937± 0.00041^{j}	0.0427± 0.0017^{l}	0.020± 0.0036^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl} amino)ethanethioate	0.216± 0.003^{b}	0.204± 0.0032^{c}	0.193± 0.0015^{d}	0.197± 0.0037^{d}	0.177± 0.0026^{g}	0.153± 0.0025^{f}	0.174± 0.0015^{g}	0.158± 0.0073^{f}	0.135± 0.0015^{h}	0.0956± 0.0003^{j}	0.0468± 0.00055^{l}	0.042± 0.0016^{m}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.219± 0.0040^{b}	0.205± 0.002^{c}	0.197± 0.0026^{d}	0.193± 0.0040^{d}	0.183± 0.0015^{e}	0.163± 0.005^{f}	0.185± 0.0032^{e}	0.153± 0.0047^{f}	0.135± 0.0015^{h}	0.1004± 0.00026^{n}	0.0927± 0.00072^{j}	0.073± 0.0010^{o}
Total Chlorophyll	**Control**	0.581± 0.0010^{a}	0.581± 0.0010^{a}	0.581± 0.0010^{a}	0.564± 0.0025^{b}	0.581± 0.0010^{b}	0.564± 0.0025^{b}	0.505± 0.0015^{d}	0.505± 0.0015^{d}	0.505± 0.0015^{d}	0.496± 0.0030^{f}	0.496± 0.0030^{f}	0.496± 0.0030^{f}
	Glyphosate	0.552± 0.0010^{b}	0.542± 0.0015^{c}	0.492± 0.0011^{f}	0.446± 0.0040^{g}	0.397± 0.0020^{i}	0.394± 0.0015^{h}	0.308± 0.0096^{i}	0.283± 0.0025^{j}	0.252± 0.0026^{j}	0.242± 0.0130^{j}	0.213± 0.0015^{k}	0.194± 0.0020^{m}

	O-methyl {[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.548± 0.004^{c}	0.536± 0.0171^{c}	0.514± 0.003^{e}	0.441± 0.0132^{g}	0.450± 0.0206^{g}	0.412± 0.0035^{i}	0.303± 0.0079^{i}	0.258± 0.0124^{j}	0.248± 0.0066^{j}	0.262± 0.0058^{j}	0.218± 0.0015^{k}	0.205± 0.002^{l}
	O-ethyl{[(diethoxyphosphorothioyl)methyl] amino}ethanethioate	0.508± 0.0096^{e}	0.465± 0.0291^{g}	0.433± 0.0032^{g}	0.409± 0.0075^{h}	0.400± 0.003^{h}	0.358± 0.0077^{n}	0.251± 0.0015^{j}	0.242± 0.0121^{j}	0.154± 0.0020^{m}	0.222± 0.0036k	0.192± 0.0055l	0.136± 0.003^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl} amino)ethanethioate	0.533± 0.0035^{c}	0.519± 0.0045^{e}	0.507± 0.004^{e}	0.444± 0.0030^{g}	0.445± 0.0189^{g}	0.399± 0.0025^{h}	0.283± 0.006^{j}	0.261± 0.0102^{j}	0.228± 0.0049^{k}	0.237± 0.00814^{k}	0.207± 0.0015^{k}	0.190± 0.0121^{m}
	O-butyl{[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.536± 0.0176^{c}	0.518± 0.0025^{e}	0.511± 0.0062^{e}	0.478± 0.0537^{g}	0.424± 0.0015^{g}	0.406± 0.0052^{h}	0.270± 0.0168^{j}	0.263± 0.0132^{j}	0.242± 0.0032^{k}	0.256± 0.0090^{k}	0.229± 0.0055^{k}	0.201± 0.0075^{l}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.1.6 Metal Complexes of Glyphosate: Five different metal complexes of glyphosate were synthesized and their herbicidal activity was checked in context of decrease in the chlorophyll content in the leaves of weed (*Parthenium hysterophorus*). Also the lethal effect of these derivatives on the plants was measured by counting the number of plants killed with respect to time and concentration. As compared to control all the metal complexes have shown decrease in photosynthetic pigments ('Chl a', 'Chl b'and 'Chl a+b') but no considerable decrease was found in comparison to glyphosate. No significant trend in the decrease of chlorophyll content was observed. Out of all the synthesized metal complexes of glyphosate, greater decrease in chlorophyll pigments was observed in case of **Co-glyphosate complex** and **Cu-glyphosate complex** at all test concentrations. In case of **Co-glyphosate complex**, at 0.25X, 'chlorophyll a' left in the plant after 15 days was found to be 0.215µg/gFW in comparison to control ('chlorophyll a' =0.355 µg/gFW). Similarly in case of **Cu-glyphosate complex** 'chlorophyll a' left was 0.33 µg/gFW **(Figure 6.48 (a))**. Similar results were noticed with 0.5X and 1X after 15 days (**Figure 6.48 (b) ,(c)**). In case of **Co-glyphosate complex**, 'chlorophyll b' left in the leaves of the treated plants was found to 0.144 µg/gFW at 0.25X, 0.125 µg/gFW at 0.5X and 0.0846 µg/gFW at 1X (**Figure 6.49 (a-c)**). However similar outcome was also perceived in case of 'total chlorophyll' (**Figure 6.50 (a-c)**). **Cu-glyphosate complex** has also shown its herbicidal effects by decreasing the chlorophyll content of the plant. 'Chl a,'Chl b' and 'Chl a+b'content was significantly reduced with increase in concentration. At 0.25X 'chlorophyll a' was 0.300 µg/gFW which get reduced to 0.136 µg/gFW at 1X after 15 days. At 0.25X 'chlorophyll b' was 0.172 µg/gFW and at IX it was further reduced to 0.0732 µg/gFW) and 'total chlorophyll' at 0.25X was found to be 0.297 µg/gFW and at 1X it was 0.202 µg/gFW).

Herbicidal effect of the synthesized metal complexes of glyphosate was also evaluated by counting the number of plants killed by these derivatives. Maximum number of plants were killed only at the recommended dose (1X) by the **Co-glyphosate complex** (7 out of 10) (**Figure 6.51**). Other derivatives didn't show any momentous reduction in the number of plants upon their exposure to these derivatives. No major difference in the results with respect to each other and control were obtained at the lower concentrations of these derivatives.

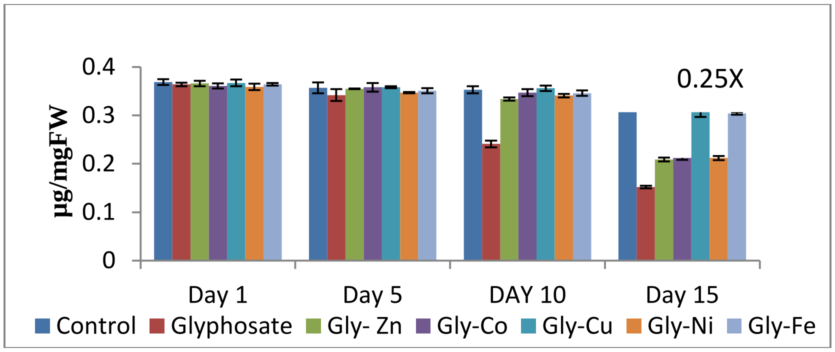


Figure a

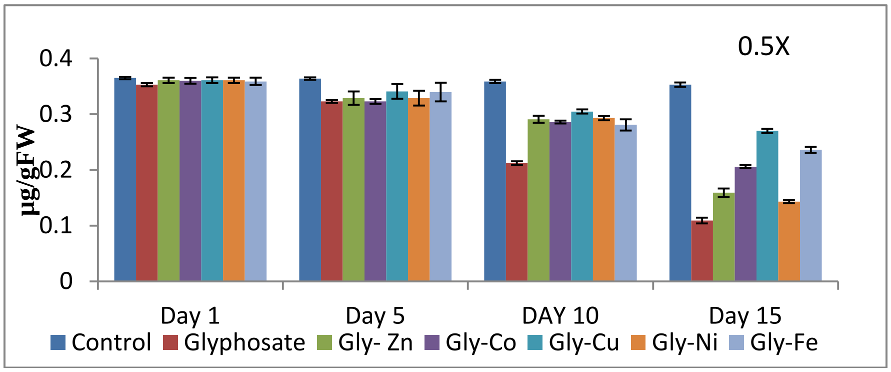


Figure b

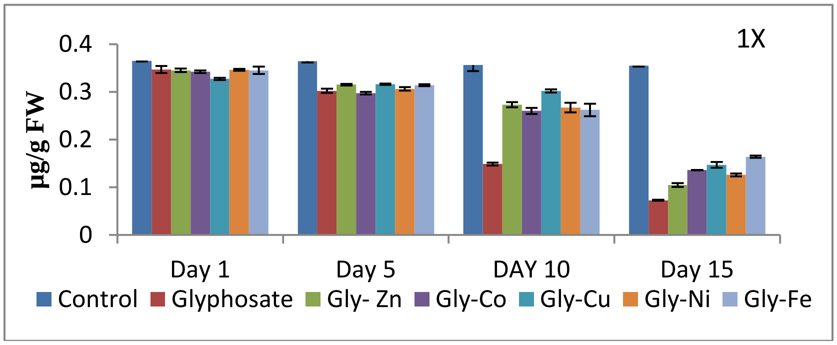


Figure c

Figure 6.48(a)-(c) Effect of metal complexes of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X, 0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Gly-Zn= Glyphosate-Zn complex; Gly-Co= Glyphosate-Co complex; Gly-Cu= Glyphosate-Cu complex; Gly-Ni= Glyphosate-Ni complex; Gly-Fe= Glyphosate-Fe complex

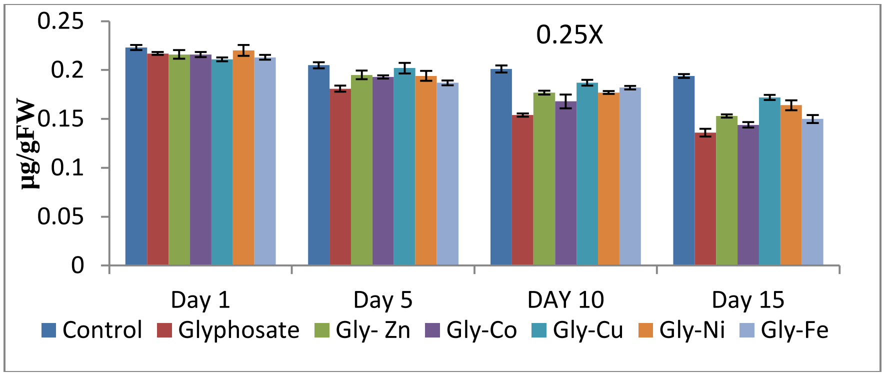


Figure a

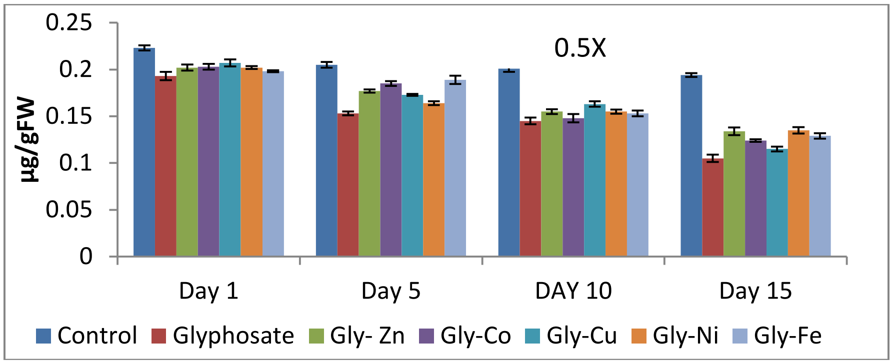


Figure b

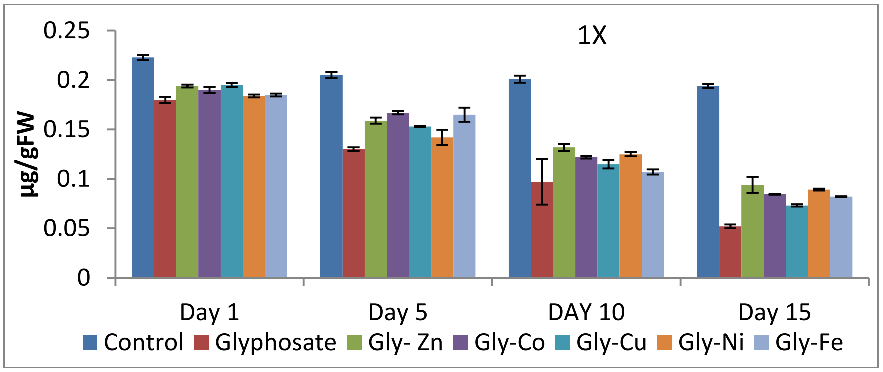


Figure c

Figure 6.49(a)-(c) Effect of metal complexes of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X, 0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Gly-Zn= Glyphosate-Zn complex; Gly-Co= Glyphosate-Co complex; Gly-Cu= Glyphosate-Cu complex; Gly-Ni= Glyphosate-Ni complex; Gly-Fe= Glyphosate-Fe complex

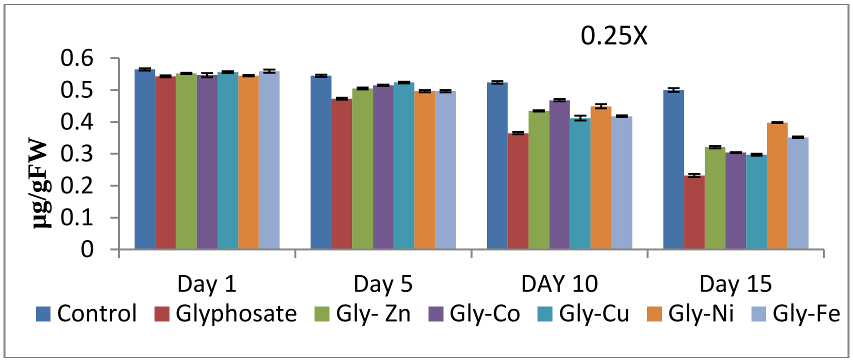


Figure a

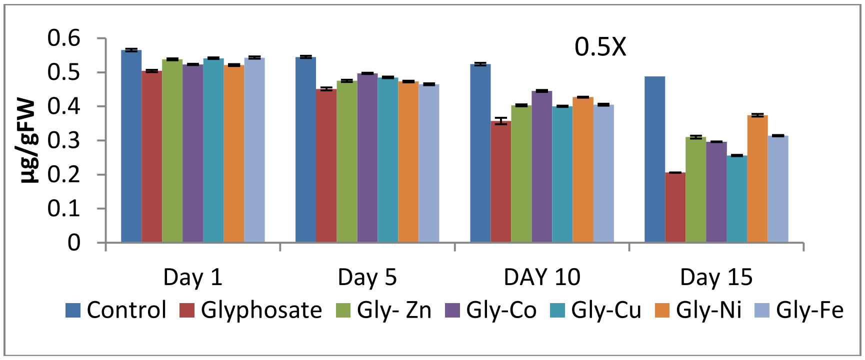


Figure b

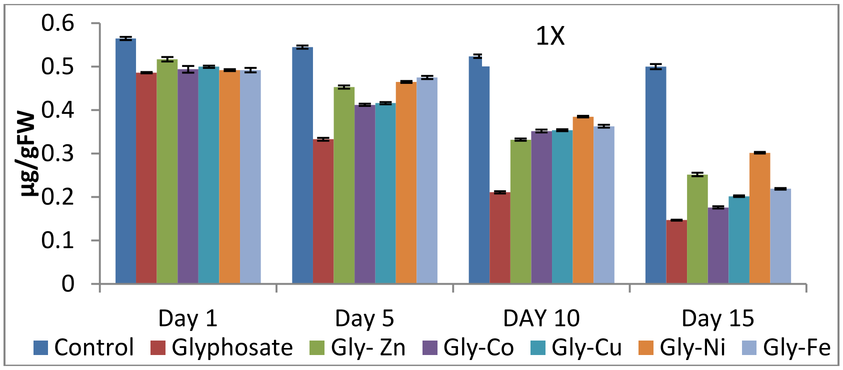


Figure c

Figure 6.50 (a)-(c) Effect of metal complexes of glyphosate on total chlorophyll content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Gly-Zn= Glyphosate-Zn complex; Gly-Co= Glyphosate-Co complex; Gly-Cu= Glyphosate-Cu complex; Gly-Ni= Glyphosate-Ni complex; Gly-Fe= Glyphosate-Fe complex

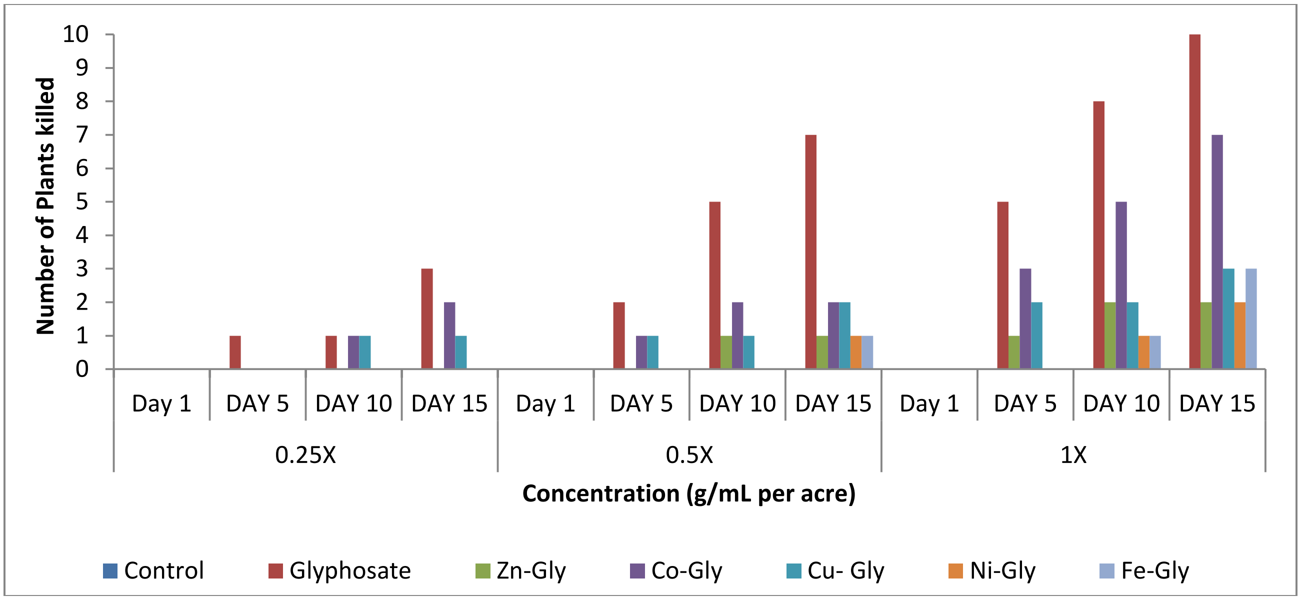
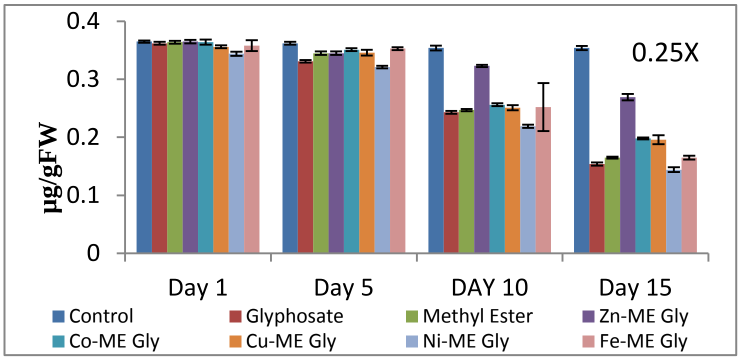


Figure 6.51 Lethal effect of exposure of Metal complexes of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3)
where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose. Zn-Gly= Glyphosate-Zn complex; Co-Gly = Glyphosate-Co complex; Cu-Gly = Glyphosate-Cu complex; Ni-Gly = Glyphosate-Ni complex; Fe-Gly = Glyphosate-Fe complex

6.3.1.7 Metal Complexes of Methyl Ester Glyphosate: A series of five different metal complexes of methyl ester derivative of glyphosate were synthesized. Their herbicidal effect was checked by determining the chlorophyll content left in the weed (*Parthenium hysterophorus*) after their exposure to the synthesized derivatives and the mortality rate of plant (weed) with respect to time and concentration. All the synthesized derivatives showed moderate to good herbicidal effects on the plant. Out of these derivatives, **Ni-methyl ester derivative of glyphosate complex** (Ni-ME) has shown preeminent herbicidal activity against the weed (*Parthenium hysterophorus*). At 0.25X 'chlorophyll a' left in the plant (after 15 days treatment) was 0.144µg/gFW whereas 'chlorophyll a' left in the plant treated with glyphosate was 0.154 µg/gFW. Similarly at 0.5X, 'chlorophyll a' was reduced to 0.098 µg/gFW in case of plants processed with Ni-ME and 0.107 µg/gFW in case of glyphosate. Major decrease in 'chlorophyll a' was recorded at the highest dose of 1X (0.0552 µg/gFW in case of Ni-ME complex and 0.0730 µg/gFW in case of glyphosate) (**Figure 6.52 (a-c))** . Diminution in 'chlorophyll b' content at all test concentrations was also recorded in case of Ni-ME as compared to control and glyphosate **(Figure 6.53 (a-c)).** Major decrease in' total chlorophyll' content of the weed was also observed at the highest dose of 1X after 15 days treatment with Ni-ME complex.' Total chlorophyll' left was found to be 0.126 µg/gFW in case of plant treated with Ni-ME complex and 0.146 µg/gFW in case of glyphosate (**Figure 6.54 (a-c)**).

Detrimental effects of the synthesized metal complexes of methyl ester derivatives of glyphosate on the weed were also scrutinized from the mortality rate of the plants. After 15 days treatment it has been found that Ni-ME complex has killed highest number of weed plants as compared to glyphosate. Even at 0.5X it has killed 9 out of 10 plants whereas glyphosate killed only7 plants. Even at 1X, all plants were killed after 10 days in case of Ni-ME complex whereas glyphosate killed only 8 plants (**Figure 6.55**). From the comparison between the herbicidal effects of both these herbicides, it has been found that Ni-ME complex was more effective in decreasing the chlorophyll content and killing maximum number of weed plants as compared to the glyphosate.



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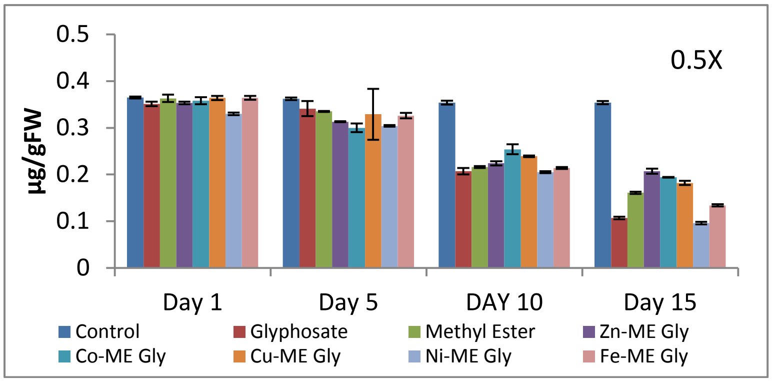


Figure b

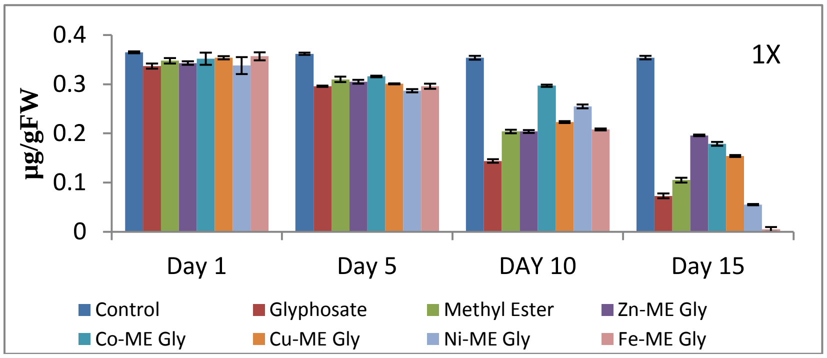
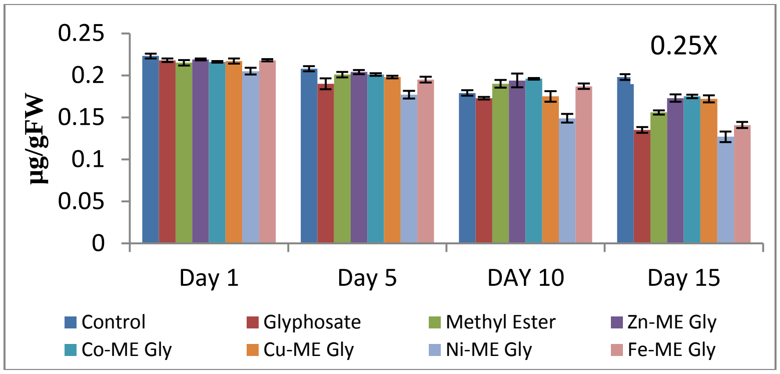


Figure c

Figure 6.52 (a)-(c) Effect of metal complexes of methyl ester derivative of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X, 0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-ME Gly=Zn-Methyl ester derivative of glyphosate; Co-ME Gly= Co-Methyl ester derivative of glyphosate; Cu-ME Gly= Cu-Methyl ester derivative of glyphosate; Ni-ME Gly= Ni-Methyl ester derivative of glyphosate; Fe-ME Gly=Fe-Methyl ester derivative of glyphosate



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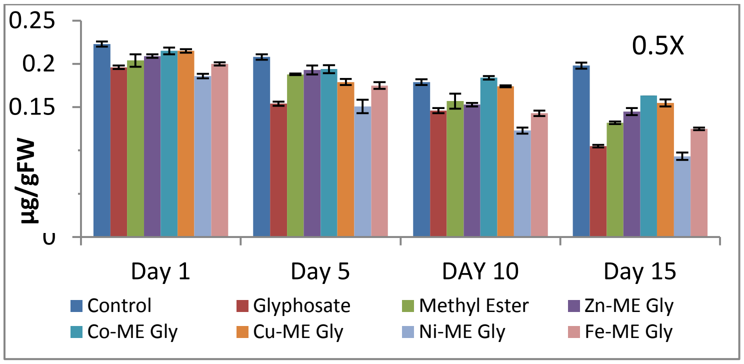

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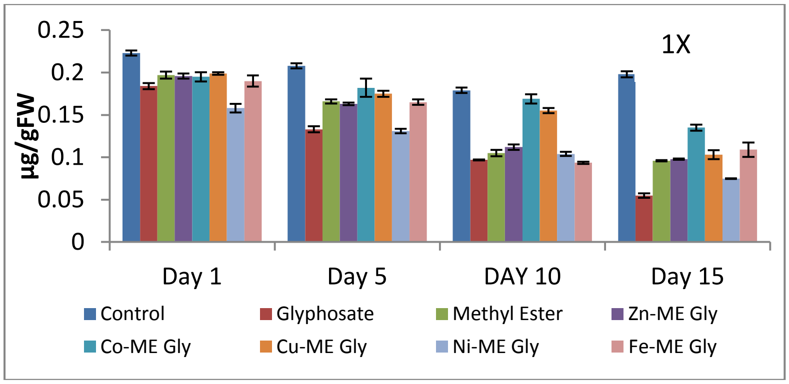
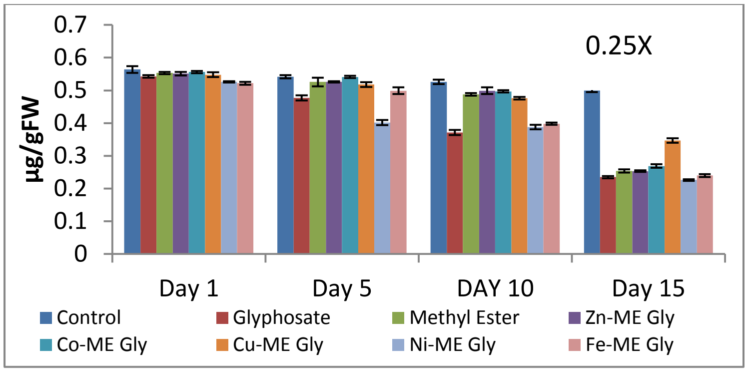


Figure c

Figure 6.53(a)-(c) Effect of metal complexes of methyl ester derivative of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X, 0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-ME Gly=Zn-Methyl ester derivative of glyphosate; Co-ME Gly= Co-Methyl ester derivative of glyphosate; Cu-ME Gly= Cu-Methyl ester derivative of glyphosate; Ni-ME Gly= Ni-Methyl ester derivative of glyphosate; Fe-ME Gly=Fe-Methyl ester derivative of glyphosate



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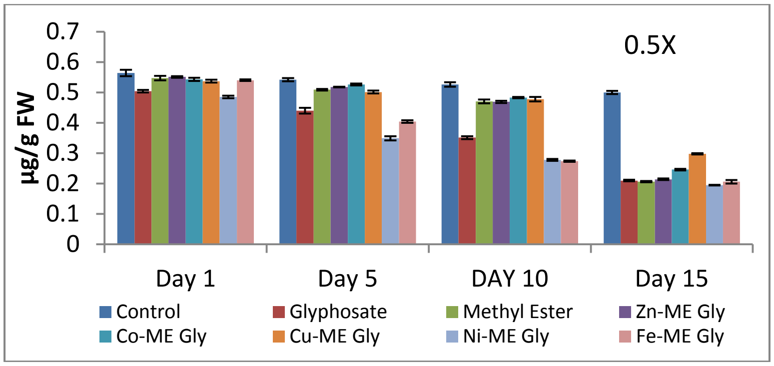

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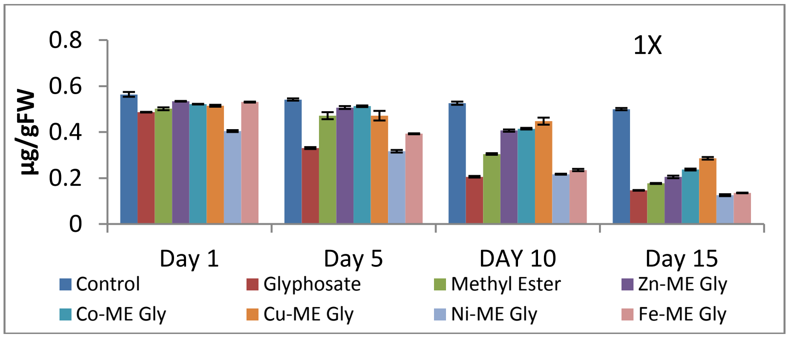


Figure c

Figure 6.54 (a)-(c) Effect of metal complexes of methyl ester derivative of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X, 0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-ME Gly=Zn-Methyl ester derivative of glyphosate; Co-ME Gly= Co-Methyl ester derivative of glyphosate; Cu-ME Gly= Cu-Methyl ester derivative of glyphosate; Ni-ME Gly= Ni-Methyl ester derivative of glyphosate; Fe-ME Gly=Fe-Methyl ester derivative of glyphosate

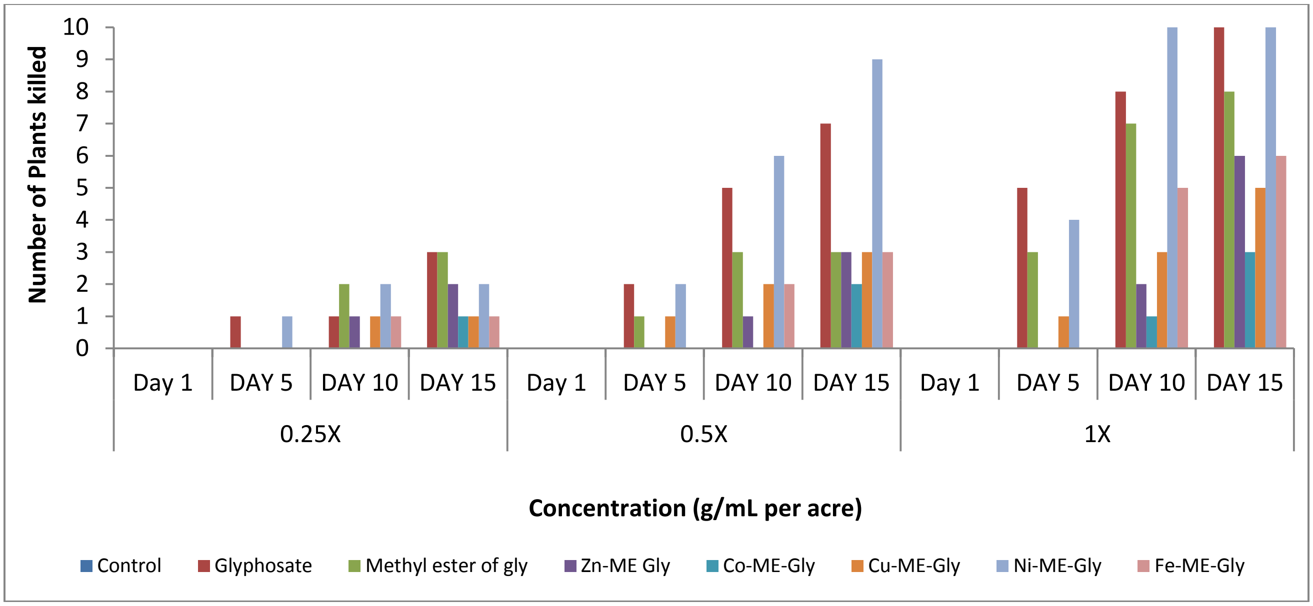


Figure 6.55 Lethal effect of exposure of Metal complexes of methyl ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3)

where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

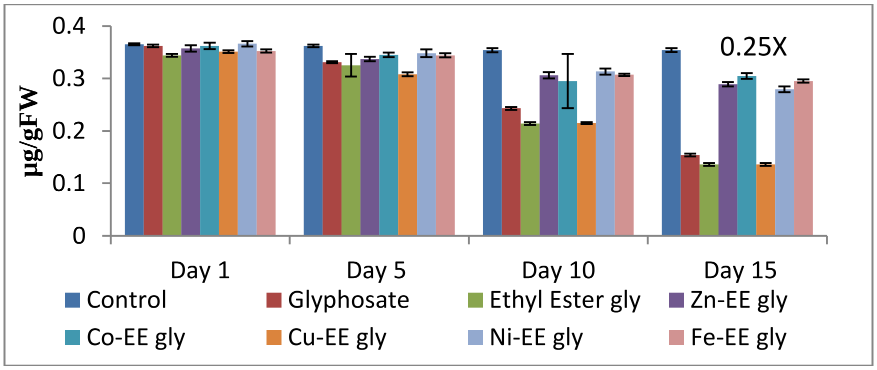
Zn-ME Gly=Zn-Methyl ester derivative of glyphosate; Co-ME Gly= Co-Methyl ester derivative of glyphosate; Cu-ME Gly= Cu-Methyl ester derivative of glyphosate; Ni-ME Gly= Ni-Methyl ester derivative of glyphosate; Fe-ME Gly=Fe-Methyl ester derivative of glyphosate

6.3.1.8 Metal Complexes of Ethyl ester of glyphosate:

Herbicidal effects of the synthesized metal complexes of ethyl ester derivative of glyphosate was evaluated on the common weed (*Parthenium hysterophorus*). Decrease in the content of photosynthetic pigments was recorded with respect to concentration of the derivative and time. In comparison to control (water treatment) all the five metal complexes showed diminution in the chlorophyll content. However this decrease in the chlorophyll content of treated plants was not comparable to glyphosate. Ethyl ester derivative of glyphosate has caused tremendous reduction in the chlorophyll content of treated plants at all the test concentrations (0.25X, 0.5X and 1X). Out of all the five metal complexes of ethyl ester derivative of glyphosate, **Cu-ethyl ester derivative of glyphosate complex** (Cu-EE gly) has momentously decreased the amount of 'Chl a,'Chl b' and 'Chl a+b'at all the three concentrations. At 0.25X 'chlorophyll a' left in the plant after 15 days treatment was 0.136µg/g FW and it get further reduced to 0.123 µg/g FW at 0.5X . By using the highest concentration of this derivative (i.e., at 1X) large decrease in the 'chlorophyll a' content was observed (0.105 µg/g FW) (**Figure 6.56 (a-c))**.'Chlorophyll b' left in the plants (after 15 days) treated with 0.25X of Cu-EE gly was 0.154 µg/g FW, with 0.5X of Cu-EE gly was 0.149 µg/g FW and with 1X of Cu-EE gly was 0.125 µg/g FW **(Figure 6.57 (a-c))**. Similar kind of reduction was also noticed in case of 'total chlorophyll' content of the treated plants **(Figure 6.58 (a-c))**.

Apart from Cu-EE gly, other metal complexes have also caused reduction in the chlorophyll content of the weed but their results are less significant as compared to glyphosate and Cu-EE gly.

In spite of reduction in the photosynthetic pigments, herbicidal activity of the metal complexes of ethyl ester derivative of glyphosate was also assessed based on the number of plants killed after 15 days. In comparison to glyphosate, ethyl ester of glyphosate has killed maximum number of plants . However the **Cu-ethyl ester derivative of glyphosate** has also shown analogous results. It has killed 5 out of 10 plants at 0.5X after 10 days of treatment. However at the same concentration after 15 days, 6 plants were killed. At the highest concentration (1X) 9 plants out of 10 were killed by the complex **(Figure 6.59).** These results showed that apart from ethyl ester derivative of glyphosate, Cu-EE gly has also showed good herbicidal effects on the weed plants.



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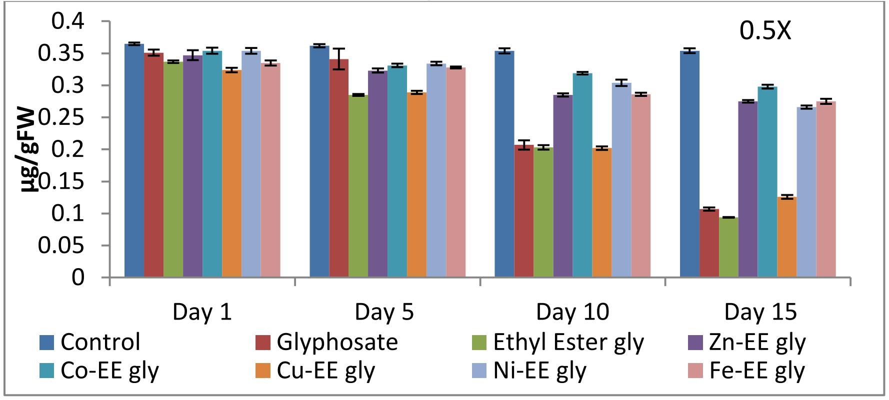


Figure b

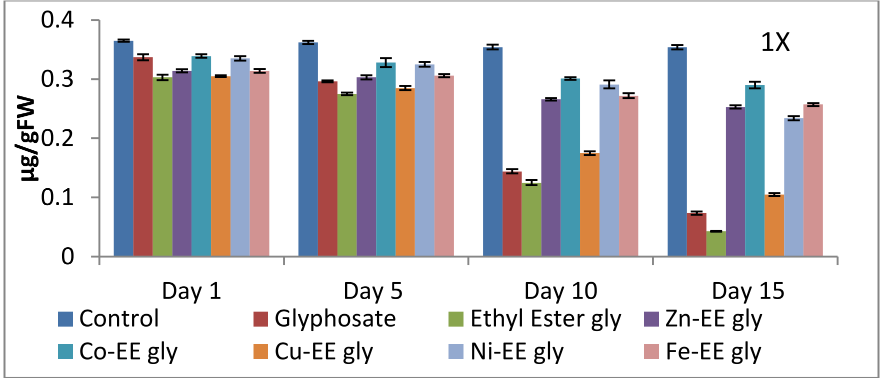


Figure c

Figure 6.56 (a)-(c) Effect of metal complexes of ethyl ester derivative of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-EE Gly=Zn-Ethyl ester derivative of glyphosate; Co-EE Gly= Co-Ethyl ester derivative of glyphosate; Cu-EE Gly= Cu-Ethyl ester derivative of glyphosate; Ni-EE Gly= Ni-Ethyl ester derivative of glyphosate; Fe-EE Gly=Fe-Ethyl ester derivative of glyphosate

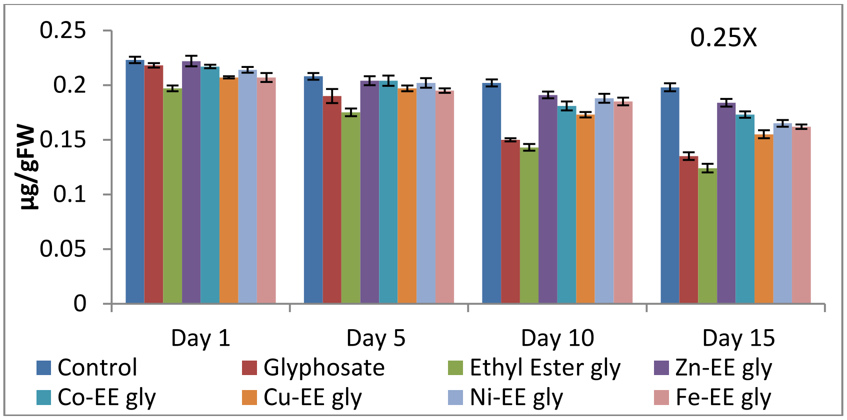
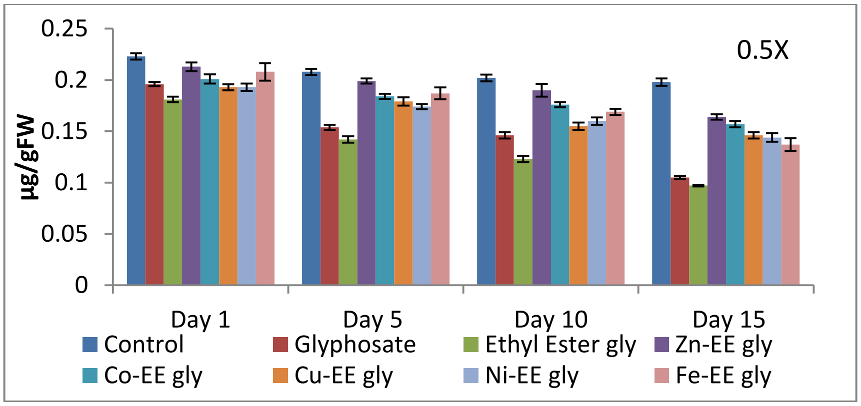


Figure a



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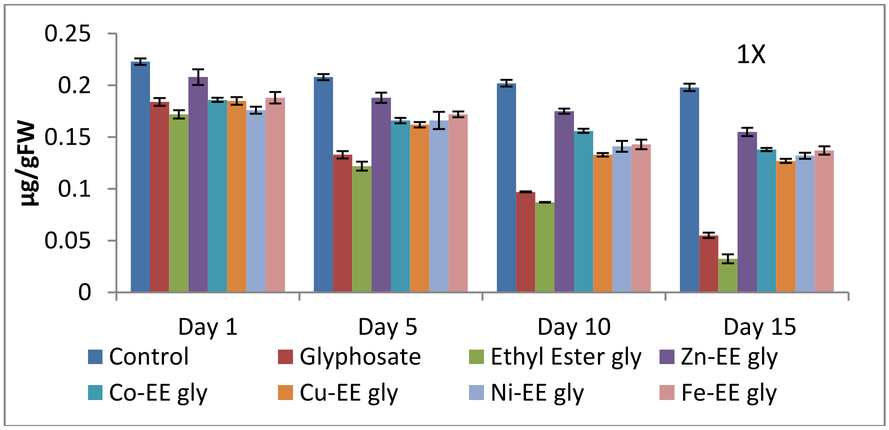
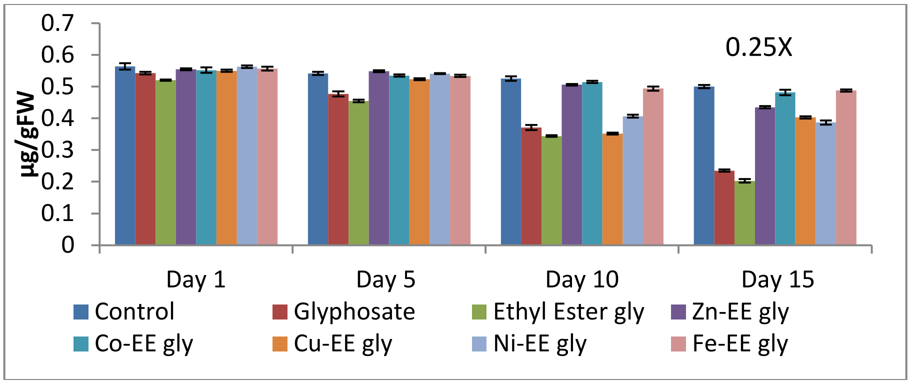


Figure c

Figure 6.57 (a)-(c) Effect of metal complexes of ethyl ester derivative of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-EE Gly=Zn-Ethyl ester derivative of glyphosate; Co-EE Gly= Co-Ethyl ester derivative of glyphosate; Cu-EE Gly= Cu-Ethyl ester derivative of glyphosate; Ni-EE Gly= Ni-Ethyl ester derivative of glyphosate; Fe-EE Gly=Fe-Ethyl ester derivative of glyphosate



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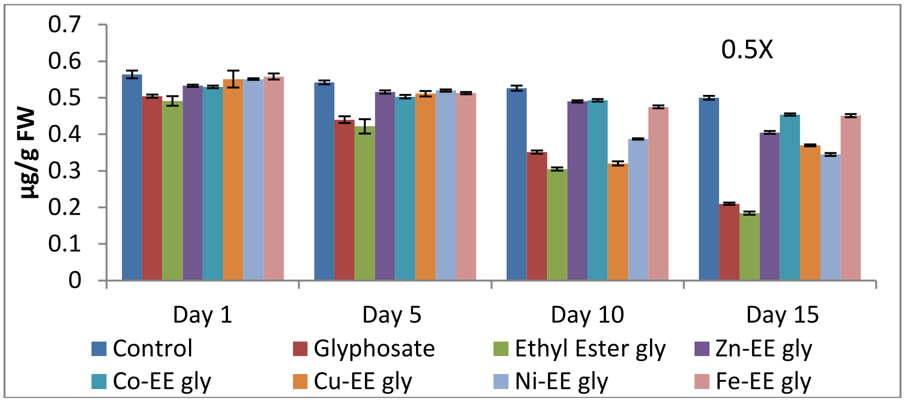

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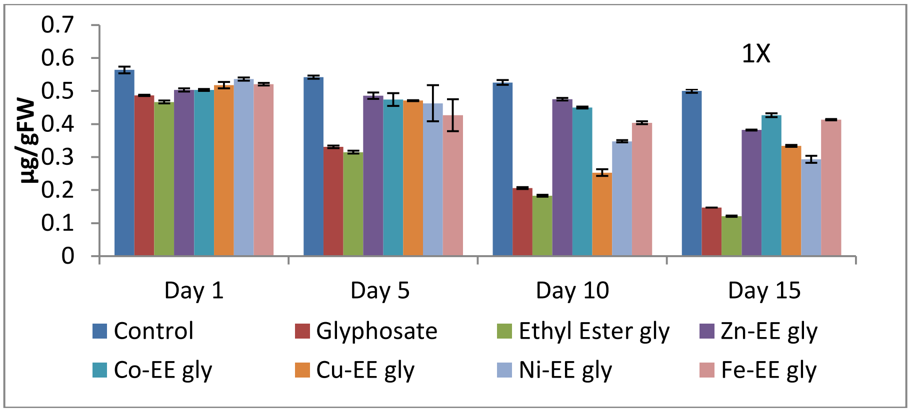


Figure c

Figure 6.58 (a)-(c) Effect of metal complexes of ethyl ester derivative of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-EE Gly=Zn-Ethyl ester derivative of glyphosate; Co-EE Gly= Co-Ethyl ester derivative of glyphosate; Cu-EE Gly= Cu-Ethyl ester derivative of glyphosate; Ni-EE Gly= Ni-Ethyl ester derivative of glyphosate; Fe-EE Gly=Fe-Ethyl ester derivative of glyphosate

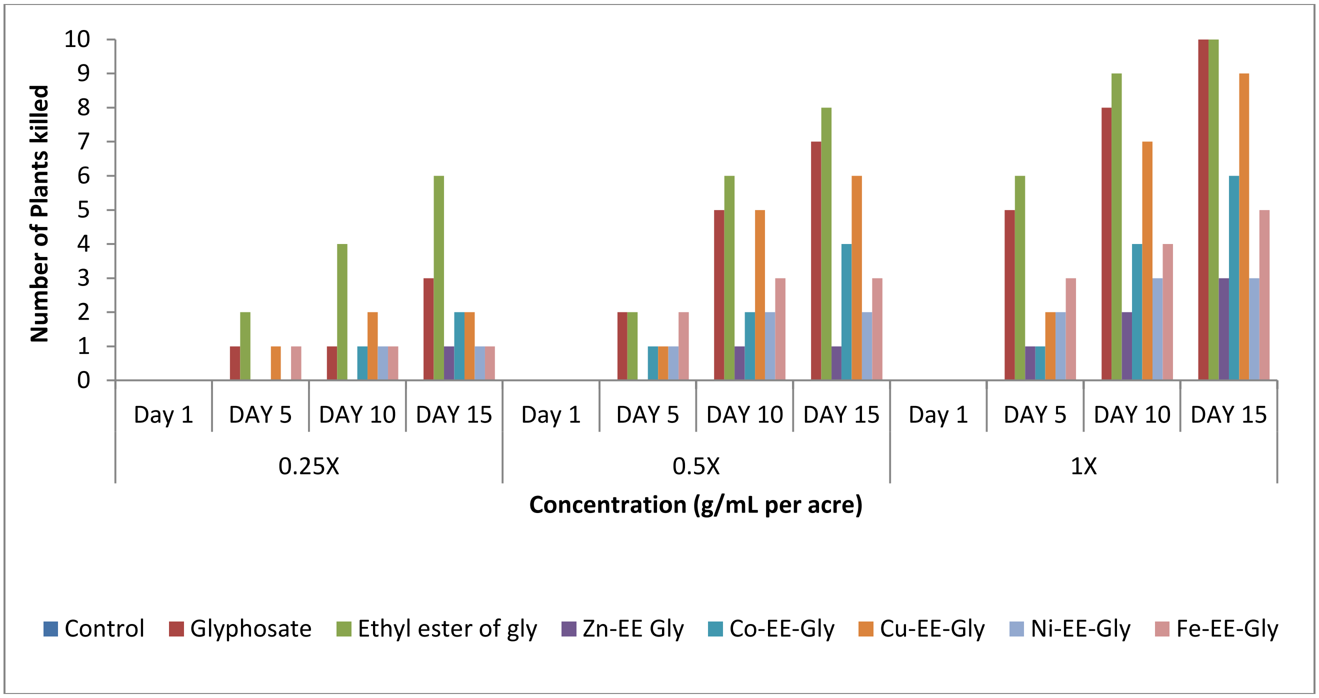


Figure 6.59 Lethal effect of exposure of metal complexes of ethyl ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3)

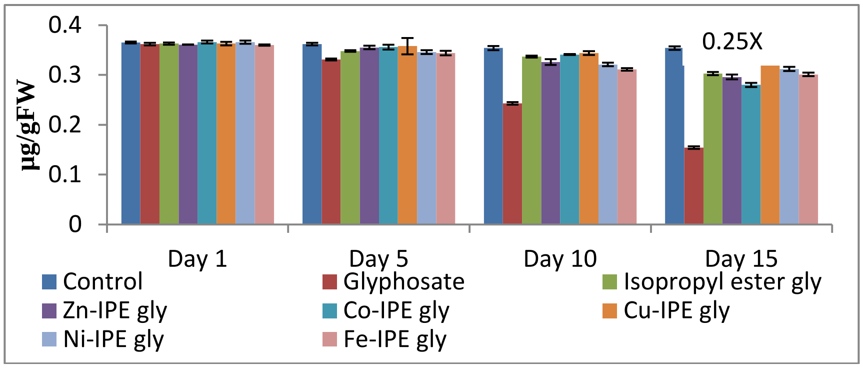
where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). Zn-EE Gly=Zn-Ethyl ester derivative of glyphosate; Co-EE Gly= Co-Ethyl ester derivative of glyphosate; Cu-EE Gly= Cu-Ethyl ester derivative of glyphosate; Ni-EE Gly= Ni-Ethyl ester derivative of glyphosate; Fe-EE Gly=Fe-Ethyl ester derivative of glyphosate

6.3.1.9 Metal Complexes of Isopropyl ester of glyphosate:

Herbicidal effect of five different metal complexes of isopropyl ester derivative of glyphosate was scrutinized on the weed (*Parthenium hysterophorus*). Plants were treated with three different concentrations (0.25X, 0.5X and 1X) of the derivatives and chlorophyll content from their leaves was evaluated. None of the derivative have shown better results as compared to glyphosate. Very little decrease in the chlorophyll pigments has been noticed in all cases. In comparison to control, only a slight reduction in 'chlorophyll a' was noticed at all the test concentrations even after 15 days. Amongst all the derivatives, **Fe-Isopropyl ester derivative of glyphosate** (Fe-IPE gly), has shown slight reduction in 'chlorophyll a' content (0.311µg/g FW) in comparison to control (0.354µg/g FW)(**Figure 6.60 (a-c)**). However no significant reduction was further noticed even at higher concentrations [at 0.5X (0.293 µg/gFW) and at 1X (0.262 µg/gFW). 'Chlorophyll b' and 'total chlorophyll' content also depicted similar kind of trend. For Fe-IPE gly at 0.25X, 'chlorophyll b' found was 0.148 µg/gFW and it was further reduced to

0.122 µg/gFW at 1X **(Figure 6.61 (a-c)) (Figure 6.62 (a-c))**. Not much change in the photosynthetic pigments was observed in other metal complexes of iso propyl ester derivative of glyphosate.

Thus, its noteworthy to mention here that none of the metal complexes of isopropyl ester of glyphosate has good herbicidal properties and none of them was effective in killing the weeds **(Figure 6.63)**.



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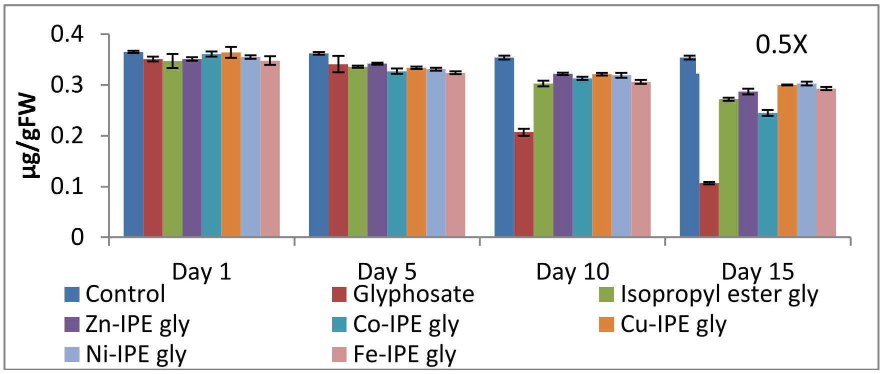

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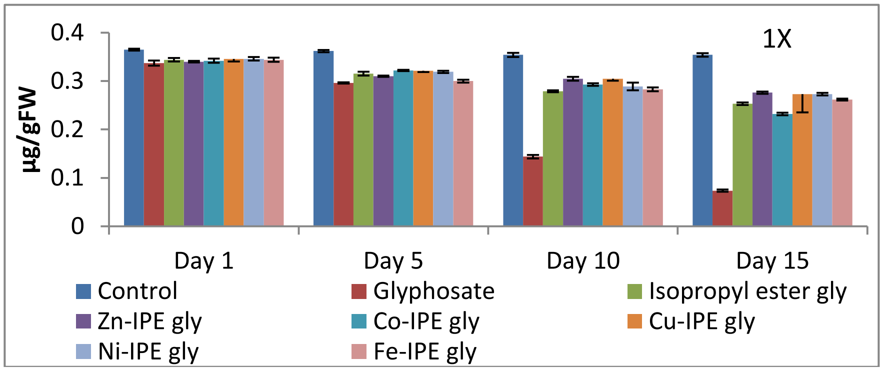
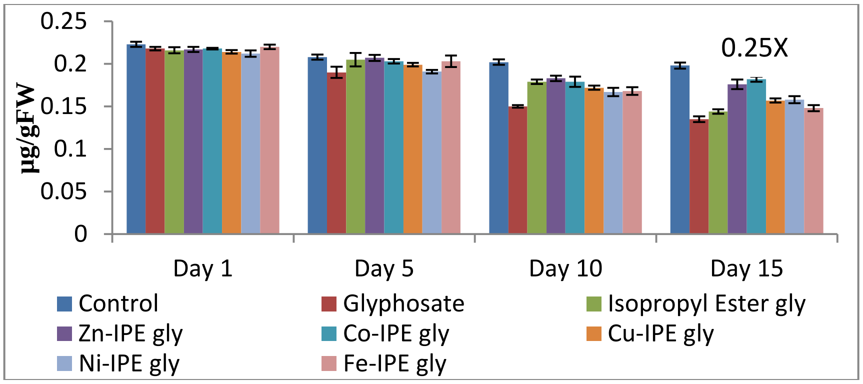


Figure c

Figure 6.60 (a)-(c) Effect of metal complexes of Isopropyl ester derivative of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-IPE Gly=Zn-Isopropyl ester derivative of glyphosate; Co-IPE Gly= Co-Isopropyl ester derivative of glyphosate; Cu-IPE Gly= Cu-Isopropyl ester derivative of glyphosate; Ni-IPE Gly= Ni-Isopropyl ester derivative of glyphosate; Fe-IPE Gly=Fe-Isopropyl ester derivative of glyphosate



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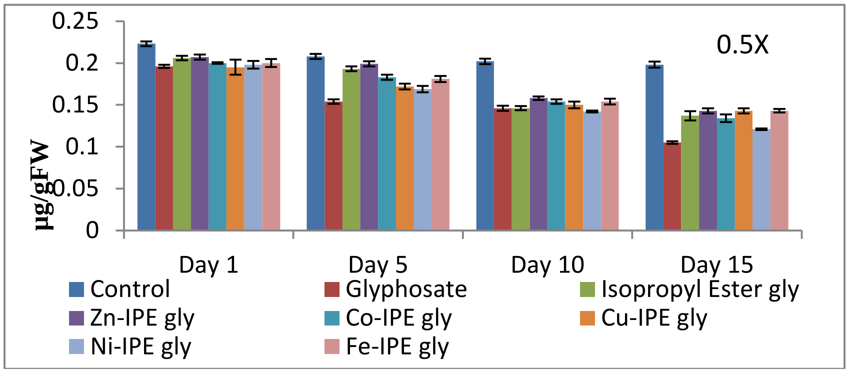

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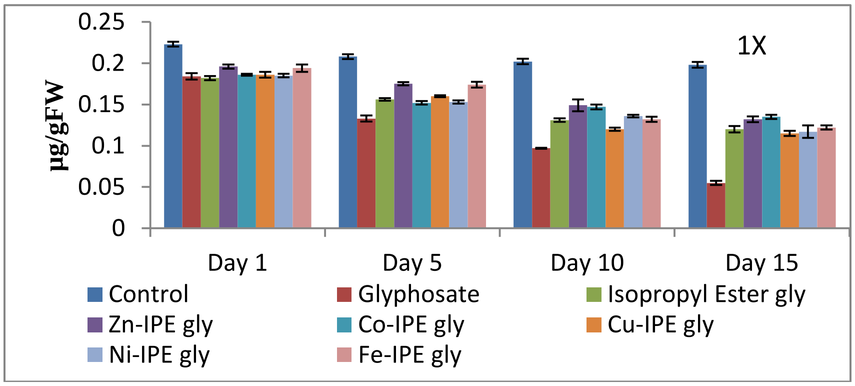
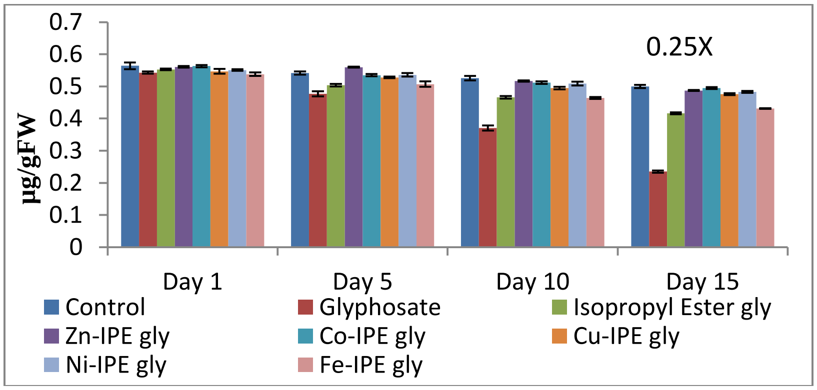


Figure c

Figure 6.61(a)-(c) Effect of metal complexes of Isopropyl ester derivative of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-IPE Gly=Zn-Isopropyl ester derivative of glyphosate; Co-IPE Gly= Co-Isopropyl ester derivative of glyphosate; Cu-IPE Gly= Cu-Isopropyl ester derivative of glyphosate; Ni-IPE Gly= Ni-Isopropyl ester derivative of glyphosate; Fe-IPE Gly=Fe-Isopropyl ester derivative of glyphosate



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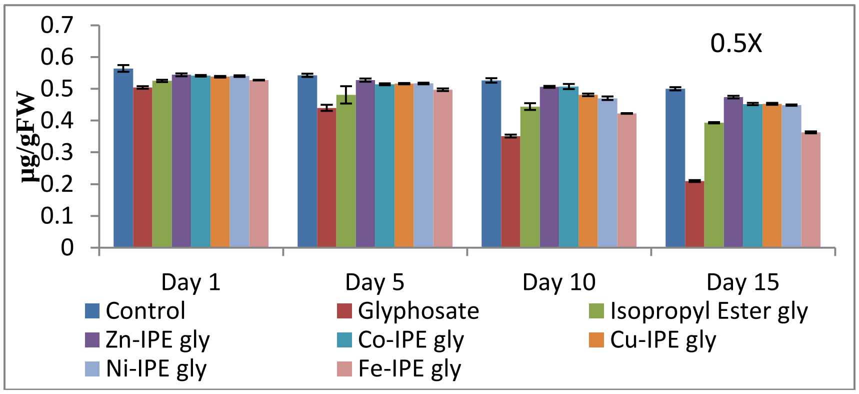

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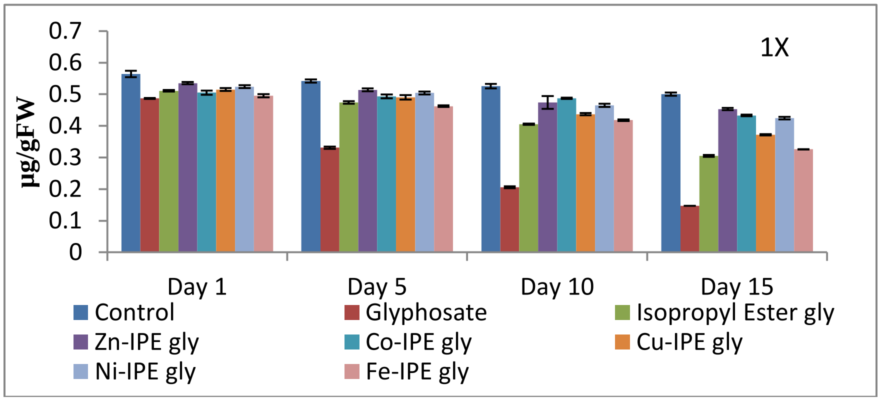


Figure c

Figure 6.62 (a)-(c) Effect of metal complexes of Isopropyl ester derivative of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-IPE Gly=Zn-Isopropyl ester derivative of glyphosate; Co-IPE Gly= Co-Isopropyl ester derivative of glyphosate; Cu-IPE Gly= Cu-Isopropyl ester derivative of glyphosate; Ni-IPE Gly= Ni-Isopropyl ester derivative of glyphosate; Fe-IPE Gly=Fe-Isopropyl ester derivative of glyphosate

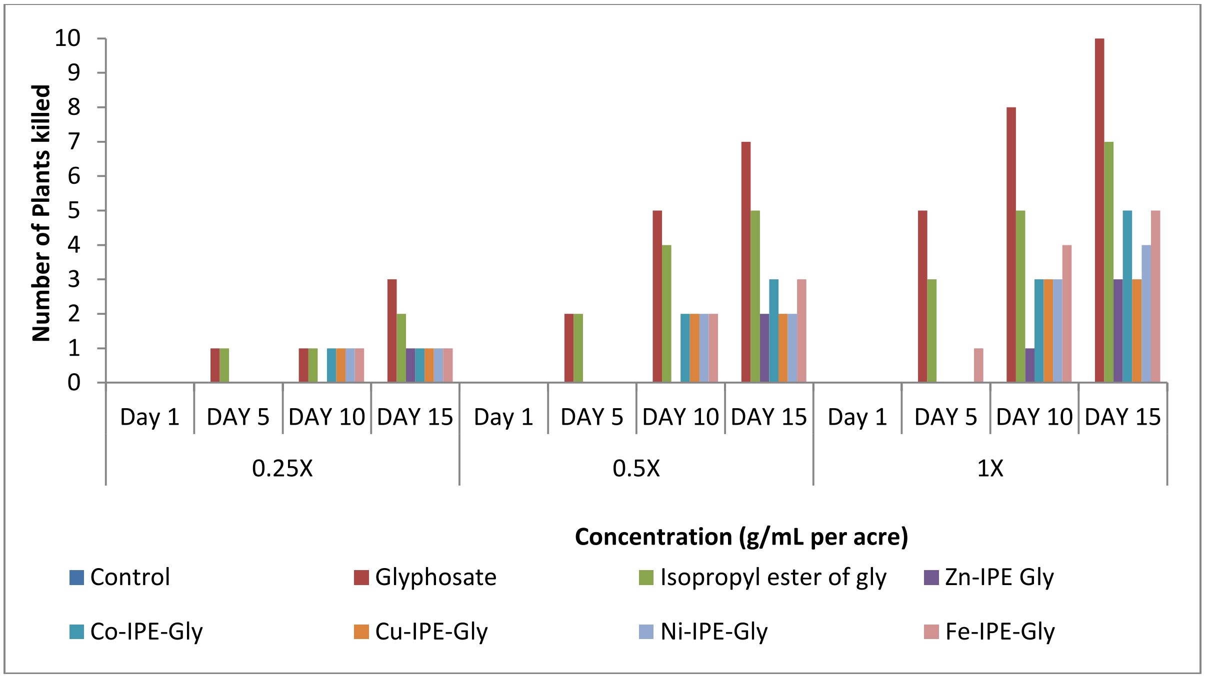


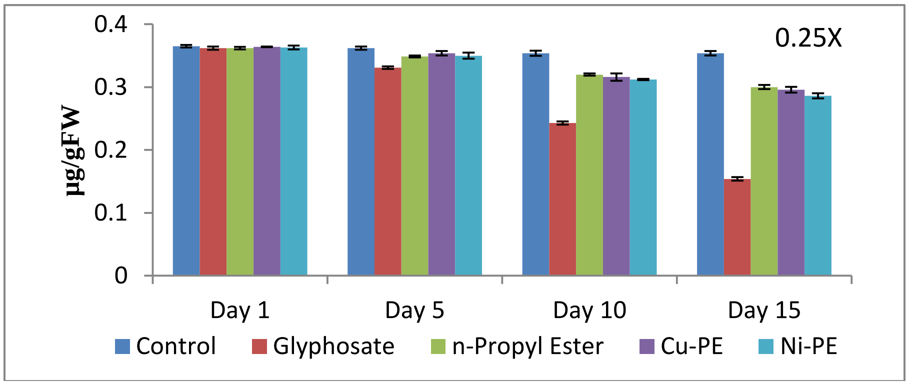
Figure 6.63 Lethal effect of exposure of metal complexes of Isopropyl ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3)

where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). Zn-IPE Gly=Zn-Isopropyl ester derivative of glyphosate; Co-IPE Gly= Co-Isopropyl ester derivative of glyphosate; Cu-IPE Gly= Cu-Isopropyl ester derivative of glyphosate; Ni-IPE Gly= Ni-Isopropyl ester derivative of glyphosate; Fe-IPE Gly=Fe-Isopropyl ester derivative of glyphosate

6.3.1.10 Metal Complexes of n-Propyl ester of glyphosate:

Herbicidal effects of the metal complexes of n-Propyl ester derivative of glyphosate was also studied on *Parthenium hysterophorus*. Reduction in the chlorophyll content was recorded in the treated plants for 15 days and was compared with control and glyphosate treatment. A remarkable decline of photosynthetic pigments was recorded in the plants treated with the derivatives. Also a notable decrease in the number of weed plants (plants killed) was recorded after their treatment with the derivatives at different concentrations. After 15 days, at 0.25X **Ni-Propyl ester of glyphosate** (Ni-PE) has effectively decreased the 'chlorophyll a' (0.286µg/gFW), 'chlorophyll b' (0.136 µg/gFW) and 'total chlorophyll' (0.473 µg/gFW) **(Figure 6.64 (a-c), Figure 6.65 (a-c) and (Figure 6.65 (a-c))**. Significant decline was also observed at 0.5X and 1X concentrations ['chlorophyll b' left in the plant was found to be 0.109 µg/gFW at 0.5X and 0.0904 µg/gFW at 1X]**.** However, Cu complex of n-propyl ester of glyphosate (Cu-PE) has also caused noteworthy decrease in chlorophyll content but its effect is less pronounced than glyphosate.

Thus, its noteworthy to mention here that Ni-propyl ester of glyphosate (Ni-PE) has good herbicidal properties and was effective in killing the weed. It effectively killed all the plants (10 out of 10) at the recommended dose even after 10 days of its application **(Figure 6.67)**.



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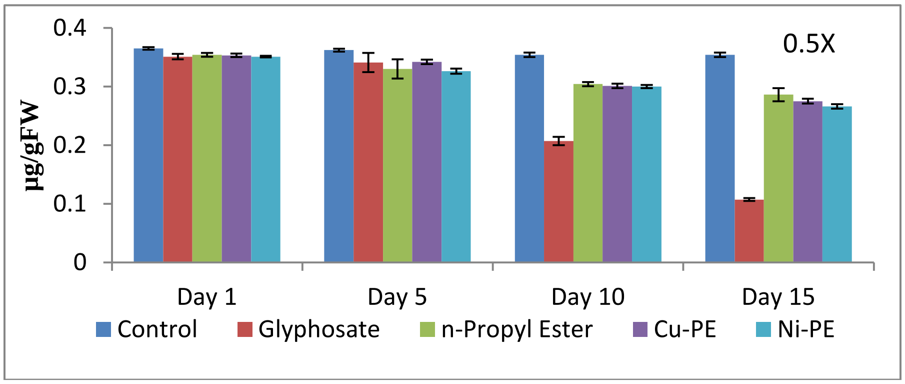

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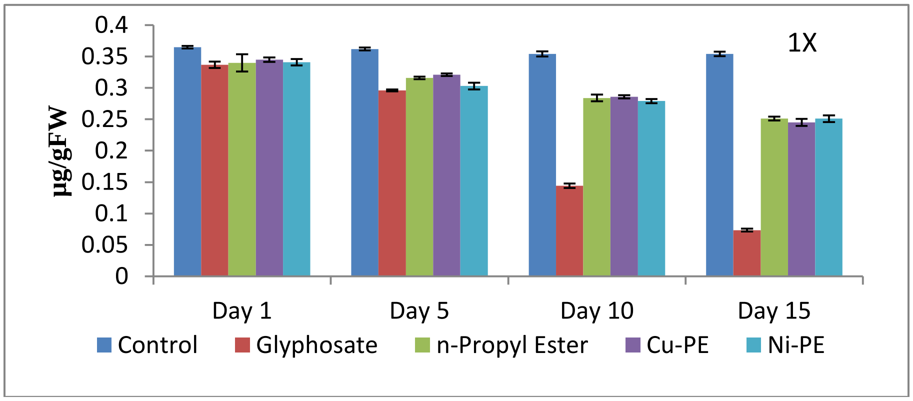
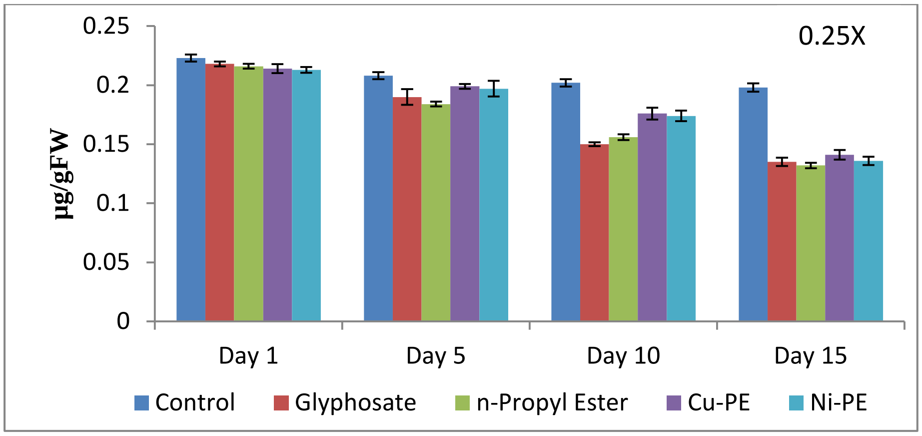


Figure c

Figure 6.64(a)-(c) Effect of metal complexes of propyl ester derivative of glyphosate on 'chlorophyll a' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Cu-PE Gly= Cu-Propyl ester derivative of glyphosate; Ni-PE Gly=Ni-Propyl ester derivative of glyphosate



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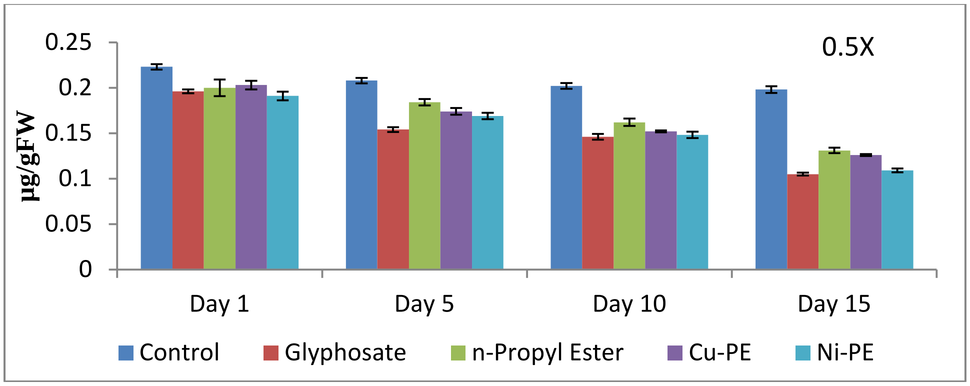

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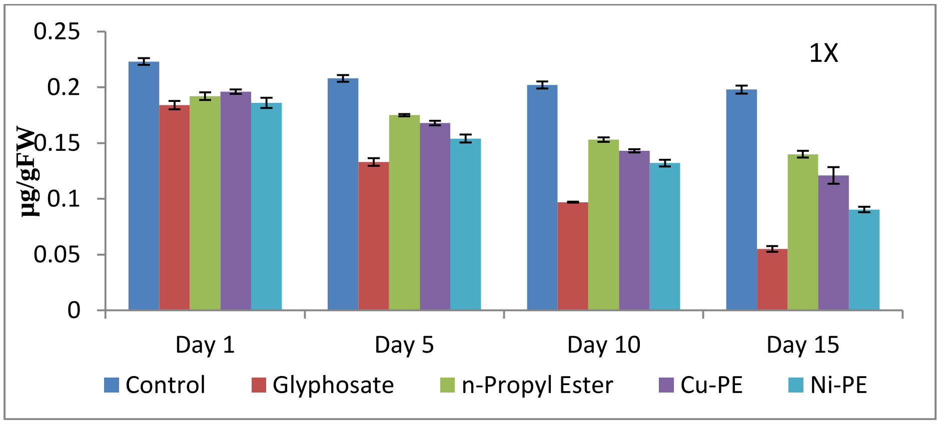
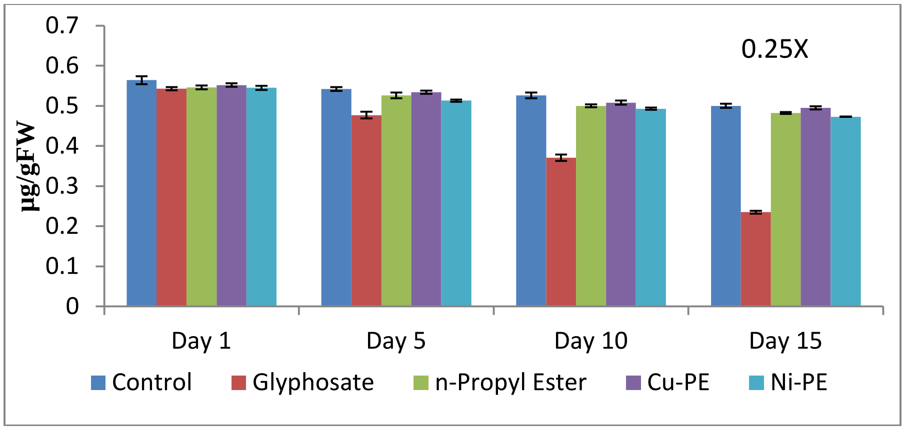


Figure c

Figure 6.65 (a)-(c) Effect of metal complexes of propyl ester derivative of glyphosate on 'chlorophyll b' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Cu-PE Gly= Cu-Propyl ester derivative of glyphosate; Ni-PE Gly=Ni-Propyl ester derivative of glyphosate



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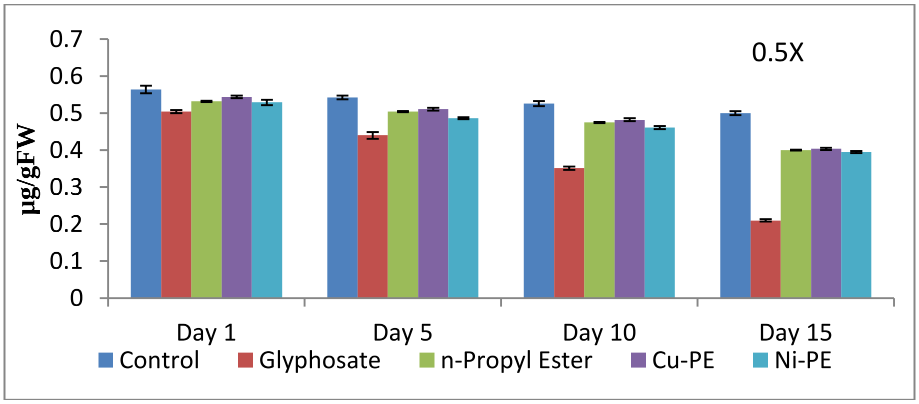

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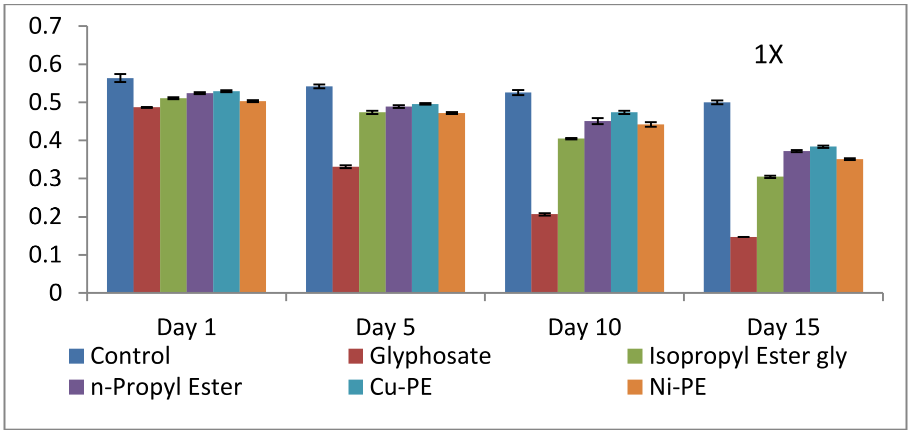


Figure c

Figure 6.66 (a)-(c) Effect of metal complexes of propyl ester derivative of glyphosate on 'total chlorophyll' content of *Parthenium hysterophorus* at different concentrations(0.25X,0.5X and 1X where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Cu-PE Gly= Cu-Propyl ester derivative of glyphosate; Ni-PE Gly=Ni-Propyl ester derivative of glyphosate

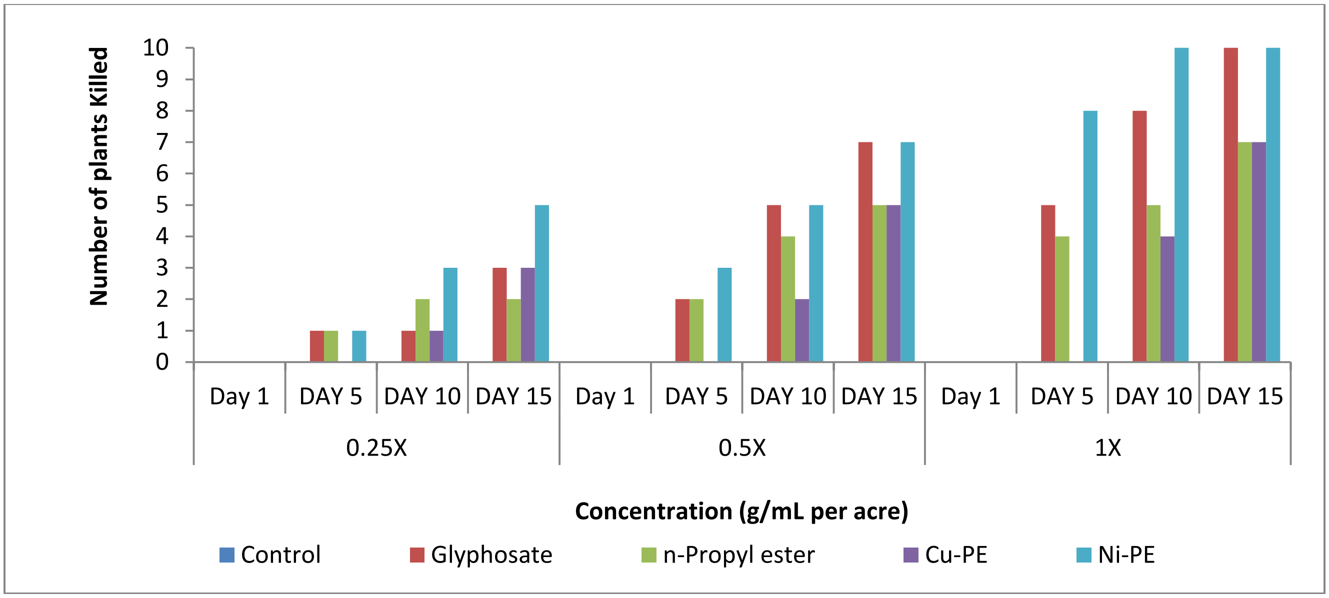


Figure 6.67 Lethal effect of exposure of metal complexes of n-propyl ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3)

where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). Cu-PE Gly= Cu-Propyl ester derivative of glyphosate; Ni-PE Gly=Ni-Propyl ester derivative of glyphosate

Decrease in chlorophyll content (Chl a , Chl b and Chl a+b) and increase in the number of plants (weed) killed after treatment to different concentrations of the synthesized derivatives of glyphosate with respect to time has proved their herbicidal effects. Good to moderate results were obtained from the herbicidal activity carried out on the common weed *Parthenium hysterophorus*. Different derivatives gave different type of response in comparison to glyphosate and control. Amongst the all, ester derivatives and their thioxylated analogues showed the best herbicidal effects on the weed plants. Thioxylated ethyl ester derivative of glyphosate (*O*-ethyl{[(diethoxyphosphorothioyl)methyl]amino}ethanethioate) when sprayed at concentartion of 0.110kg/L per acre effectually lowered the chlorophyll content and killed the plant completely (after 48 hours). At the same dose ethyl ester derivative of glyphosate (Ethyl 2-{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride) has also diminished the amount of photosynthetic pigments in the leaves of the weed (*Parthenium hysterophorus*). Several similar type of studies were carried out to determine the reduction in the chlorophyll content of the plant.

Sreenivasulu et al. carried out similar experiment on the *Parthenium hysterophorus* and *Cyperus rotundus* and determined the chlorophyll content on exposure to glyphosate. A tremendous decrease in chlorophyll content ('Chl a', 'Chl b'and 'Chl a+b') was observed in both the plants[160]. Krenchinski et al. also evaluated the ocomes of glyphosate on the chlorophyll content of RR2 Intacta soyabean plants. Glyphosate had a negative linear effect on the chlorophyll content of the plants. A significant decline in the chlorophyll index was also noted with rise in the glyphosate dose for all the cultivars[161]. Reddy et al. also examined the physiological changes that occurred in the soyabean plant after their exposure to glyphosate. It was found that spraying the compound for only single time at the concentration of 0.28kg/ha of glyphosate has decreased the chlorophyll content to 49% after 2 weeks[162]. In another study by Kitchen et al. a significant decrease in the photosynthetic pigments of field grown soyabeans was also found after application of 2.24kg/ha of glyphosate within 48 hours[163].

From all these studies it was found that use of glyphosate had linearly decreased the chlorophyll content of different plants. These results are in concurrence with the results shown by the synthesized derivatives of glyphosate.

6.3.2 Herbicidal Activity Of Synthesized glyphosate derivatives on *Cyperus rotundus*:

6.3.2.1 Ester Derivatives Of Glyphosate:

Similar type of results were obtained in case of *Cyperus rotundus* also. Ethyl 2-{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride (**Ethyl ester of glyphosate**) have shown improved herbicidal activity than the glyphosate even on one fourth of the recommended dose after first day of its application. However, the compounds Methyl 2-{[(dimethoxyphosphoryl)methyl]amino}acetate hydrochloride (**Methyl ester of glyphosate**), Isopropyl 2- ({[diisopropoxyphosphoryl] methyl}amino) acetate hydrochloride (**Isopropyl ester of glyphosate**) and Butyl 2-{[(dibutoxyphosphoryl)methyl]amino}acetate hydrochloride (**Butyl ester of glyphosate**) manifested herbicidal effects only at the recommended doses after fifth day of their application. Significant reduction of chlorophyll content in the leaves of *Cyperus rotundus* was observed in all the cases after 15 days. Use of these ester derivatives of glyphosate on the *Cyperus rotundus* had a linear effect in decreasing the 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll'. In comparison to control (water treatment) and glyphosate, ethyl ester of glyphosate at all the three test concentrations 0.25X. 0.5 X and 1X had shown much enhanced effect in decreasing the chlorophyll content (**Table 6.13**). After 15 days of the treatment, the 'chlorophyll a', was found to be 0.325 µg/g FW at 0.25X, 0.142 µg/g FW at 0.5X and 0.0971 +µg/g FW at 1X concentration of ethyl ester of glyphosate in comparison to glyphosate (0.675

µg/g FW at 0.25X, 0.582 µg/g FW at 0.5X and 0.484 µg/g FW at 1X), Similar results were also obtained in 'chlorophyll b' and 'total chlorophyll' as shown in **Table 6.13**. The chlorophyll b in *Cyperus rotundus* was also reduced with the application of ethyl ester of glyphosate, it was found to be 0.0942 µg/g FW at 0.25X , 0.0821 µg/g FW at 0.5X and 0.0639 µg/g FW at 1X. Similar reduction was also observed in 'total chlorophyll' content [0.529 µg/g FW at 0.25X, 0.210 µg/g FW at 0.5X and 0.179 µg/g FW at 1X. However other ester derivatives of glyphosate have also reduced the chlorophyll content in comparison to control but in comparison to glyphosate their effect is less pronounced as shown in **Table 6.13**.

Apart from chlorophyll content determination in the leaves of *Cyperus rotundus*, herbicidal activity was also measured on the basis of number of plants dead after their exposure to the ester derivatives of glyphosate. **Figure 6.68** shows the number of plants that become dead after the application of different concentrations of the synthesized ester derivatives of glyphosate. At 0.25X in comparison to glyphosate, ethyl ester of glyphosate has resulted in the death of maximum number of plants (2 after 5 days, 5 after 10 days and 7 after 15 days of exposure). However with increase in the concentration (0.5X), the number of dead plants has increased (4 after 5days, 6 after 10 days and 9 after 15 days). In comparison to glyphosate at recommended dose (1X) ethyl ester of glyphosate has shown enhanced herbicidal activity, it has killed all the plants after 15 days exposure. With the analysis of above data, it could be concluded that, the rate of killing of plants is more in case of ethyl ester of glyphosate (7 after 5days, 9 after 10 days and 10 after 15 days).

Table 6.13 Effect of Ester derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b' (µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		1			5			10			15		
Concentration →		0.25X	0.5X	1X	0.25X	0.5X	1X	0.25X	0.5X	1X	0.25X	0.5X	1X
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}
	Glyphosate	0.690± 0.0036^{c}	0.650± 0.0030^{d}	0.603± 0.00737^{d}	0.683± 0.0012^{d}	0.634± 0.00264^{f}	0.594± 0.0015^{g}	0.680± 0.0070^{f}	0.600± 0.0036^{h}	0.560± 0.0056^{h}	0.675± 0.0025^{i}	0.582± 0.0050^{i}	0.484± 0.009^{i}
	O-methyl {[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.697± 0.0010^{c}	0.662± 0.004^{c}	0.631± 0.003^{d}	0.689± 0.003^{b}	0.654± 0.00755^{d}	0.607± 0.0351^{g}	0.685± 0.00152^{c}	0.621± 0.0058^{b}	0.574± 0.002^{g}	0.677± 0.0171^{g}	0.594± 0.00157^{h}	0.503± 0.003^{i}
	O-ethyl {[(diethoxyphosphorothioy) methyl]amino}ethanethioate	0.620± 0.0005^{f}	0.594± 0.0096^{f}	0.362± 0.0032^{f}	0.586± 0.0102^{f}	0.304± 0.0132^{f}	0.242± 0.0077^{g}	0.472± 0.0055^{i}	0.204± 0.00360^{i}	0.132± 0.0049^{i}	0.325± 0.0291j	0.142± 0.006^{j}	0.0971± 0.0121^{j}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioylmethyl}amino) ethanethioate	0693± 0.0291^{c}	0.672± 0.0035^{e}	0.648± 0.004^{d}	0.691± 0.01216^{d}	0.646± 0.0040^{d}	0.597± 0.0025^{f}	0.682± 0.00152^{g}	0.615± 0.00814^{h}	0.562± 0.0032^{h}	0.672± 0.003^{i}	0.589± 0.0168^{i}	0.493± 0.0075^{i}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.695± 0.0045^{c}	0.659± 0.0176^{e}	0.624± 0.006^{d}	0.687± 0.0055^{e}	0.639± 0.0537^{d}	0.603± 0.0529^{f}	0.0.682± 0.01242^{g}	0.629± 0.00901^{g}	0.577± 0.0066^{i}	0.676± 0.0015^{i}	0.599± 0.00755^{i}	0.509± 0.0015^{i}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0030^{a}	0.452± 0.0015^{a}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015	0.400± 0.0045	0.360± 0.0032	0.205± 0.0040	0.385± 0.0020	0.300± 0.003	0.385± 0.0015	0.310± 0.0034	0.232± 0.0046	0.301± 0.004	0.296± 0.004	0.109± 0.0030
	O-methyl {[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.431± 0.003^{a}	0.419± 0.0037^{c}	0.372± 0.0062^{d}	0.196± 0.0026^{d}	0.392± 0.0026^{e}	0.310± 0.0032^{f}	0.399± 0.0015^{e}	0.324± 0.0068^{f}	0.254± 0.0025^{i}	0.321± 0.0015^{j}	0.300± 0.0003^{m}	0.125± 0.00624^{l}
	O-ethyl {[(diethoxyphosphorothioyl) methyl]amino}ethanethioate	0.309± 0.0047^{c}	0.205± 0.0015^{d}	0.172± 0.0045^{e}	0.242± 0.0015^{e}	0.149± 0.007^{f}	0.083± 0.002^{h}	0.105± 0.002^{f}	0.093± 0.0015^{i}	0.0714± 0.0025^{j}	0.0942± 0.00083^{j}	0.0427± 0.0017^{l}	0.0639± 0.0045^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl} amino) ethanethioate	0.429± 0.0035^{b}	0.409± 0.0015^{c}	0.366± 0.0032^{d}	0.417± 0.001^{d}	0.389± 0.0037^{g}	0.309± 0.0025^{f}	0.389± 0.0010^{g}	0.312± 0.00152^{f}	0.240± 0.0026^{h}	0.353± 0.0047^{j}	0.0298± 0.00041^{l}	0.119± 0.0032^{m}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.446± 0.003^{b}	0.429± 0.0047^{c}	0.379± 0.002^{d}	0.428± 0.0036^{d}	0.399± 0.0040^{e}	0.317± 0.0073^{f}	0.401± 0.0025^{e}	0.331± 0.00321^{f}	0.263± 0.0017^{h}	0.367± 0.003^{n}	0.315± 0.0040^{j}	0.137± 0.002^{o}

Total Chlorophyll	**Control**	0.963 ± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.925± 0.0024^{f}	0.925± 0.0024^{f}	0.925± 0.0024^{f}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{f}	0.905± 0.003^{g}	0.852± 0.0020^{i}	0.721± 0.0041^{h}	0.862± 0.00461^{i}	0.724± 0.0034^{j}	0.690± 0.0005^{j}	0.832± 0.0030^{j}	0.632± 0.0040^{k}	0.514± 0.0025^{m}
	O-methyl {[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.947± 0.006^{c}	0.917± 0.004^{c}	0.784± 0.0171^{e}	0.917± 0.002^{g}	0.874± 0.0075^{g}	0.732± 0.0206^{i}	0.884± 0.00105^{i}	0.756± 0.006^{j}	0.704± 0.0124^{j}	0.845± 0.001^{j}	0.651± 0.0058^{k}	0.613± 0.0015^{l}
	O-ethyl{[(diethoxyphosphorothioyl) methyl] amino}ethanethioate	0.900± 0.0035^{e}	0.763± 0.0096^{g}	0.629± 0.0291^{g}	0.803± 0.0025^{h}	0.521± 0.003^{h}	0.414± 0.003^{n}	0.743± 0.0016^{j}	0.342± 0.0036^{j}	0.295± 0.0121^{m}	0.529± 0.0016k	0.210± 0.0036^{l}	0.179± 0.005^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl} amino)ethanethioate	0.936± 0.0015^{c}	0.907± 0.0035^{e}	0.796± 0.004^{e}	0.907± 0.0050^{g}	0.862± 0.053^{g}	0.729± 0.0189^{h}	0.872± 0.0032^{j}	0.731± 0.0081^{j}	0.697± 0.0102^{k}	0.839± 0.00326^{k}	0.642± 0.0081^{k}	0.519± 0.0015^{m}
	O-butyl{[(dibutoxyphosphorothioyl) methyl]amino}ethanethioate	0.952± 0.002^{c}	0.923± 0.0176^{e}	0.789± 0.0025^{e}	0.923± 0.0015^{g}	0.889± 0.0015^{g}	0.739± 0.0015^{h}	0.889± 0.0015^{j}	0.763± 0.0090^{j}	0.713± 0.0132^{k}	0.852± 0.0015^{k}	0.663± 0.0090^{k}	0.624± 0.0055^{l}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

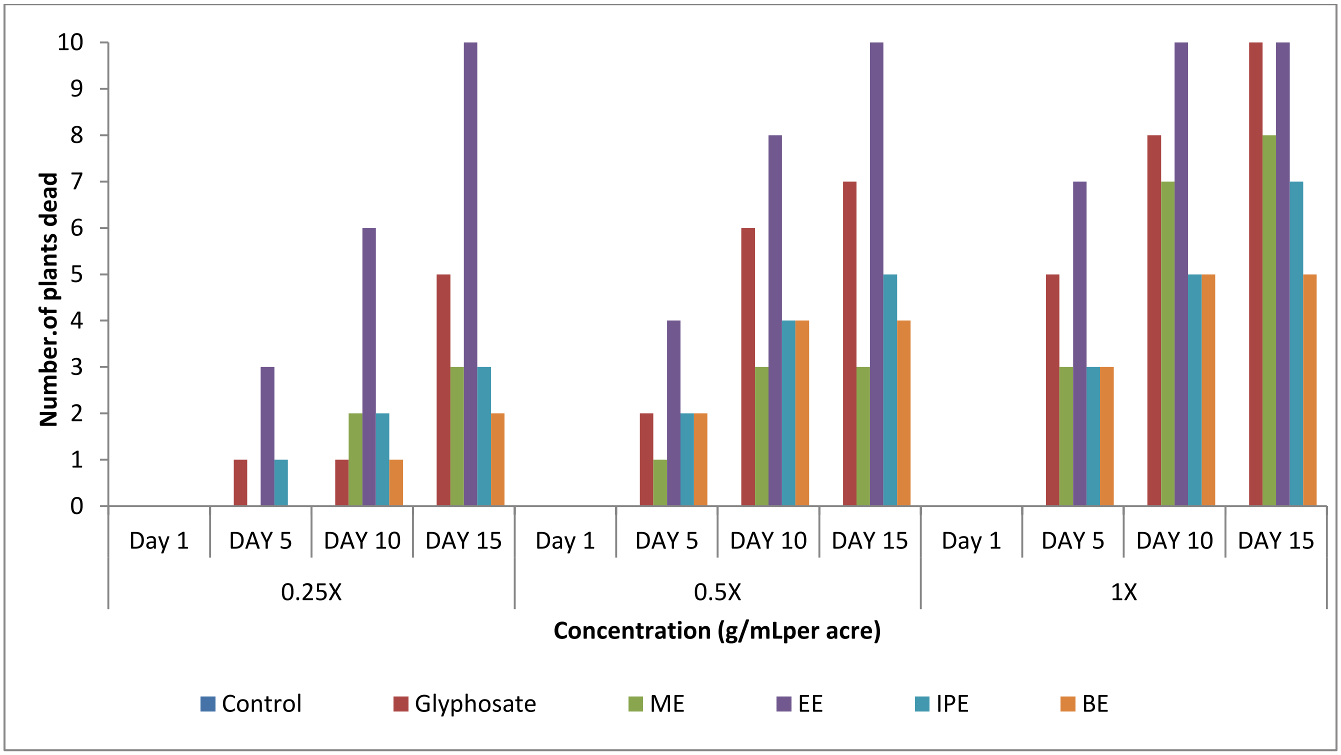


Figure 6.68 Lethal effect of exposure of Ester derivatives of glyphosate on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) (Where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). ME-Methyl ester of glyphosate; EE- Ethyl ester of glyphosate; IPE-Isopropyl ester of glyphosate; BE- Butyl ester of glyphosate

6.3.2.2 Amide Derivatives Of Glyphosate:

Amide derivatives of glyphosate have also shown similar type of the herbicidal effects on the weed *Cyperus rotundus*. Significant reduction in the photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) have been observed in all cases with respect to time and concentration as compared to normal control (**Table 6.14**). But this decrease was not comparable to glyphosate. Out of the three different synthesized amide derivatives, **Isopropyl amide derivative of glyphosate** [({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid] is more effective in decreasing the 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' content in the plant at all the three concentrations. At 0.25X concentration, an effective decrease was observed in 'chlorophyll a' (0.676µg/gFW), 'chlorophyll b' (0.382µg/gFW) and 'total chlorophyll' (0.845µg/gFW) However with increase in concentration of isopropyl amide derivative i.e. at 1X concentration, further noticeable drop off in the chlorophyll pigments have been noticed ['chlorophyll a' (0.520µg/gFW); 'chlorophyll b' (0.209µg/gFW); 'total chlorophyll' (0.734µg/gFW)].

Other two amide derivatives also follow the same trend of reduction in chlorophyll content with increase in concentration of the derivative with respect to time. After 15 days of application, a momentous change in the amount of photosynthetic pigments have been observed in comparison to control. In case of **methyl amide of glyphosate** [({[2-oxo-2-(propan-2-ylamino) ethyl] amino}methyl)phosphonic acid] and **butyl amide of glyphosate** [({[2-(butylamino)-2-oxoethyl] amino}methyl)phosphonic acid] at 0.25X concentration 'chlorophyll a' found was 0.680µg/gFW and 0.682µg/gFW respectively. While at 1X, 'chlorophyll a' get reduced to 0.531µg/gFW and 0.524µg/gFW respectively. Similarly, amount of 'chlorophyll b' left at 0.25X (after 15 days) was 0.394µg/gFW in case of **methyl amide of glyphosate** and 0.390µg/gFW in case of **butyl amide of glyphosate**. This reduction is more manifested at higher concentration of 1X (**Table 6.14**).

Along with chlorophyll content determination, a significant increase in the number of dead plants was observed with exposure of *Cyperus rotundus* to the synthesized amide derivatives of glyphosate. This increase was manifested with increase in the concentration of the derivative and time. At 0.25X, in case **of methyl amide of glyphosate** only 2 plants were dead even after 15 days. At 0.5X, not much change was noticed even after 15 days of treatment. But at 1X, half of the plant population (5 out of 10) was dead. **Isopropyl amide of glyphosate** also followed the similar trend at all the three test concentrations (**Figure 6.69**). However **butyl amide of glyphosate** killed the minimum number of plants (only 3 after 15 days at 1X) and does not show good herbicidal activity.

Table 6.14 Effect of synthesized amide derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll' content (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699 ± 0.0061^{a}	0.699 ± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.011^{b}	0.693± 0.011^{b}	0.693± 0.0115^{b}	0.687± 0.0073^{b}	0.687± 0.0073^{b}	0.687± 0.0073^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}
	Glyphosate	0.690± 0.003^{c}	0.650± 0.003^{e}	0.603± 0.0073^{e}	0.683± 0.001^{h}	0.634± 0.0026^{i}	0.594± 0.0015^{h}	0.680± 0.007^{h}	0.600± 0.0036^{h}	0.560± 0.0056^{i}	0.675± 0.0025^{m}	0.582± 0.0050^{j}	0.484± 0.009^{m}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.697± 0.006^{b}	0.668± 0.0021^{c}	0.634± 0.0012^{e}	0.690± 0.0045^{e}	0.652± 0.0016^{l}	0.619± 0.0016^{e}	0.687± 0.0063^{d}	0.622± 0.0283^{f}	0.582± 0.00163^{g}	0.680± 0.0005^{d}	0.603± 0.003^{h}	0.531± 0.0012^{h}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid	0693 0.0048^{e}	0.657± 0.0024^{f}	0.615± 0.0016^{g}	0.685± 0.0057^{f}	0.642± 0.0038^{p}	0.602± 0.0021^{g}	0.681± 0.0050^{e}	0.612± 0.0012^{g}	0.571± 0.0012^{h}	0.676± 0.002^{g}	0.594± 0.001^{h}	0.520± 0.0012^{h}
	({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.695± 0.0016^{c}	0.665± 0.0020^{e}	0.630± 0.0020^{f}	0.692± 0.0028^{f}	0.650± 0.002^{l}	0.614± 0.0020^{g}	0.685± 0.0049^{e}	0.619± 0.002^{f}	0.579± 0.0012^{h}	0.682± 0.0049^{f}	0.599± 0.002^{g}	0.524± 0.0012^{h}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{a}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.0030^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{f}	0.301± 0.004^{e}	0.296± 0.004^{m}	0.109± 0.003^{n}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.444±0. 0008^{a}	0.432± 0.0015^{b}	0.399± 0.0015^{b}	0.440± 0.0021^{c}	0.401± 0.0032^{c}	0.354± 0.0016^{c}	0.416± 0.0020^{d}	0.376± 0.0020^{f}	0.305± 0.0032^{d}	0.394± 0.0026^{g}	0.352± 0.0015^{f}	0.284± 0.0024^{g}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid	0.432± 0.0016^{d}	0.416± 0.0012^{b}	0.378± 0.0026^{c}	0.420± 0.0021^{g}	0.393± 0.0020^{d}	0.319± 0.0016^{d}	0.403± 0.0012^{i}	0.300± 0.0024^{d}	0.264± 0.0028^{d}	0.382± 0.002^{b}	0.298± 0.0026^{f}	0.209± 0.0028^{h}
	({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.439± 0.0008^{a}	0.430± 0.0024^{a}	0.394± 0.0009^{c}	0.434± 0.0012^{c}	0.413± 0.0012^{d}	0.349± 0.0021^{d}	0.411± 0.0003^{d}	0.366± 0.0032^{e}	0.298± 0.0024^{e}	0.390± 0.0024^{f}	0.345± 0.0024^{j}	0.271± 0.0024^{g}
Total Chlorophyll	**Control**	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{a}	0.952± 0.002^{b}	0.940± 0.0230^{d}	0.940± 0.023^{d}	0.940± 0.023^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{c}	0.905± 0.003^{f}	0.852± 0.0020^{j}	0.721± 0.0041^{j}	0.862± 0.0046^{j}	0.724± 0.0034^{n}	0.690± 0.0005^{j}	0.832± 0.0030^{m}	0.632± 0.0040^{p}	0.514± 0.0025^{m}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.954± 0.0010^{a}	0.931± 0.0016^{b}	0.904± 0.0024^{c}	0.915± 0.0012^{c}	0.901± 0.0026^{c}	0.876± 0.0020^{d}	0.915± 0.0028^{f}	0.876± 0.0012^{d}	0.839± 0.0021^{d}	0.892± 0.00249^{d}	0.839± 0.002^{e}	0.800± 0.0012^{f}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid	0.935± 0.0024^{c}	0.917± 0.0016^{c}	0.864± 0.0012^{d}	0.926± 0.002^{c}	0.884± 0.0081^{d}	0.803± 0.0012^{d}	0.900± 0.0008^{d}	0.832± 0.0026^{e}	0.752± 0.00169^{f}	0.845± 0.0016^{e}	0.784± 0.002^{f}	0.734± 0.0024^{f}

	({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.943± 0.0024^{b}	0.929± 0.0024^{b}	0.887± 0.002^{c}	0.533± 0.0020^{b}	0.897± 0.002^{b}	0.851± 0.0024^{d}	0.908± 0.0012^{d}	0.859± 0.0024^{d}	0.806± 0.0047^{e}	0.884± 0.0008^{h}	0.813± 0.0015^{e}	0.771± 0.002^{g}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

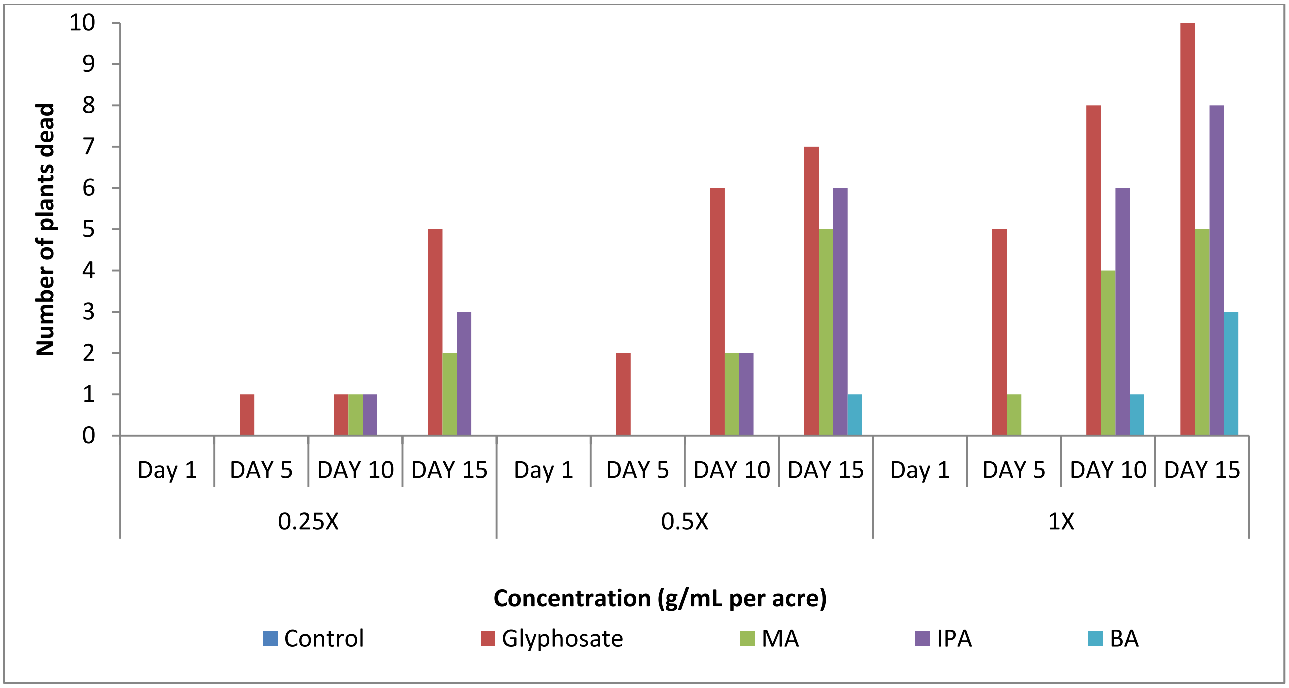


Figure 6.69 Lethal effect of exposure of Amide derivatives of glyphosate on *Cyperus rotundus* after 15 days Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
MA = ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid; IPA = ({[2-oxo-2-(propan-2-ylamino)ethyl] amino}methyl)phosphonic acid ; BA = ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid.

6.3.2.3 Boc-protected glyphosate derivative:

Boc-Protected glyphosate derivative also showed similar kind of results of herbicidal activity as shown by amide derivatives of glyphosate. As compared to control (water treatment), the plants treated with Boc-protected glyphosate have shown momentous reduction in photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll). A considerable decrease in the amount of 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' has been observed at all test concentrations with respect to time (**Table 6.15**). After 15 days treatment at 0.25X concentration no significant change in the 'chlorophyll a' content was found in the control and treated plant. However at 0.5X concentration 'chlorophyll a' was reduced to 0.624µg/gFW and at 1X it was further reduced to 0.583µg/gFW. Analogous trend was also scrutinized in case of 'chlorophyll b' and 'total chlorophyll' content of the *Cyperus rotundus*. 'Chlorophyll b' content present in control plant after 15 days was found to be 0.431 µg/gFW however, after the application of the Boc-protected glyphosate on the weed plant the 'chlorophyll b' was reduced to 0.403 µg/gFW at 0.25

X, 0.393 µg/gFW at 0.5X and 0.331 µg/gFW at 1X concentration. Similar results were observed with 'total chlorophyll' content.

Mortality rate of *Cyperus rotundus* with respect to concentration of Boc- protected glyphosate and time was perceived by using 10 plants in each pot. It was observed that Boc-protected glyphosate did not show any significant effect on the mortality of the plants. This compound has shown its herbicidal activity only at higher concentration (0.5X and 1X) . Foliar spray of Boc-protected glyphosate at 0.5X has resulted in death of 4 plants after 15 days, whereas glyphosate has shown significant reduction in the number of plants even after 10th day (7 plants were dead out of 10). At 1X concentration of Boc-protected glyphosate, more than half of the plants (6 out of 10) has become dead (In case of glyphosate at 1X concentration, 8 out of 10 plants were dead) (**Figure 6.70**). This data illustrates that application of Boc-protected glyphosate on the weed significantly reduced the content of its photosynthetic pigments at all the three test concentrations, but it killed the plant completely only at highest concentrations (1X).

Table 6.15 Effect of Boc-protected glyphosate derivative on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll' content (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0693.± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.0073^{b}	0.687± 0.0073^{b}	0.0.687± 0.0073^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}
	Glyphosate	0.690± 0.003^{c}	0.650± 0.003^{e}	0.603± 0.0073^{e}	0.683± 0.0012^{e}	0.634± 0.002^{h}	0.594± 0.0015^{h}	0.680± 0.007^{h}	0.600± 0.0036^{h}	0.560± 0.0056^{i}	0.675± 0.0025^{m}	0.582± 0.005^{j}	0.484± 0.009^{m}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.698± 0.0021^{c}	0.681± 0.002^{f}	0.652± 0.0045^{d}	0.691± 0.0026^{f}	0.664± 0.0020^{d}	0.639± 0.002^{d}	0.686± 0.001^{d}	0.648± 0.001^{e}	0.605± 0.002^{f}	0.683± 0.0005^{d}	0.624± 0.0015^{f}	0.583± 0.0032^{e}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{f}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.451± 0.0015^{a}	0.449± 0.0043^{c}	0.417± 0.0010^{b}	0.432± 0.0011^{c}	0.431± 0.0025^{d}	0.405± 0.0035^{e}	0.423± 0.0025^{d}	0.420± 0.0026^{e}	0.354± 0.0096^{f}	0.403± 0.0036^{e}	0.393± 0.002^{h}	0.331± 0.0025^{i}
Total Chlorophyll	**Control**	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.023^{d}	0.940± 0.0023^{d}	0.940± 0.0023^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{c}	0.905± 0.003^{f}	0.852± 0.0020^{j}	0.721± 0.0041^{j}	0.862± 0.0046^{i}	0.724± 0.0034^{k}	0.690± 0.0005^{l}	0.832± 0.003^{m}	0.632± 0.0040^{n}	0.514± 0.0025^{o}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.954± 0.0036^{h}	0.939± 0.003^{h}	0.915± 0.002^{d}	0.941± 0.002^{b}	0.914± 0.0015^{c}	0.893± 0.002^{f}	0.924± 0.0025^{f}	0.897± 0.0062^{d}	0.874± 0.0035^{f}	0.903± 0.0036^{g}	0.864± 0.0020^{f}	0.853± 0.0050^{e}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

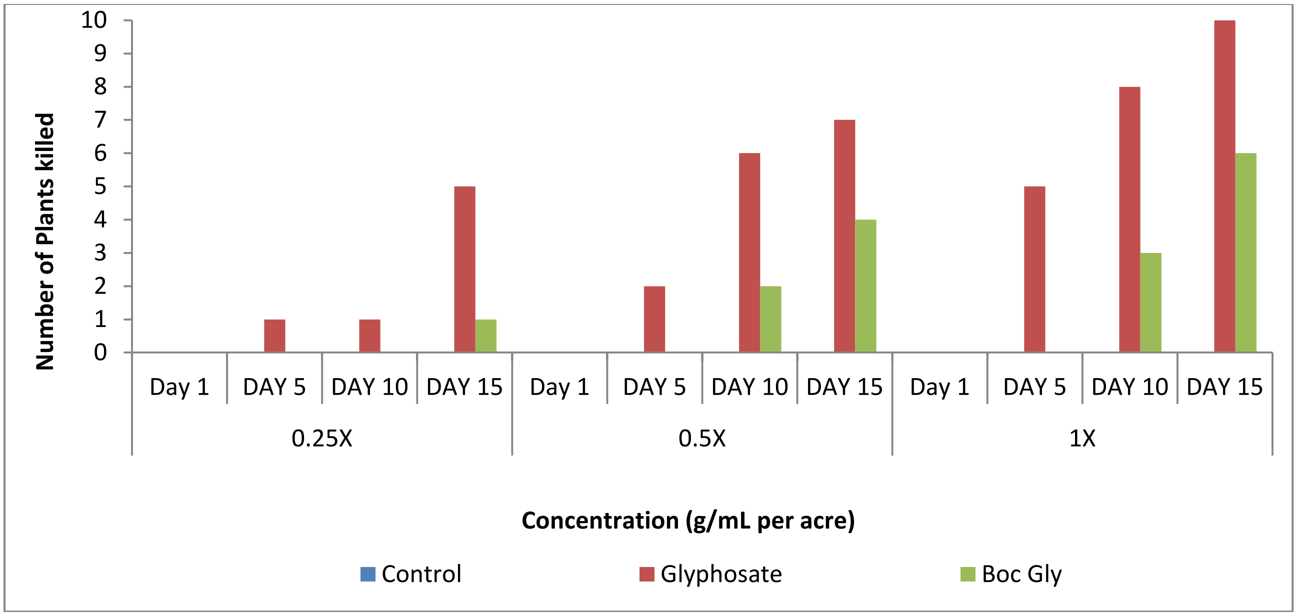


Figure 6.70 Lethal effect of exposure of Boc- Protected glyphosate on *Cyperus rotundus* after 15 days Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-Protected Gly= (2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid

6.3.2.4 Boc-protected guanidine derivative of glyphosate and its de-protected analogue:

Boc-protected guanidine derivative of glyphosate and its de-protected analogue (guanidine derivative of glyphosate) have also shown herbicidal properties on the common weed *Cyperus rotundus*. A regular decrease in the chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) was found in both the cases at all the test concentrations. In comparison to control (water treatment) both of these newly synthesized derivatives are effective in controlling the weed population.

In case of **Boc-protected guanidine derivative of glyphosate**, at 0.25X after 15 days, 'chlorophyll a' has been reduced to 0.679µg/gFW in comparison to control (0.683µg/gFW). However at 0.5X, reduction in' chlorophyll a' was 0.619 µg/gFW and at 1X, 'chlorophyll a' left behind was 0.544µg/gFW. In case of 'chlorophyll b', at 0.25X significant reduction was observed after 15 days treatment. 'Chlorophyll b' was reduced from 0.431µg/gFW (control) to 0.394µg/gFW. However the reduction in 'chlorophyll b' content also became significant at 0.5X ['chlorophyll b' (treated plant=0.381µg/gFW)] and at 1X ['chlorophyll b' (treated plant=0.293µg/gFW)]. Significant reduction in 'total chlorophyll' content of plant was also observed at all test concentrations.

The **guanidine derivative of glyphosate** (de-protected guanidine derivative of glyphosate) is more effective in showing its herbicidal effects in comparison to the Boc-protected guanidine derivative of glyphosate. It has shown greater herbicidal effects by decreasing the content of photosynthetic pigments in the plant. At all the test concentrations a marked reduction in the 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' has been observed. At 0.25X 'chlorophyll a' left in the plant was 0.678µg/gFW which get further reduced 0.603µg/gFW and 0.515µg/gFW at 0.5X and 1X respectively. Similarly a considerable decrease in 'chlorophyll b' and 'total chlorophyll' (**Table 6.16**) was also examined in the weed plant at all the test concentrations (0.25X, 0.5X and 1X).

Herbicidal activity of the synthesized Boc-protected guanidine derivative of glyphosate and guanidine derivative of glyphosate was also established on the basis of the total number of plants killed after their exposure to these derivatives. It was found that in comparison to glyphosate no considerable decrease in the number of plants was found in both these derivatives. However at higher concentration (1X) guanidine derivative of glyphosate was effective in killing the plants (after 10 days and after 15 days). But these results are not noteworthy with respect to glyphosate. Use of Boc-protected guanidine derivative of glyphosate and guanidine derivative of glyphosate on the weed (*Cyperus rotundus*), at all the three test concentrations has effectively decreased the amount of photosynthetic pigments in the plant. But the number of deaths in the plant was fewer (At the highest concentration of 1X, Boc-protected guanidine derivative of glyphosate has killed 3 plants after 10 days and 6 plants after 15 days whereas the guanidine derivative of glyphosate has killed 4 plants after 10 days and 7 plants after 15 days). (**Figure 6.71**).

Table 6.16. Effect Boc-protected guanidine derivative of glyphosate and its deprotected analogue (guanidine derivative of glyphosate) on the chlorophyll content a (µg/gFW), chlorophyll content b(µg/gFW) and total chlorophyll content (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0693.± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.0073^{b}	0.687± 0.0073^{b}	0.0.687± 0.0073^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}
	Glyphosate	0.690± 0.003^{c}	0.650± 0.003^{e}	0.603± 0.0073^{e}	0.683± 0.0012^{e}	0.634± 0.002^{h}	0.594± 0.0015^{h}	0.680± 0.007^{h}	0.600± 0.0036^{h}	0.560± 0.0056^{i}	0.675± 0.0025^{m}	0.582± 0.005^{j}	0.484± 0.009^{m}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	0.696± 0.0032^{c}	0.672± 0.00152^{d}	0.643± 0.0017^{f}	0.689± 0.0015^{d}	0.659± 0.002^{h}	0.625± 0.0026^{d}	0.683± 0.0025^{e}	0.633± 0.0036^{j}	0.598± 0.0025^{h}	0.679± 0.0050^{h}	0.619± 0.0015^{h}	0.544± 0.0032^{i}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.694± 0.001^{d}	0.664± 0.004^{f}	0.615± 0.0017^{e}	0.687± 0.0015^{d}	0.648± 0.002^{d}	0.601± 0.005^{d}	0.681 ± 0.00152^{h}	0.624± 0.0041^{h}	0.583± 0.002^{h}	0.678± 0.002^{h}	0.603± 0.00013^{i}	0.515± 0.0015^{e}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{f}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	0.448± 0.003^{e}	0.434± 0.0058^{a}	0.403± 0.0015^{e}	0.425± 0.0072^{a}	0.416± 0.0064^{e}	0.371± 0.0035^{e}	0.412± 0.0025^{e}	0.408± 0.002^{g}	0.314± 0.0025^{g}	0.394± 0.0035^{j}	0.381± 0.001^{i}	0.293± 0.013^{h}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.431± 0.002^{f}	0.418± 0.0015^{b}	0.384± 0.0058^{e}	0.419± 0.004^{b}	0.396± 0.0015^{e}	0.359± 0.0040^{j}	0.400± 0.0015^{j}	0.354± 0.0052^{i}	0.305± 0.0030^{i}	0.382± 0.003^{g}	0.309± 0.0015^{h}	0.286± 0.004^{h}
Total Chlorophyll	**Control**	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.023^{d}	0.940± 0.0023^{d}	0.940± 0.0023^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{c}	0.905± 0.003^{f}	0.852± 0.0020^{j}	0.721± 0.0041^{j}	0.862± 0.0046^{i}	0.724± 0.0034^{k}	0.690± 0.0005^{l}	0.832± 0.003^{m}	0.632± 0.0040^{n}	0.514± 0.0025^{o}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	0.947± 0.0055^{b}	0.921± 0.0015^{b}	0.903± 0.0015^{e}	0.932± 0.0035^{c}	0.901± 0.0032^{e}	0.887± 0.0020^{c}	0.914± 0.0025^{e}	0.883± 0.0020^{g}	0.851± 0.0041^{g}	0.896± 0.0030^{d}	0.851± 0.0030^{g}	0.832± 0.002^{f}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.921± 0.0015^{b}	0.901± 0.001^{e}	0.871±0. 004^{c}	0.912± 0.0035^{f}	0.884± 0.0028^{c}	0.832± 0.0015^{d}	0.894± 0.023^{c}	0.851± 0.0026^{f}	0.804± 0.0015^{f}	0.871± 0.0015^{g}	0.832± 0.0020^{f}	0.761± 0.0050^{h}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

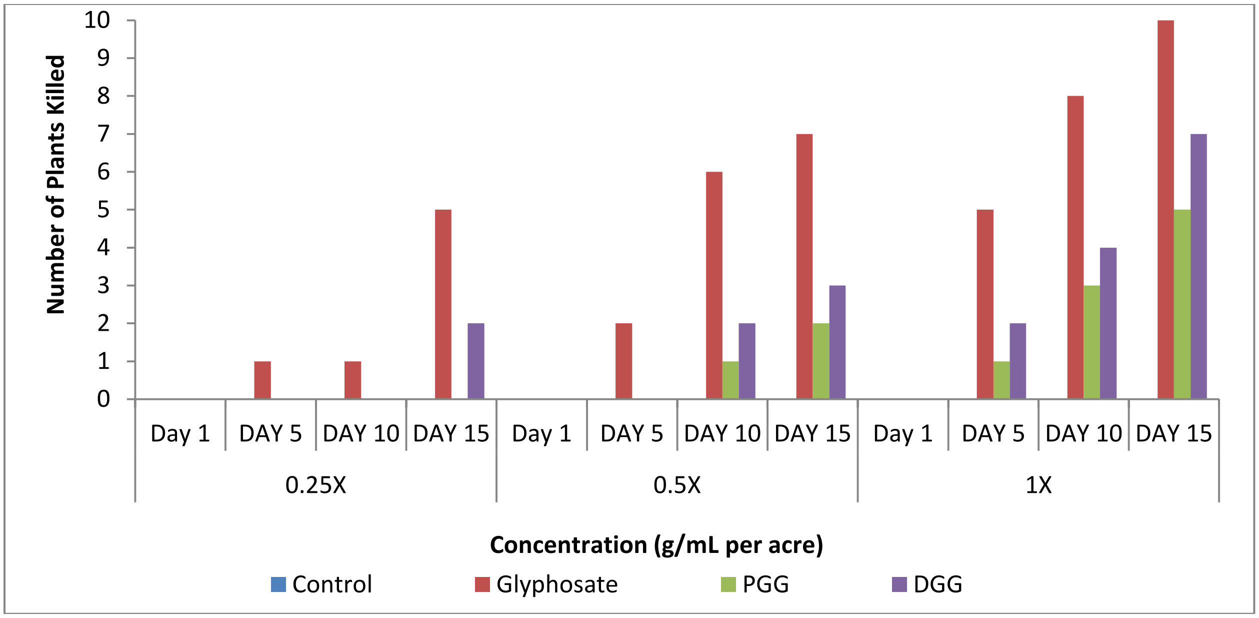


Figure 6.71 Lethal effect of exposure of Boc-protected guanidine glyphosate and De-protected guanidine glyphosate on *Cyperus royundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

PGG(Boc-protected guanidine gly) = 2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid ; DGG (De- protected guanidine gly) = 2-(1-(Phosphonomethyl)guanidino) ethanoic acid

6.3.2.5 Thioxylated ester derivatives of glyphosate:

A series of four new thioxylated ester derivatives of glyphosate were synthesized and their herbicidal activity was compared with control (water treatment) and glyphosate. These newly synthesized derivatives have shown excellent herbicidal effects on the weed *Cyperus rotundus* after 15 days treatment with three test concentrations (0.25X, 0.5X and 1X). Among these thioxylated esters of glyphosate, **thioxylated ethyl ester of glyphosate** (*O*-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate) have shown best herbicidal effects. It has significantly decreased the chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) and killed the plant. At 0.25X after 15 days, a considerable decline in 'chlorophyll a' (0.179µg/gFW), 'chlorophyll b'(0.0937 µg/gFW), 'total chlorophyll' (0.224 µg/gFW) was observed in the treated plant as compared to control ['chlorophyll a' (0.683 µg/gFW), 'chlorophyll b'(0.431 µg/gFW), 'total chlorophyll' (0.925 µg/gFW] and glyphosate ['chlorophyll a' (0.675 µg/gFW), 'chlorophyll b' (0.301 µg/gFW), 'total chlorophyll' (0.832 µg/gFW)]. Similar significant reduction was observed at 0.5X and a remarkable decline in chlorophyll content was observed at 1X (**Table 6.17**). Thioxylated ethyl ester of glyphosate behave as a potent herbicide and is extremely effective in decreasing the content of photosynthetic pigments of plant in comparison to glyphosate. Plants treated with thioxylated ethyl ester derivative of glyphosate at 1X have shown tremendous decrease in 'chlorophyll a' (0.0072 µg/gFW), 'chlorophyll b' (0.009 µg/gFW) and 'total chlorophyll' (0.0014 µg/gFW) in contrast to glyphosate. Other thioxylated ester derivatives of glyphosate are also effectual in killing the weed. They have also reduced the content of photosynthetic pigments in the plant at all the test concentrations. Major diminution in 'chlorophyll a' (0.238 µg/gFW) , 'chlorophyll b' (0.107µg/gFW) and 'total chlorophyll' (0.349 µg/gFW) was found in the plants treated with 0.25X of **thioxylated isopropyl ester of glyphosate** (O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl] methylamino)ethanethioate). However this decrease became much pronounced with increase in the concentration of the thioxylated isopropyl ester of glyphosate. At 1X chlorophyll content was greatly reduced ['chlorophyll a' (0.0942 µg/gFW), 'chlorophyll b' (0.0140µg/gFW) and 'total chlorophyll' (0.176 µg/gFW) in the plant and it exterminate the plant completely.

Transience of plant is the main criterion for determining the herbicidal effects of any pesticide. Glyphosate is a well known herbicide that effectually killed the plant. Synthesized Thioxylated ester derivatives of glyphosate also act as potent herbicides and competently killed the plant. Thioxylated ethyl ester of glyphosate is more effectual in killing the plant in contrast to glyphosate. Even at the lowest concentration(0.25X) half of the plants were exterminated by this compound after 10 days of the treatment. The number of plants killed by the thioxylated ethyl ester of glyphosate was increased with increase in the concentration of the compound. At 0.5X, 6 plants were killed after 5 days and 9 plants were killed after 15 days. The rate at which the plants become dead after their exposure to this compound was greatly increased with increase in concentration and time. However at 1X all the plants (10) were dead even after 10 days exposure. Whereas, glyphosate killed 8 out of 10 plants even after 10 days exposure. Similar results were obtained in case of Thioxylated isopropyl ester of glyphosate. It has also shown its herbicidal effects even at the lowest concentration of 0.25X. It effectively killed 7 out of 10 plants after 15 days **(Figure 6.72)**. At 1X more than half of the plants were killed by this compound after 10 days exposure. All these findings showed that thioxylated ester derivatives of glyphosate are potent herbicides and effectively killed the plant.

Table 6.17 Effect Thioxylated Ester derivatives of glyphosate on the chlorophyll content a (µg/gFW), chlorophyll content b(µg/gFW) and total chlorophyll content (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0693.± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.0073^{b}	0.687± 0.0073^{b}	0.0.687± 0.0073^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}
	Glyphosate	0.690± 0.003^{c}	0.650± 0.003^{e}	0.603± 0.0073^{e}	0.683± 0.0012^{e}	0.634± 0.002^{h}	0.594± 0.0015^{h}	0.680± 0.007^{h}	0.600± 0.0036^{h}	0.560± 0.0056^{i}	0.675± 0.0025^{m}	0.582± 0.005^{j}	0.484± 0.009^{m}
	O-methyl{[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.695± 0.0025^{c}	0.663± 0.0015^{c}	0.625± 0.002^{d}	0.687± 0.002^{b}	0.648± 0.0015^{d}	0.603± 0.001^{g}	0.680± 0.0040^{c}	0.613± 0.0036^{b}	0.572± 0.0010^{g}	0.679± 0.0025^{g}	0.594± 0.0015^{h}	0.498± 0.0014^{i}
	O-ethyl{[(diethoxyphosphorothioy) methyl]amino}ethanethioate	0.430± 0.0025^{f}	0.406± 0.002^{f}	0.321± 0.0035^{f}	0.372± 0.0015^{f}	0.351± 0.0035^{f}	0.175± 0.0036^{g}	0.236± 0.0030^{i}	0.203± 0.004^{i}	0.0932± 0.002^{i}	0.0179± 0.0025^{j}	0.156± 0.003^{j}	0.0072± 0.0030^{j}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl}amino) ethanethioate	0.543± 0.0032^{c}	0.521± 0.001^{e}	0.469± 0.003^{d}	0.496± 0.0041^{d}	0.396± 0.0015^{d}	0.249± 0.001^{f}	0.321± 0.0025^{g}	0.276± 0.0015^{h}	0.163± 0.002^{h}	0.238± 0.0015^{i}	0.204± 0.002^{i}	0.0942± 0.002^{i}
	O-butyl{[(dibutoxyphosphorothioyl) methyl]amino}ethanethioate	0.693± 0.0020^{c}	0.654± 0.0028^{e}	0.612± 0.036^{d}	0.684± 0.0020^{e}	0.645± 0.0057^{d}	0.598± 0.002^{f}	0.681± 0.001^{g}	0.609± 0.001^{g}	0.566± 0.0030^{i}	0.678± 0.004^{i}	0.589± 0.001^{i}	0.489± 0.0001^{i}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{f}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	O-methyl{[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.432± 0.0015^{a}	0.415± 0.00624^{c}	0.376± 0.0015^{d}	0.429± 0.0026^{d}	0.397± 0.0026^{e}	0.329± 0.003^{f}	0.394± 0.0007^{e}	0.325± 0.0035^{f}	0.240± 0.0055^{i}	0.326± 0.0032^{j}	0.315± 0.0015^{m}	0.124± 0.0013^{l}
	O-ethyl{[(diethoxyphosphorothioyl) methyl] amino}ethanethioate	0.362± 0.0083^{c}	0.243± 0.0045d	0.169± 0.0171^{e}	0.276± 0.006^{e}	0.142± 0.0035^{f}	0.093± 0.0189^{h}	0.150± 0.0004^{f}	0.098± 0.0015^{i}	0.014± 0.0015^{j}	0.093± 0.0070^{j}	0.003± 0.0015^{l}	0.009± 0.014^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl}amino) ethanethioate	0.394± 0.0047^{b}	0.324± 0.0032^{c}	0.211± 0.0291^{d}	0.296± 0.0015^{d}	0.208± 0.0025^{g}	0.164± 0.0124^{f}	0.203± 0.0003^{g}	0.159± 0.0026^{f}	0.099± 0.0015^{h}	0.107± 0.0026^{j}	0.096± 0.00105^{l}	0.014± 0.0058^{m}
	O-butyl{[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.429± 0.003^{b}	0.409± 0.002^{c}	0.366± 0.0045^{d}	0.419± 0.0015^{d}	0.389± 0.0073^{e}	0.317± 0.0121^{f}	0.389± 0.0032^{e}	0.314± 0.0050^{f}	0.236± 0.0055^{h}	0.319± 0.0084^{n}	0.309± 0.0036^{j}	0.116± 0.0036^{o}

Total Chlorophyll	**Control**	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.023^{d}	0.940± 0.0023^{d}	0.940± 0.0023^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{c}	0.905± 0.003^{f}	0.852± 0.0020^{j}	0.721± 0.0041^{j}	0.862± 0.0046^{i}	0.724± 0.0034^{k}	0.690± 0.0005^{l}	0.832± 0.003^{m}	0.632± 0.0040^{n}	0.514± 0.0025^{o}
	O-methyl {[(dimethoxyphosphorothioyl) methyl]amino}ethanethioate	0.946 ± 0.0171^{c}	0.931± 0.0010^{c}	0.894± 0.0083^{e}	0.917± 0.003^{g}	0.903± 0.0052^{g}	0.854± 0.0015^{i}	0.876± 0.0015^{i}	0.862± 0.0015^{j}	0.783± 0.002^{j}	0.848± 0.0055^{j}	0.831± 0.0025^{k}	0.741± 0.0047^{l}
	O-ethyl{[(diethoxyphosphorothioyl)methyl] amino}ethanethioate	0.768± 0.0291^{e}	0.632± 0.003^{g}	0.476± 0.0047^{g}	0.553± 0.018^{h}	0.486± 0.0032^{h}	0.232± 0.007^{n}	0.321± 0.0055^{j}	0.339± 0.0093^{j}	0.063± 0.003^{m}	0.224± 0.0015^{k}	0.154± 0.0051^{m}	0.0014± 0.0083^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl} amino)ethanethioate	0.836± 0.0045^{c}	0.802± 0.0032^{e}	0.663± 0.003^{e}	0.661± 0.0015^{g}	0.596± 0.0075^{g}	0.482± 0.0026^{h}	0.579± 0.0124^{j}	0.445± 0.0014^{j}	0.241± 0.0007^{k}	0.349± 0.0291^{k}	0.300± 0.0032^{k}	0.176± 0.0026^{m}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	0.934± 0.0025^{c}	0.924± 0.004^{e}	0.861± 0.0040^{e}	0.909± 0.0132^{g}	0.892± 0.002^{g}	0.834± 0.0052^{h}	0.866± 0.0206^{j}	0.854± 0.01322^{j}	0.769± 0.0032^{k}	0.839± 0.0171^{k}	0.811± 0.0075^{k}	0.721± 0.0068^{l}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

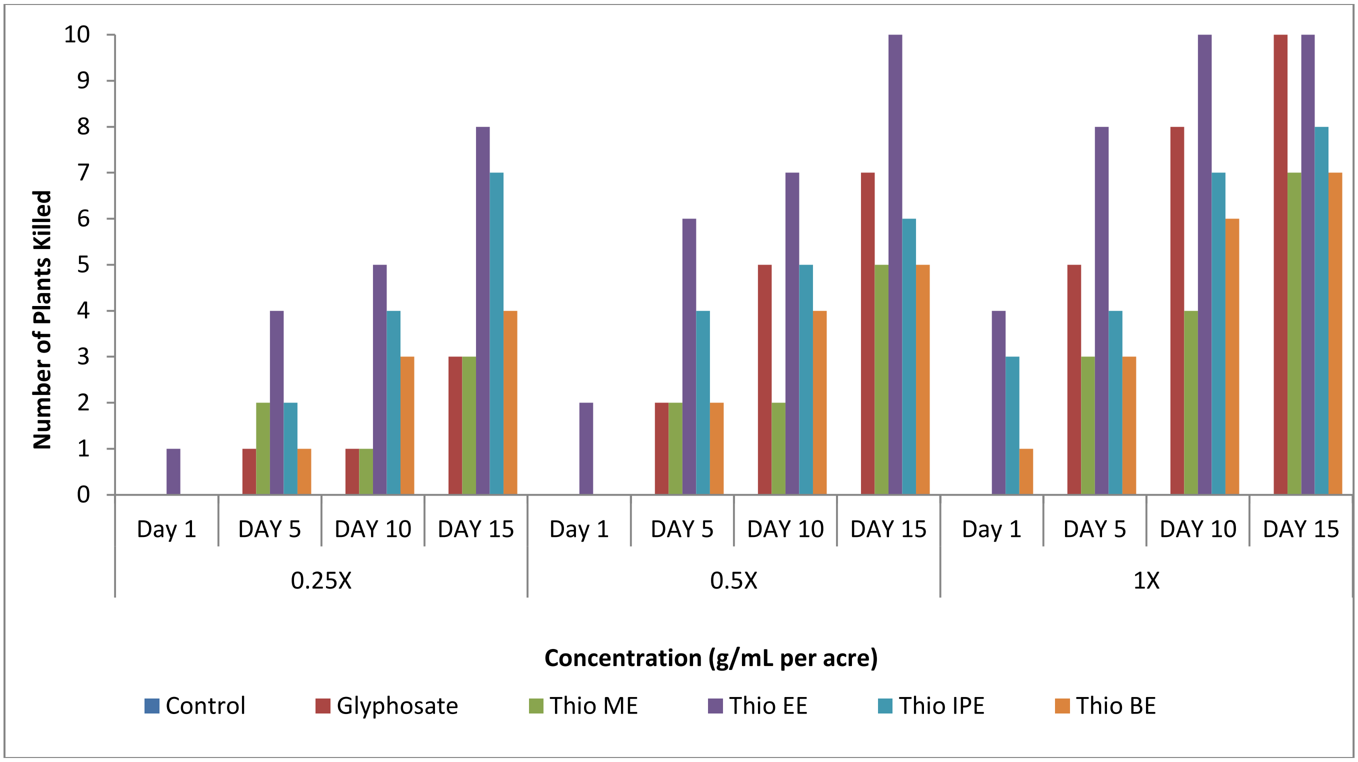


Figure 6.72 Lethal effect of exposure of Thioxylated ester derivatives of glyphosate on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Where, Thio ME = O-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate,
Thio EE = O-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate,
ThioIPE = O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl]methyl}amino)ethanethioate
Thio BE = O-butyl {[(dibutoxyphosphorothioyl)methyl]amino}ethanethioate

6.3.2.6 Metal Complexes of Glyphosate:

Five different metal complexes of glyphosate were synthesized and their herbicidal activity was checked in milieu of decrease in the chlorophyll content in the leaves of *Cyperus rotundus* on exposure to these derivatives. Also the lethal effect of these derivatives on the plants was measured by counting the number of plants killed with respect to time and concentration. As compared to control all the metal complexes have shown minimal decrease in photosynthetic pigments (Chlorophyll a, Chlorophyll b and total Chlorophyll) but no considerable decrease was found in comparison to glyphosate. No significant trend in the decrease of chlorophyll content (chlorophyll a, chlorophyll b, total chlorophyll) was observed in all the cases at all the three test concentrations (0.25X, 0.5X and 1X).

Herbicidal effect of the synthesized metal complexes of glyphosate was also evaluated by inscribing the number of plants killed by the synthesized metal complexes. Maximum number of plants were killed only at the recommended dose (1X) by the **Co-glyphosate complex** (4 out of 10) (**Figure 6.73**). Other derivatives didn't show any significant reduction in the number of plants upon their exposure to these derivatives. No major difference in the results with respect to each other and control were obtained at the lower concentrations of these derivatives.

Table 6.18 Effect of Metal complexes of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0693.± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.0073^{c}	0.687± 0.0073^{c}	0.0.687± 0.0073^{c}	0.683± 0.0051^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}
	Glyphosate	0.690± 0.003^{c}	0.650± 0.003^{e}	0.603± 0.0073^{e}	0.683± 0.0012^{e}	0.634± 0.002^{h}	0.594± 0.0015^{h}	0.680± 0.007^{h}	0.600± 0.0036^{h}	0.560± 0.0056^{i}	0.675± 0.0025^{m}	0.582± 0.005^{j}	0.484± 0.009^{m}
	Zn-Glyphosate complex	0.693± 0.0055^{b}	0.674± 0.0051^{c}	0.600± 0.0030^{d}	0.684± 0.0005^{f}	0.663± 0.0026^{d}	0.593± 0.0017^{g}	0.680± 0.0032^{c}	0.651± 0.0041^{b}	0.572± 0.0056^{g}	0.676± 0.004^{g}	0.642± 0.0025^{h}	0.544± 0.0025^{i}
	Co-Glyphosate complex	0.697± 0.0050^{f}	0.675± 0.0049^{f}	0.651± 0.0037^{f}	0.684± 0.0089^{f}	0.665± 0.0120^{f}	0.632± 0.0025^{g}	0.683± 0.0073^{i}	0.654± 0.0061^{i}	0.611± 0.0023^{i}	0.680± 0.0035^{j}	0.649± 0.0075^{j}	0.540± 0.0030^{j}
	Cu-Glyphosate complex	0.694± 0.007^{c}	0.672± 0.0066^{e}	0.649± 0.0015^{d}	0.687± 0.002^{d}	0.667± 0.0166^{d}	0.630± 0.002^{f}	0.681± 0.0056^{g}	0.653± 0.0015^{h}	0.624± 0.003^{h}	0.678± 0.0282^{i}	0.647± 0.0056^{i}	0.611± 0.0052^{i}
	Ni-Glyphosate complex	0.692± 0.0020^{c}	0.670± 0.0049^{e}	0.643± 0.001^{d}	0.689± 0.0020^{e}	0.665± 0.0134^{d}	0.626± 0.001^{f}	0.684± 0.0036^{g}	0.652± 0.0036^{g}	0.603± 0.0026^{i}	0.679± 0.004^{i}	0.641± 0.0030^{i}	0.531± 0.0043^{i}
	Fe-Glyphosate complex	0.695± 0.0026^{b}	0.671± 0.0037^{c}	0.635± 0.0045^{j}	0.690± 0.0055^{c}	0.670± 0.0026^{c}	0.610± 0.0030^{k}	0.683± 0.0055^{g}	0.648± 0.0133^{i}	0.543± 0.0017^{j}	0.676± 0.0025^{g}	0.632± 0.0052^{l}	0.510± 0.0032^{m}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{f}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	Zn-Glyphosate complex	0.436± 0.0045^{k}	0.422± 0.0032^{c}	0.384± 0.0015^{d}	0.429± 0.0043^{d}	0.410± 0.0017^{e}	0.359± 0.003^{f}	0.401± 0.077^{e}	0.362± 0.0026^{f}	0.341± 0.0035^{i}	0.342± 0.0015^{j}	0.304± 0.0015^{m}	0.294± 0.0081^{l}
	Co-Glyphosate complex	0.440± 0.0026^{c}	0.428± 0.0030^{d}	0.389± 0.0030^{e}	0.430± 0.0015^{e}	0.415± 0.0025^{f}	0.362± 0.0152^{h}	0.422± 0.068^{f}	0.375± 0.0026^{i}	0.357± 0.0014^{j}	0.351± 0.0026^{j}	0.312± 0.0026^{l}	0.297± 0.0035^{m}
	Cu-Glyphosate complex	0.451± 0.0020^{b}	0.438± 0.0037^{c}	0.390± 0.002^{d}	0.436± 0.0055^{d}	0.429± 0.001^{g}	0.385± 0.0005^{f}	0.433± 0.003^{g}	0.400± 0.0045^{f}	0.374± 0.0045^{h}	0.369± 0.0025^{j}	0.351± 0.0035^{l}	0.330± 0.0011^{m}
	Ni-Glyphosate complex	0.439± 0.003^{b}	0.426± 0.0015^{c}	0.387± 0.0015^{d}	0.425± 0.0051^{d}	0.415± 0.002^{e}	0.361± 0.007^{f}	0.369± 0.0015^{e}	0.369± 0.0028^{f}	0.344± 0.0020^{h}	0.351± 0.0051^{n}	0315.± 0.003^{j}	0.296± 0.0009^{o}

	Fe-Glyphosate complex	0.436± 0.0025^{k}	0.410± 0.0011^{k}	0.365± 0.0015^{d}	0.420± 0.0025^{d}	0.396± 0.0045	0.310± 0.007^{m}	0.390± 0.0017^{e}	0.321± 0.0023^{h}	0.241± 0.0026^{l}	0.324± 0.004^{h}	0.300± 0.0070^{k}	0.126± 0.0004^{n}
Total Chlorophyll	**Control**	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.023^{d}	0.940± 0.0023^{d}	0.940± 0.0023^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{c}	0.905± 0.003^{f}	0.852± 0.0020^{j}	0.721± 0.0041^{j}	0.862± 0.0046^{i}	0.724± 0.0034^{k}	0.690± 0.0005^{l}	0.832± 0.003^{m}	0.632± 0.0040^{n}	0.514± 0.0025^{o}
	Zn-Glyphosate complex	0.957 ± 0.0015^{c}	0.927± 0.002^{c}	0.900± 0.0030^{e}	0.943± 0.003^{g}	0.916± 0.0030^{g}	0.884± 0.0020^{i}	0.936± 0.0035^{i}	0.835± 0.0020^{j}	0.826± 0.004^{j}	0.921± 0.0035^{j}	0.830± 0.0035^{k}	0.794± 0.0020^{l}
	Co-Glyphosate complex	0.953± 0.003^{e}	0.923± 0.006^{g}	0.905± 0.0025^{g}	0.922± 0.0015^{h}	0.913± 0.002^{h}	0.891± 0.002^{n}	0.892± 0.0014^{j}	0.824± 0.0035^{j}	0.816± 0.001^{m}	0.848± 0.0011^{k}	0.796± 0.0015^{m}	0.731± 0.0035^{m}
	Cu-Glyphosate complex	0.942± 0.002^{c}	0.932± 0.0030^{e}	0.891± 0.003^{e}	0.927± 0.0005^{g}	0.925± 0.0026^{g}	0.872± 0.0026^{h}	0.886± 0.0045^{j}	0.827± 0.0075^{j}	0.804± 0.002^{k}	0.852± 0.00092^{k}	0.799± 0.003^{k}	0.750± 0.0032^{m}
	Ni-Glyphosate complex	0.956± 0.0015^{c}	0.926± 0.002^{e}	0.900± 0.002^{e}	0.934± 0.0078^{g}	0.913± 0.0036^{g}	0.879± 0.001^{h}	0.893± 0.0020^{j}	0.820± 0.0065^{j}	0.810± 0.002^{k}	0.855± 0.004^{k}	0.792± 0.0025^{k}	0.749± 0.0075^{l}
	Fe-Glyphosate complex	0.952± 0.0152^{d}	0.903± 0.005^{h}	0.894± 0.0026^{l}	0.925± 0.007^{k}	0.883± 0.0026^{n}	0.876± 0.0030^{l}	0.875± 0.0026^{l}	0.841± 0.0036^{m}	0.800± 0.0005^{f}	0.845± 0.0035^{m}	0.780± 0.0050^{k}	0.712± 0.0051^{o}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

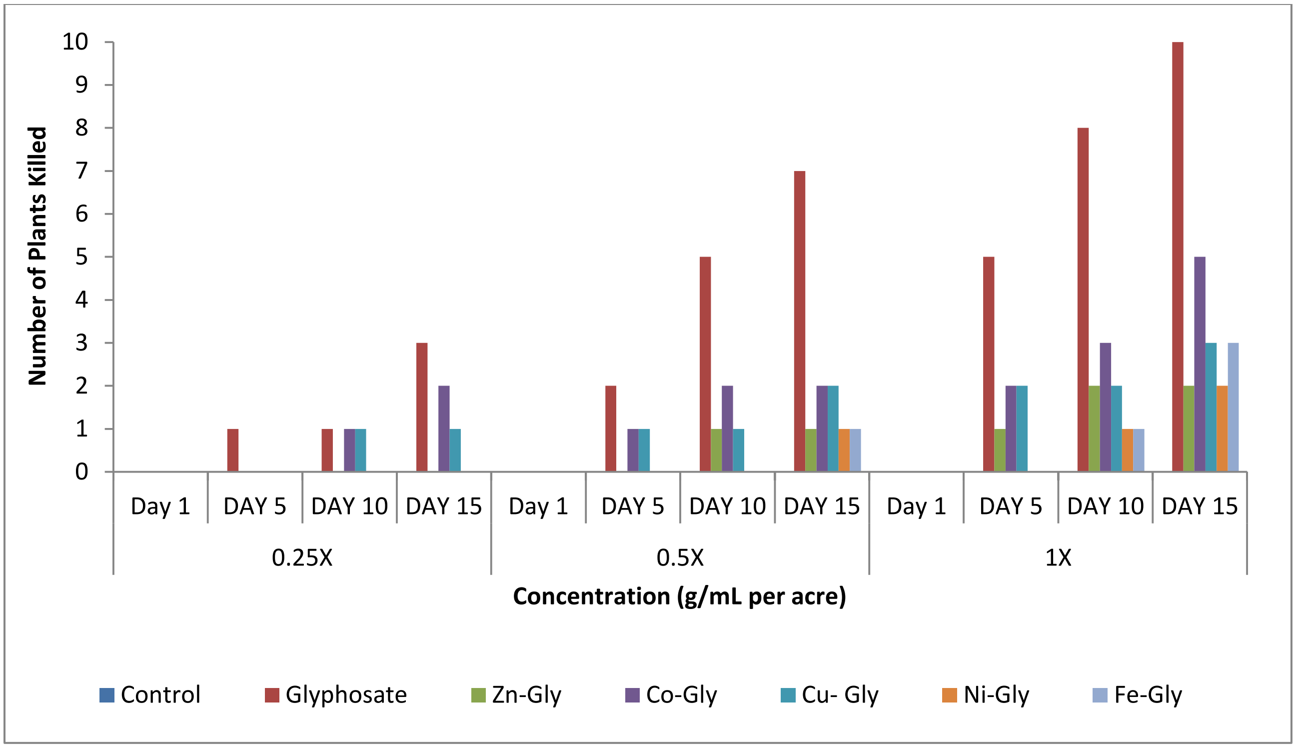


Figure 6.73 Lethal effect of exposure of Metal complexes of glyphosate on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Zn-Gly = Zinc-Glyphosate complex, Co-Gly = Cobalt-Glyphosate complex, Cu-Gly= Copper-Glyphosate complex, Ni-Gly= Nickel-Glyphosate complex, Fe-Gly= Iron-Glyphosate complex

6.3.2.7 Metal Complexes of Methyl Ester Glyphosate: A series of five different metal complexes of methyl ester derivative of glyphosate were synthesized. Their herbicidal effect was checked by determining the chlorophyll content left in the weed (*Cyperus rotundus*) after their exposure to these derivatives and the mortality rate of plant (weed) with respect to time and concentration. All the synthesized derivatives showed moderate to good herbicidal effects on the plant. Out of these derivatives, **Ni-methyl ester derivative of glyphosate complex** (Ni-ME) has shown preeminent herbicidal activity against the weed (*Cyperus rotundus*). At 0.25X 'chlorophyll a' left in the plant (after 15 days treatment) was 0.612 µg/gFW whereas 'chlorophyll a' left in the plant treated with glyphosate was 0.675 µg/gFW. Similarly at 0.5X, 'chlorophyll a' was reduced to 0.510 µg/gFW in case of plant treated with Ni-ME and 0.582 µg/gFW in case of glyphosate. Major decrease in 'chlorophyll a' was observed at the highest dose of 1X (0.400 µg/gFW in case of Ni-ME complex and 0.484 µg/gFW in case of glyphosate). Diminution in 'chlorophyll b' content at all test concentrations was also recorded in case of Ni-ME as compared to control and glyphosate**.** Major decrease in' total chlorophyll' content of the weed was also observed at the highest dose of 1X after 15 days treatment with Ni-ME complex.' Total chlorophyll' left was found to be 0.482 µg/gFW in case of plant treated with Ni-ME complex and 0.514 µg/gFW in case of glyphosate (**Table 6.19).**

Detrimental effects of the synthesized metal complexes of methyl ester derivatives of glyphosate on the weed were also scrutinized from the mortality rate of the plants. After 15 days treatment it has been found that Ni-ME complex has killed maximum number of weed plants as compared to glyphosate. Even at 0.5X it has killed 9 out of 10 plants whereas glyphosate killed only7 plants. Even at 1X, all plants were killed after 10 days in case of Ni-ME complex whereas glyphosate has killed only 8 plants **(Figure 6.74**). From the comparison between the herbicidal effects of both these herbicides, it has been found that Ni-ME complex was more effective in decreasing the chlorophyll content and killing maximum number of weed plants as compared to the glyphosate.

Table 6.19 Effect of Metal-Methyl ester of glyphosate complexes on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Cyperus rotundus*.

Days ⟶		1			5			10			15		
Concentration ⟶		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0693.± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.0073^{c}	0.687± 0.0073^{c}	0.0.687± 0.0073^{c}	0.683± 0.0051^{b}	0.683± 0.0051^{b}	0.683± 0.0051^{b}
	Glyphosate	0.690± 0.003^{c}	0.650± 0.003^{e}	0.603± 0.0073^{e}	0.683± 0.0012^{e}	0.634± 0.002^{h}	0.594± 0.0015^{h}	0.680± 0.007^{h}	0.600± 0.0036^{h}	0.560± 0.0056^{i}	0.675± 0.0025^{m}	0.582± 0.005^{j}	0.484± 0.009^{m}
	Zn-Methyl ester of Glyphosate complex	0.692± 0.0050^{b}	0.654± 0.0015^{c}	0.625± 0.0032^{d}	0.684± 0.0023^{f}	0.640± 0.0030^{d}	0.601± 0.001^{g}	0.683± 0.00208^{c}	0.631± 0.0035^{b}	0.583± 0.0026^{g}	0.680± 0.0321^{g}	0.623± 0.0081^{h}	0.492± 0.0040^{i}
	Co-Methyl ester of Glyphosate complex	0.697± 0.0075^{f}	0.657± 0.0030^{f}	0.629± 0.0030^{f}	0.689± 0.0026^{f}	0.649± 0.0015^{f}	0.609± 0.0017^{g}	0.687± 0.0036^{i}	0.632± 0.0014^{i}	0.587± 0.0045^{i}	0.684± 0.002^{j}	0.620± 0.0003^{j}	0.499± 0.0015^{j}
	Cu-Methyl ester of Glyphosate complex	0.694± 0.0025^{c}	0.654± 0.002^{e}	0.622± 0.0037^{d}	0.685± 0.0035^{d}	0.651± 0.0005^{d}	0.599± 0.0034^{f}	0.681± 0.0025^{g}	0.630± 0.0045^{h}	0.575± 0.0028^{h}	0.677± 0.0032^{i}	0.616± 0.0011^{i}	0.487± 0.0026^{i}
	Ni-Methyl ester of Glyphosate complex	0.670± 0.0051^{c}	0.650± 0.00152^{e}	0.599± 0.001^{d}	0.634± 0.0023^{e}	0.622± 0.0078^{d}	0.574± 0.0028^{f}	0.623± 0.0035^{g}	0.532± 0.002^{g}	0.493± 0.03^{i}	0.612± 0.002^{i}	0.510± 0.0009^{i}	0.400± 0.0035^{i}
	Fe-Methyl ester of Glyphosate complex	0.695± 0.0051^{b}	0.655± 0.0015^{c}	0.628± 0.0025^{j}	0.685± 0.0036^{c}	0.635± 0.0070^{c}	0.607± 0.003^{k}	0.682± 0.002^{g}	0.620± 0.0026^{i}	0.589± 0.0026^{j}	0.681± 0.0025^{g}	0.590± 0.0004^{l}	0.499± 0.003^{m}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{f}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	Zn-Methyl ester of Glyphosate complex	0.436± 0.005^{k}	0.412± 0.0061^{c}	0.372± 0.0032^{d}	0.422± 0.0026^{d}	0.394± 0.0027^{e}	0.315± 0.003^{f}	0.398± 0.0015^{e}	0.376± 0.0043^{f}	0.246± 0.0041^{i}	0.374± 0.0036^{j}	0.305± 0.005^{m}	0.111± 0.0025^{l}
	Co-Methyl ester of Glyphosate complex	0.430± 0.0075^{c}	0.419± 0.0075^{d}	0.379± 0.0025^{e}	0.426± 0.0023^{e}	0.399± 0.0026^{f}	0.312± 0.002^{h}	0.397± 0.0032^{f}	0.379± 0.0035^{i}	0.240± 0.0032^{j}	0.370± 0.0025^{j}	0.300± 0.0025^{l}	0.114± 0.0036^{m}
	Cu-Methyl ester of Glyphosate complex	0.432± 0.0025^{b}	0.417± 0.0026^{c}	0.375± 0.002^{d}	0.429± 0.0026^{d}	0.397± 0.002^{g}	0.310± 0.007^{m}	0.395± 0.002^{g}	0.384± 0.002^{f}	0.247± 0.0076^{h}	0.372± 0.001^{j}	0.310± 0.007^{l}	0.117± 0.0017^{m}
	Ni-Methyl ester of Glyphosate complex	0.400± 0.0051^{b}	0.372± 0.0052^{c}	0.301± 0.0037^{d}	0.396± 0.0035^{d}	0.351± 0.003^{e}	0.275± 0.005^{f}	0.352± 0.002^{e}	0.302± 0.001^{f}	0.205± 0.0015^{h}	0.275± 0.037^{n}	0.209.± 0.047^{j}	0.0998± 0.005^{o}
	Fe-Methyl ester of Glyphosate complex	0.424± 0.0051^{k}	0.406± 0.0052^{k}	0.364± 0.0096^{d}	0.420± 0.0035^{d}	0.387± 0.035^{k}	0.302± 0.0025^{j}	0.389± 0.0027^{e}	0.370± 0.002^{h}	0.234± 0.0055^{l}	0.365± 0.0036^{h}	0.298± 0.003^{k}	0.103± 0.0015^{n}

Total Chlorophyll	**Control**	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.023^{d}	0.940± 0.0023^{d}	0.940± 0.0023^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}	0.925± 0.0024^{d}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{c}	0.905± 0.003^{f}	0.852± 0.0020^{j}	0.721± 0.0041^{j}	0.862± 0.0046^{i}	0.724± 0.0034^{k}	0.690± 0.0005^{l}	0.832± 0.003^{m}	0.632± 0.0040^{n}	0.514± 0.0025^{o}
	Zn-Methyl ester of Glyphosate complex	0.936 ± 0.0025^{c}	0.917± 0.003^{c}	0.826± 0.0077^{e}	0.916± 0.004^{g}	0.864± 0.004^{g}	0.759± 0.0011^{i}	0.875± 0.0025^{i}	0.795± 0.002^{j}	0.694± 0.004^{j}	0.845± 0.0031^{j}	0.642± 0.0040^{k}	0.632± 0.0025^{l}
	Co-Methyl ester of Glyphosate complex	0.942± 0.007^{e}	0.913± 0.0027^{g}	0.829± 0.007^{g}	0.913± 0.0037^{h}	0.866± 0.005^{h}	0.752± 0.0095^{n}	0.872± 0.0037^{j}	0.799± 0.0023^{j}	0.699± 0.010^{m}	0.849± 0.0039^{k}	0.644± 0.0032^{m}	0.630± 0.0052^{m}
	Cu-Methyl ester of Glyphosate complex	0.949± 0.0074^{c}	0.921± 0.001^{e}	0.831± 0.0026^{e}	0.917± 0.0024^{g}	0.869± 0.0036^{g}	0.765± 0.0545^{h}	0.877± 0.0035^{j}	0.786± 0.0026^{j}	0.701± 0.002^{k}	0.852± 0.0052^{k}	0.649± 0.0040^{k}	0.639± 0.0057^{m}
	Ni-Methyl ester of Glyphosate complex	0.910± 0.0064^{c}	0.874± 0.005^{e}	0.795± 0.004^{e}	0.872± 0.0058^{g}	0.836± 0.0015^{g}	0.702± 0.0015^{h}	0.831± 0.0049^{j}	0.703± 0.0035^{j}	0.651± 0.002^{k}	0.794± 0.004^{k}	0.604± 0.0035^{k}	0.482± 0.0043^{l}
	Fe-Methyl ester of Glyphosate complex	0.935± 0.0032^{d}	0.909± 0.009^{h}	0.807± 0.0026^{l}	0.909± 0.0014^{k}	0.850± 0.0035^{n}	0.729± 0.0058^{l}	0.865± 0.0036^{l}	0.732± 0.0075^{m}	0.692± 0.0002^{f}	0.837± 0.002^{m}	0.635± 0.0036^{k}	0.519± 0.0251^{o}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

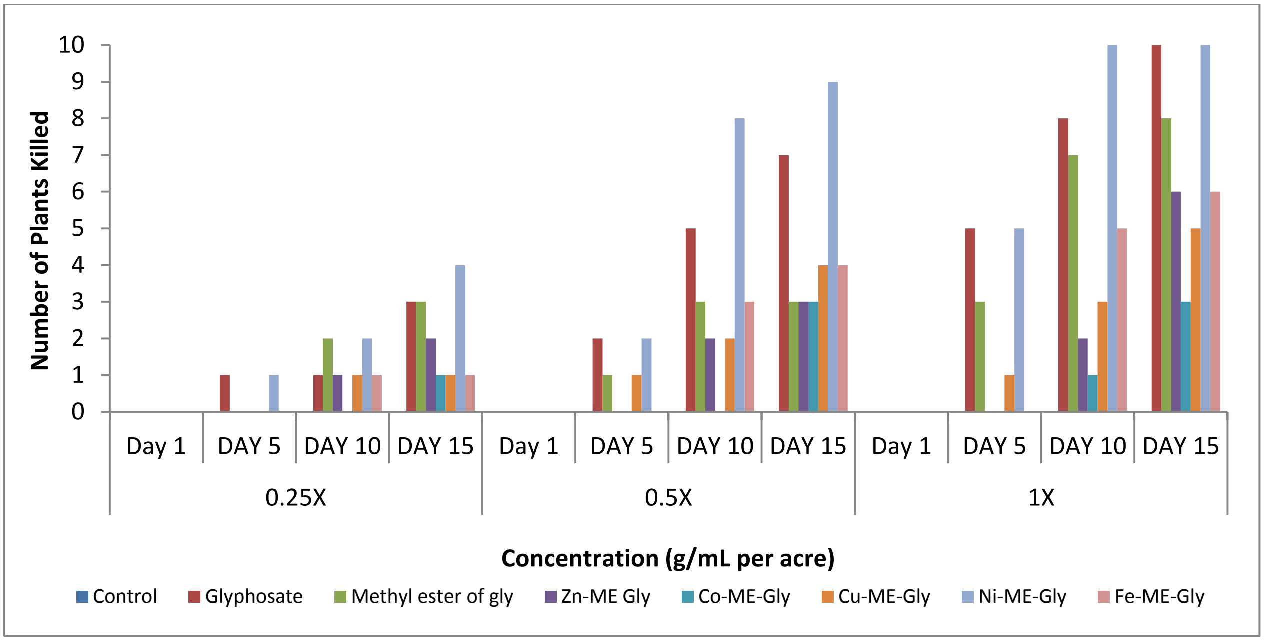


Figure 6.74 Lethal effect of exposure of Metal-Methyl ester of glyphosate complexes on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Zn-ME Gly = Zinc-Methyl ester of glyphosate complex, Co-ME Gly = Cobalt- Methyl ester of glyphosate complex, Cu-ME Gly= Copper- Methyl ester of glyphosate complex, Ni-ME Gly= Nickel- Methyl ester of glyphosate complex, Fe- ME Gly= Iron- Methyl ester of glyphosate complex

6.3.2.8 Metal Complexes of Ethyl ester of glyphosate:

Herbicidal effects of the synthesized metal complexes of ethyl ester derivative of glyphosate was evaluated on the common weed (*Cyperus rotundus*). Decrease in the content of photosynthetic pigments was recorded with respect to concentration of the derivative and time.

In comparison to control (water treatment) all the five metal complexes have shown diminution in the chlorophyll content. However this decrease in the chlorophyll content of treated plants was not comparable to glyphosate. Ethyl ester derivative of glyphosate has caused tremendous reductionin the chlorophyll content of treated plants at all the test concentrations (0.25X, 0.5X and 1X).Out of all the five metal complexes of ethyl ester derivative of glyphosate, **Ni-ethyl ester derivative** (Ni-EE gly) has momentously decreased the amount of 'Chl a,'Chl b' and 'Chl a+b' at all the three concentrations. At 0.25X 'chlorophyll a' left in the plant after 15 days treatment was 0.673 µg/g FW and it get further reduced to 0.585 µg/g FW at 0.5X . By using the highest concentration of this derivative i.e., at 1X, large decrease in the chlorophyll content was observed (0.489 µg/g FW). 'Chlorophyll b' left in the plants (after 15 days) treated with 0.25X of Ni-EE gly was 0.315 µg/g FW, with 0.5X of Ni-EE gly was 0.0.304 µg/g FW and with 1X of

Ni-EE gly was 0.191 μg/g FW. Similar kind of reduction was also noticed in case of 'total chlorophyll' content of the treated plants. Apart from Ni-EE gly, other metal complexes have also caused reduction in the chlorophyll content of the weed but their results are less significant as compared to glyphosate and Ni-EE gly (**Table 6.20).**

In spite of reduction in the photosynthetic pigments, herbicidal activity of the metal complexes of ethyl ester derivative of glyphosate was also assessed based on the number of plants killed after 15 days. In comparison to glyphosate, ethyl ester of glyphosate has killed maximum number of plants . However the **Ni-ethyl ester derivative of glyphosate** has also shown analogous results. It has killed 5 out of 10 plants at 0.5X after 10 days of treatment. However at the same concentration after 15 days, 6 plants were killed. At the highest concentration (1X) 9 plants out of 10 were killed by the complex (**Figure 6.75).** These results showed that apart from ethyl ester derivative of glyphosate, Ni-EE gly has also showed good herbicidal effects on the weed plants.

Table 6.20 Effect of Metal- Ethyl ester of glyphosate complexes on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b' (µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.00611^{a}	0.699± 0.00611^{a}	0.699± 0.00611^{a}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}
	Glyphosate	0.690± 0.0036^{c}	0.650± 0.00305^{d}	0.603± 0.00737^{d}	0.683± 0.0012^{d}	0.634± 0.00264^{f}	0.594± 0.0015^{g}	0.680± 0.0070^{f}	0.600± 0.0036^{h}	0.560± 0.0056^{h}	0.675± 0.0025^{i}	0.582± 0.0050^{i}	0.484± 0.009^{i}
	Zn- Ethyl ester of Glyphosate complex	0.698± 0.0036^{c}	0.669± 0.0035^{c}	0.623± 0.0073^{d}	0.690± 0.0041^{b}	0.642± 0.004^{d}	0.604± 0.0094^{j}	0.685± 0.0025^{c}	0.615± 0.003^{b}	0.584± 0.0040^{g}	0.679± 0.0036^{g}	0.596± 0.0045^{h}	0.504± 0.0055^{i}
	Co- Ethyl ester of Glyphosate complex	0.699± 0.0032^{f}	0.672± 0.0020^{f}	0.617± 0.0028^{f}	0.688± 0.003 ^{f}	0.658± 0.0032^{f}	0.603± 0.019^{g}	0.683± 0.0035^{i}	0.625± 0.0035^{i}	0.574± 0.0030^{i}	0.677± 0.0087j	0.599± 0.0035^{j}	0.497± 0.003^{j}
	Cu- Ethyl ester of Glyphosate complex	0694± 0.0086^{c}	0.662± 0.0085^{e}	0.631± 0.0096^{d}	0.685± 0.0035^{d}	0.649± 0.0026^{d}	0.612± 0.0015^{f}	0.682± 0.0041^{g}	0.629± 0.0058^{h}	0.579± 0.010^{h}	0.678± 0.0036^{i}	0.594± 0.002^{i}	0.500± 0.01069^{i}
	Ni- Ethyl ester of Glyphosate complex	0.691± 0.0035^{c}	0.654± 0.030^{e}	0.609± 0.0049^{d}	0.687± 0.0015^{e}	0.639± 0.004^{d}	0.597± 0.0546^{f}	0.681± 0.0041^{g}	0.604± 0.002^{g}	0.569± 0.0045^{i}	0.673± 0.0065^{i}	0.585± 0.004^{i}	0.489± 0.002^{i}
	Fe- Ethyl ester of Glyphosate complex	0.695± 0.0043^{e}	0.668± 0.0036^{c}	0.614± 0.0037^{g}	0.689± 0.0035^{b}	0.645± 0.0041^{d}	0.600± 0.0485^{k}	0.684± 0.0065^{i}	0.615± 0.0234^{g}	0.572± 0.002	0.677± 0.0035^{i}	0.589± 0.0041^{j}	0.495± 0.003^{i}
Chlorophyll b	**Control**	0.452± 0.00264^{a}	0.452± 0.00305^{a}	0.452± 0.0015^{a}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{m}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	Zn- Ethyl ester of Glyphosate complex	0.432± 0.002^{a}	0.424± 0.0026^{c}	0.410± 0.0075^{d}	0.420± 0.0078^{d}	0.396± 0.0025^{e}	0.319± 0.005^{f}	0.400± 0.0026^{e}	0.324± 0.0061^{f}	0.294± 0.0040^{i}	0.357± 0.0023^{j}	0.314± 0.0030^{m}	0.201± 0.0040^{l}
	Co- Ethyl ester of Glyphosate complex	0.446± 0.003^{c}	0.419± 0.0041^{d}	0.398± 0.0020^{e}	0.429± 0.0036^{e}	0.399± 0.0025^{f}	0.324± 0.0026^{h}	0.410± 0.0032^{f}	0.329± 0.0025^{i}	0.314± 0.0025^{j}	0.340± 0.0041^{j}	0.317± 0.0041^{l}	0.285± 0.0015^{m}
	Cu- Ethyl l ester of Glyphosate complex	0.429± 0.001^{b}	0.415± 0.0045^{c}	0.394± 0.0036^{d}	0.419± 0.0025^{d}	0.394± 0.004^{g}	0.316± 0.0083^{f}	0.399± 0.0058^{g}	0.318± 0.0036^{f}	0.301± 0.0020^{h}	0.332± 0.0035^{j}	0.309± 0.0062^{l}	0.272± 0.002^{m}
	Ni- Ethyl ester of Glyphosate complex	0.428± 0.002^{b}	0.410± 0.003^{c}	0.365± 0.0035^{d}	0.417± 0.0020^{d}	0.390± 0.0025^{e}	0.310± 0.0026^{f}	0.398± 0.0025^{e}	0.315± 0.0035^{f}	0.254± 0.0017^{h}	0.315± 0.005^{n}	0.304± 0.0007^{j}	0.191± 0.003^{o}
	Fe- Ethyl ester of Glyphosate complex	0.431± 0.0037^{c}	0.418± 0.0035^{d}	0.372± 0.0055^{k}	0.422± 0.0066^{d}	0.392± 0.0056^{h}	0.315± 0.0035^{f}	0.395± 0.005^{i}	0.314± 0.003	0.282± 0.0052^{l}	0.320± 0.0041^{m}	0.307± 0.0015^{j}	0.198± 0.004^{0}

Total Chlorophyll	**Control**	0.963 ± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.925± 0.0024^{f}	0.925± 0.0024^{f}	0.925± 0.0024^{f}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{f}	0.905± 0.003^{g}	0.852± 0.0020^{i}	0.721± 0.0041^{h}	0.862± 0.00461^{i}	0.724± 0.0034^{j}	0.690± 0.0005^{j}	0.832± 0.0030^{j}	0.632± 0.0040^{k}	0.514± 0.0025^{m}
	Zn- Ethyl ester of Glyphosate complex	0.962± 0.002^{c}	0.942± 0.0032^{c}	0.902± 0.0045^{e}	0.925± 0.003^{g}	0.900± 0.0197^{g}	0.871± 0.0094^{i}	0.896± 0.0020^{i}	0.856± 0.004^{j}	0.805± 0.0040^{j}	0.855± 0.0036^{j}	0.742± 0.0045^{k}	0.708± 0.0055^{l}
	Co- Ethyl l ester of Glyphosate complex	0.956± 0.0086^{e}	0.934± 0.0234^{g}	0.893± 0.049^{g}	0.931± 0.003^{h}	0.895± 0.0047^{h}	0.851± 0.0192^{n}	0.881± 0.0037^{j}	0.849± 0.0035^{j}	0.824± 0.0030^{m}	0.850± 0.0087^{k}	0.731± 0.0032^{l}	0.694± 0.003^{m}
	Cu- Ethyl ester of Glyphosate complex	0.942± 0.0035^{c}	0.921± 0.0020^{e}	0.897± 0.0028^{e}	0.926± 0.0015^{g}	0.881± 0.0072^{g}	0.842± 0.0015^{h}	0.892± 0.0035^{j}	0.842± 0.0058^{j}	0.811± 0.0102^{k}	0.857± 0.0036^{k}	0.725± 0.0026^{k}	0.709± 0.0106^{m}
	Ni- Ethyl l ester of Glyphosate complex	0.939± 0.0043^{c}	0.914± 0.0102^{e}	0.826± 0.0096^{e}	0.911± 0.0035^{g}	0.872± 0.003^{g}	0.713± 0.0546^{h}	0.874± 0.004^{j}	0.832± 0.002^{j}	0.702± 0.0030^{k}	0.846± 0.0065^{k}	0.704± 0.004^{k}	0.631± 0.002^{l}
	Fe- Ethyl ester of Glyphosate complex	0.945± 0.0062^{c}	0.919± 0.0085	0.871± 0.004	0.919± 0.0065^{h}	0.879± 0.003^{h}	0.803± 0.0485	0.889± 0.0065^{k}	0.837± 0.0036	0.752± 0.0045	0.849± 0.0035^{l}	0.714± 0.0041^{k}	0.712± 0.0015^{k}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

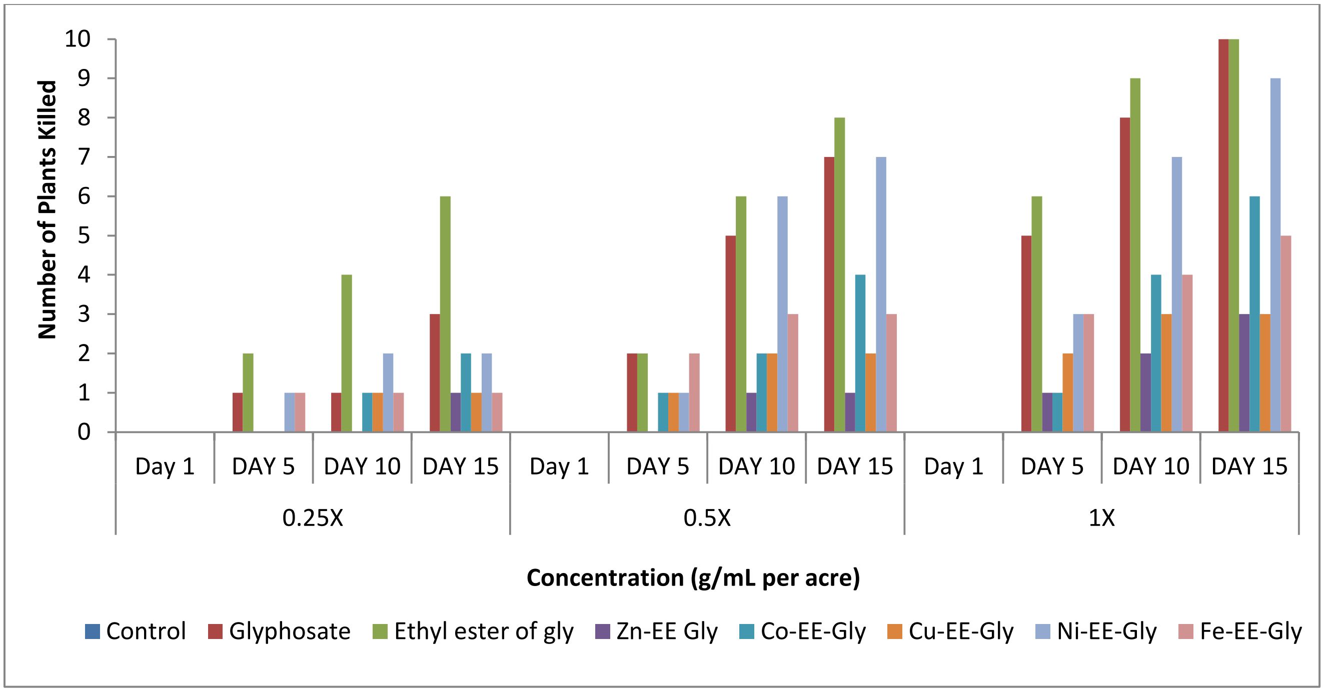


Figure 6.75 Lethal effect of exposure of Metal-Ethyl ester of glyphosate complexes on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Zn-EE Gly = Zinc-Ethyl ester of glyphosate complex, Co-EE Gly = Cobalt- Ethyl ester of glyphosate complex, Cu-EE Gly= Copper- Ethyl ester of glyphosate complex, Ni-EE Gly= Nickel- Ethyl ester of glyphosate complex, Fe- EE Gly= Iron- Ethyl ester of glyphosate complex

6.3.2.9 Metal Complexes of Isopropyl ester derivative of glyphosate:

Herbicidal effect of five different metal complexes of isopropyl ester derivative of glyphosate was scrutinized on the weed (*Cyperus rotundus*). Plants were treated with three different concentrations (0.25X, 0.5X and 1X) of the derivatives and chlorophyll content from their leaves was evaluated. None of the derivative have shown better results as compared to glyphosate. Little decrease in the chlorophyll pigments has been noticed in all cases (**Table 6.21**). In comparison to control, only a slight decrease in 'chlorophyll a' was observed at all the test concentrations even after 15 days. Amongst all the derivatives, **Fe-Isopropyl ester derivative of glyphosate** (Fe-IPE gly), at 0.25X has shown reduction in 'chlorophyll a' content (0.676µg/g FW) in comparison to control (0.675µg/g FW). However no significant reduction was further noticed even at higher concentrations [at 0.5X (0.585 µg/gFW) and at 1X (0.489 µg/gFW). 'Chlorophyll b' and 'total chlorophyll' content also depicted similar kind of trend. For Fe-IPE gly at 0.25X, 'chlorophyll b' found was 0.311 µg/gFW and it was further reduced to

0.121µg/gFW at 1X **(Figure 6.76)**. Not significant decrease in the photosynthetic pigments was observed in other metal complexes of iso propyl ester derivative of glyphosate.

Table 6.21 Effect of Metal-Isopropyl ester of glyphosate complexes on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b' (µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.00611^{a}	0.699± 0.00611^{a}	0.699± 0.00611^{a}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}
	Glyphosate	0.690± 0.0036^{c}	0.650± 0.00305^{d}	0.603± 0.00737^{d}	0.683± 0.0012^{d}	0.634± 0.00264^{f}	0.594± 0.0015^{g}	0.680± 0.0070^{f}	0.600± 0.0036^{h}	0.560± 0.0056^{h}	0.675± 0.0025^{i}	0.582± 0.0050^{i}	0.484± 0.009^{i}
	Zn-Isopropyl ester of Glyphosate complex	0.695± 0.002^{c}	0.655± 0.0034^{c}	0.627± 0.0037^{d}	0.687± 0.0015^{b}	0.639± 0.002^{d}	0.615± 0.001^{j}	0.683± 0.0025^{c}	0.624± 0.005^{b}	0.579± 0.002^{g}	0.680± 0.0032^{g}	0.594± 0.0030^{h}	0.513± 0.0025^{i}
	Co-Isopropyl ester of Glyphosate complex	0.698± 0.0005^{f}	0.672± 0.005^{f}	0.611± 0.0015^{f}	0.690± 0.0035^{f}	0.653± 0.002^{f}	0.598± 0.015^{g}	0.685± 0.0058^{i}	0.631± 0.0025^{i}	0.582± 0.004^{i}	0.679± 0.0045j	0.599± 0.0056^{j}	0.524± 0.002^{j}
	Cu-Isopropyl ester of Glyphosate complex	0697± 0.003^{c}	0.684± 0.0105^{e}	0.631± 0.0043^{d}	0.691± 0.0047^{d}	0.662± 0.0052^{d}	0.612± 0.002^{f}	0.684± 0.001^{g}	0.627± 0.0032^{h}	0.595± 0.002^{h}	0.677± 0.004^{i}	0.598± 0.0055^{i}	0.494± 0.002^{i}
	Ni-Isopropyl ester of Glyphosate complex	0.699± 0.0035^{c}	0.664± 0.003^{e}	0.655± 0.0052^{d}	0.689± 0.016^{e}	0.642± 0.0026^{d}	0.604± 0.002^{f}	0.684± 0.0037^{g}	0.615± 0.002^{g}	0.583± 0.0045^{i}	0.680± 0.0110^{i}	0.587± 0.001^{i}	0.503± 0.037^{i}
	Fe-Isopropyl ester of Glyphosate complex	0.694± 0.0030^{e}	0.652± 0.0085^{c}	0.612± 0.0035^{g}	0.685± 0.004^{b}	0.635± 0.0026^{d}	0.596± 0.003^{k}	0.682± 0.0036^{i}	0.610± 0.0045^{g}	0.574± 0.008	0.676± 0.0041^{i}	0.585± 0.0031^{j}	0.489± 0.002^{i}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.450± 0.0030^{b}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{b}	0.400± 0.0045^{b}	0.360± 0.0032^{c}	0.414± 0.0030^{c}	0.385± 0.0020^{d}	0.300± 0.003^{d}	0.385± 0.0015^{e}	0.310± 0.0034^{g}	0.232± 0.0046^{m}	0.301± 0.004^{j}	0.296± 0.0040^{k}	0.109± 0.0030^{k}
	Zn-Isopropyl ester of Glyphosate complex	0.431± 0.003^{a}	0.417± 0.0025^{c}	0.396± 0.0025^{d}	0.426± 0.0078^{d}	0.399± 0.0025^{e}	0.341± 0.0015^{f}	0.397± 0.0026^{e}	0.354± 0.0025^{f}	0.332± 0.0020^{i}	0.375± 0.002^{j}	0.300± 0.005^{m}	0.289± 0.0040^{l}
	Co-Isopropyl ester of Glyphosate complex	0.446± 0.003^{c}	0.427± 0.0030^{d}	0.381± 0.0025^{e}	0.432± 0.0036^{e}	0.405± 0.002^{f}	0.332± 0.002^{h}	0.411± 0.0032^{f}	0.368± 0.002^{i}	0.315± 0.0072^{j}	0.362± 0.002^{j}	0.325± 0.003^{l}	0.291± 0.0036^{m}
	Cu-Isopropyl ester of Glyphosate complex	0.439± 0.001^{b}	0.422± 0.001^{c}	0.371± 0.001^{d}	0.421± 0.0025^{d}	0.401± 0.0026^{g}	0.325± 0.002^{f}	0.399± 0.0058^{g}	0.359± 0.0026^{f}	0.301± 0.0030^{h}	0.340± 0.004^{j}	0.317± 0.0045^{l}	0.275± 0.002^{m}
	Ni-Isopropyl ester of Glyphosate complex	0.432± 0.002^{b}	0.420± 0.009^{c}	0.399± 0.0020^{d}	0.425± 0.0020^{d}	0.390± 0.0011^{e}	0.321± 0.001^{f}	0.405± 0.0025^{e}	0.382± 0.004^{f}	0.299± 0.002^{h}	0.311± 0.0035^{n}	0.314± 0.003^{j}	0.252± 0.003^{o}
	Fe-Isopropyl ester of Glyphosate complex	0.427± 0.0037^{c}	0.412± 0.0047^{d}	0.369± 0.0035^{k}	0.419± 0.0066^{d}	0.392± 0.0056^{h}	0.310± 0.002^{f}	0.389± 0.005^{i}	0.321± 0.0011^{i}	0.241± 0.00152^{l}	0.320± 0.0041^{m}	0.301± 0.001^{j}	0.121± 0.007^{0}

Total Chlorophyll	**Control**	0.963 ± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.925± 0.0024^{f}	0.925± 0.0024^{f}	0.925± 0.0024^{f}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{f}	0.905± 0.003^{g}	0.852± 0.0020^{i}	0.721± 0.0041^{h}	0.862± 0.00461^{i}	0.724± 0.0034^{j}	0.690± 0.0005^{j}	0.832± 0.0030^{j}	0.632± 0.0040^{k}	0.514± 0.0025^{m}
	Zn- Isopropyl ester of Glyphosate complex	0.949± 0.002^{c}	0.924± 0.0045^{c}	0.903± 0.0036^{e}	0.929± 0.004^{g}	0.906± 0.0274^{g}	0.864± 0.0045^{i}	0.915± 0.0043^{i}	0.835± 0.003^{j}	0.752± 0.0020^{j}	0.874± 0.0030^{j}	0.797± 0.0040^{k}	0.671± 0.0034^{l}
	Co-Isopropyl ester of Glyphosate complex	0.952± 0.002^{e}	0.936± 0.0251^{g}	0.911± 0.0068^{g}	0.931± 0.0017^{h}	0.912± 0.003^{h}	0.872± 0.007^{n}	0.912± 0.002^{j}	0.874± 0.008^{j}	0.764± 0.0020^{m}	0.862± 0.0015^{k}	0.803± 0.0032^{l}	0.693± 0.0026^{m}
	Cu-Isopropyl ester of Glyphosate complex	0.962± 0.0036^{c}	0.951± 0.0026^{e}	0.932± 0.0045^{e}	0.929± 0.0037^{g}	0.926± 0.0020^{g}	0.899± 0.0041^{h}	0.916± 0.0037^{j}	0.894± 0.0040^{j}	0.771± 0.0035^{k}	0.869± 0.003^{k}	0.823± 0.0030^{k}	0.682± 0.0023^{m}
	Ni- Isopropyl ester of Glyphosate complex	0.945± 0.007^{c}	0.931± 0.0264^{e}	0.909± 0.0045^{e}	0.922± 0.0025^{g}	0.903± 0.002^{g}	0.869± 0.0025^{h}	0.903± 0.004^{j}	0.830± 0.0055^{j}	0.752± 0.0051^{k}	0.883± 0.003^{k}	0.786± 0.002^{k}	0.679± 0.004^{l}
	Fe-Isopropyl ester of Glyphosate complex	0.937± 0.0020^{c}	0.921± 0.001	0.851± 0.0051	0.916± 0.005^{h}	0.884± 0.004^{h}	0.756± 0.0035	0.893± 0.0060^{k}	0.764± 0.001	0.703± 0.0025	0.851± 0.001^{l}	0.700± 0.0032^{k}	0.601± 0.0005^{k}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

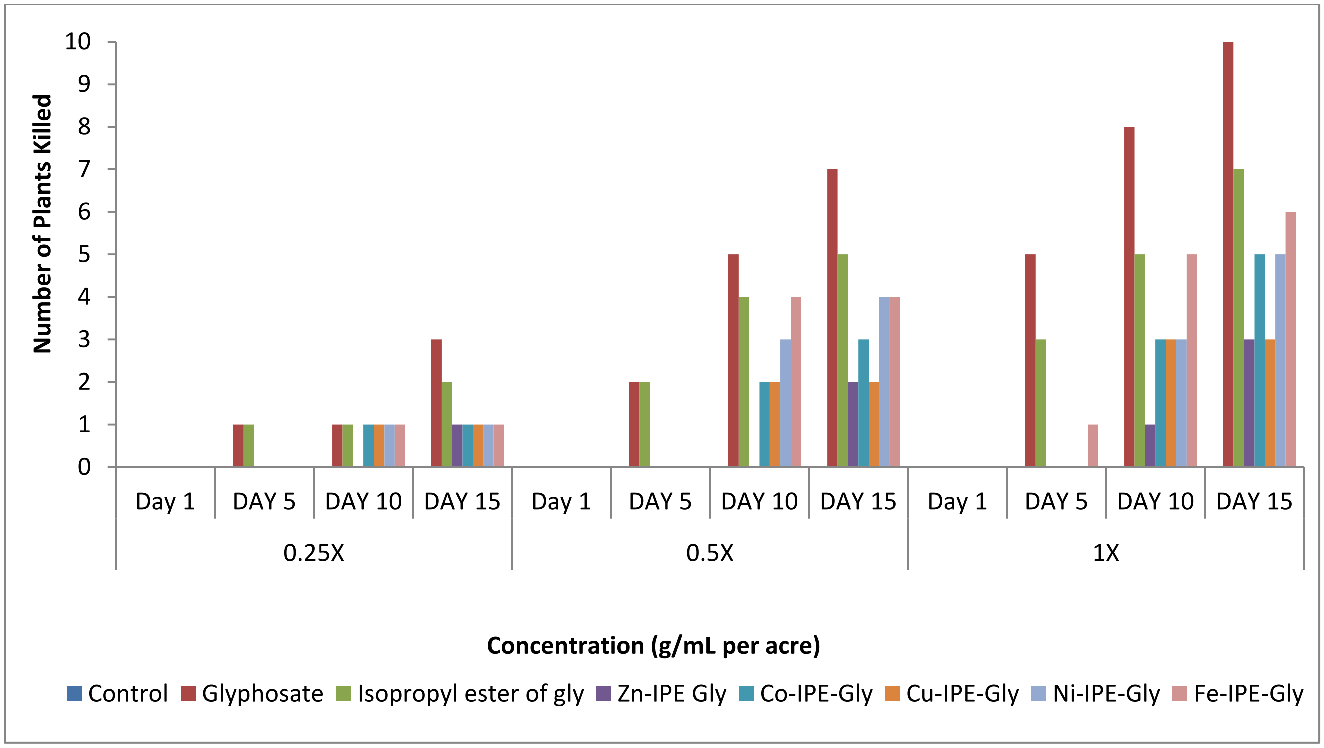


Figure 6.76 Lethal effect of exposure of Metal-Isopropyl ester of glyphosate complexes on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Zn-IPE Gly = Zinc-Isopropyl ester of glyphosate complex, Co-IPE Gly = Cobalt-Isopropyl ester of glyphosate complex, Cu-IPE Gly= Copper-Isopropyl ester of glyphosate complex, Ni-IPE Gly= Nickel-Isopropyl ester of glyphosate complex, Fe-IPE Gly= Iron-Isopropyl ester of glyphosate complex

6.3.2.10 Metal Complexes of n-Propyl ester derivative of glyphosate:

Herbicidal effects of the metal complexes of n-Propyl ester derivative of glyphosate was also studied on *Cyperus rotundus*. Reduction in the chlorophyll content was recorded in the treated plants for 15 days and was compared with control and glyphosate treatment. A remarkable decline of photosynthetic pigments was recorded in the plants treated with the derivatives. Also a notable decrease in the number of weed plants (plants killed) was recorded after their treatment with the derivatives at different concentrations. After 15 days, at 0.25X **Ni-Propyl ester derivative of glyphosate** (Ni-PE) has effectively decreased the 'chlorophyll a' (0.657 µg/gFW), 'chlorophyll b' (0.291 µg/gFW) and 'total chlorophyll' (0.822 µg/gFW) (**Table 6.23**). Significant decline was also observed at 0.5X and 1X concentrations ['chlorophyll b' left in the plant was found to be 0.202 µg/gFW at 0.5X and 0.102 µg/gFW at 1X and 'total chlorophyll found was 0.628 µg/gFW at 0.5X and 0.509 µg/gFW at 1X]. However, Cu complex of n-propyl ester derivative of glyphosate (Cu-PE) has also caused noteworthy decrease in chlorophyll content but its effect is less pronounced than glyphosate.

Thus, its noteworthy to mention here that Ni-propyl ester derivative of glyphosate (Ni-PE) had good herbicidal properties and effectively killed the weed. It effectively killed all the plants (10 out of 10) at the recommended dose after 10 days of its application (**Figure 6.77**).

Table 6.22 Effect of Metal-Propyl ester of glyphosate complexes on the chlorophyll content a (µg/gFW), chlorophyll content b(µg/gFW) and total chlorophyll content (µg/gFW) in the leaves of *Cyperus rotundus*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.699± 0.0061^{a}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.693± 0.0115^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.687± 0.00737^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}	0.683± 0.00513^{b}
	Glyphosate	0.690± 0.0036^{c}	0.650± 0.00305^{d}	0.603± 0.0073^{d}	0.683± 0.0012^{d}	0.634± 0.00264^{f}	0.594± 0.0015^{g}	0.680± 0.0070^{f}	0.600± 0.0036^{h}	0.560± 0.0056^{h}	0.675± 0.0025^{k}	0.582± 0.0050^{i}	0.484± 0.009^{m}
	Propyl ester of Glyphosate	0.695± 0.0041^{c}	0.658± 0.0075^{d}	0.621± 0.0032^{h}	0685± 0.0251^{d}	0.639± 0.005^{f}	0.610± 0.0041^{h}	0.682± 0.0061^{g}	0.610± 0.0036^{h}	0.586± 0.0025^{h}	0.676± 0.0030^{e}	0.595± 0.002^{h}	0.515± 0.0036^{i}
	Cu-Propyl ester of Glyphosate complex	0.698± 0.0045^{a}	0.660± 0.002^{j}	0.615± 0.0086^{h}	0.692± 0.004^{e}	0.642± 0.0026^{j}	0.613± 0.003^{h}	0.687± 0.0025^{d}	0.621± 0.0026^{h}	0.592± 0.003^{h}	0.679± 0.0041^{h}	0.590± 0.003^{i}	0.510± 0.0087^{p}
	Ni-Propyl ester of Glyphosate complex	0.684± 0.003^{d}	0.645± 0.0036^{l}	0.597± 0.0035^{h}	0.674± 0.0030^{e}	0.627± 0.0026^{h}	0.588± 0.0035^{k}	0.662± 0.0035^{j}	0.596± 0.00173^{j}	0.552± 0.0035^{l}	0.657± 0.0062^{j}	0.577± 0.0040^{n}	0.478± 0.0036^{o}
Chlorophyll b	**Control**	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.452± 0.0026^{a}	0.450± 0.0030^{b}	0.450± 0.0030^{a}	0.450± 0.0030^{a}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.445± 0.0036^{c}	0.431± 0.002^{d}	0.431± 0.002^{d}	0.431± 0.002^{d}
	Glyphosate	0.420± 0.0015^{g}	0.400± 0.0045^{h}	0.360± 0.0032^{i}	0.414± 0.0030^{g}	0.385± 0.0020^{j}	0.300± 0.003^{l}	0.385± 0.0015^{j}	0.310± 0.0034^{k}	0.232± 0.0046^{m}	0.301± 0.004^{l}	0.296± 0.0040^{n}	0.109± 0.0030^{o}
	Propyl ester of Glyphosate	0.438± 0.003^{e}	0.405± 0.0049^{h}	0.375± 0.0096^{i}	0.426± 0.0047^{g}	0.395± 0.0094^{f}	0.329± 0.054^{g}	0.399± 0.0032^{h}	0.329± 0.0030^{g}	0.256± 0.0055^{g}	0.335± 0.0045^{j}	0.311± 0.0055^{i}	0.208± 0.003^{h}
	Cu-Propyl ester of Glyphosate complex	0.435± 0.023^{f}	0.409± 0.0028^{h}	0.384± 0.0049^{j}	0.421± 0.003^{b}	0.400± 0.0192^{h}	0.331± 0.0485^{g}	0.395± 0.002^{h}	0.330± 0.0030^{g}	0.249± 0.003^{p}	0.321± 0.004^{g}	0.309± 0.003^{h}	0.197± 0.0035^{q}
	Ni-Propyl ester of Glyphosate complex	0.417± 0.0020^{b}	0.393± 0.0096^{e}	0.349± 0.0037^{k}	0.409± 0.002^{h}	0.372± 0.0015^{i}	0.298± 0.0015^{n}	0.370± 0.004^{i}	0.305± 0.0045^{l}	0.220± 0.0106^{m}	0.291± 0.0058^{n}	0.202± 0.0106^{f}	0.102± 0.0005^{r}
Total Chlorophyll	**Control**	0.963 ± 0.0037^{a}	0.963± 0.0037^{a}	0.963± 0.0037^{a}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.952± 0.002^{b}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.940± 0.0230^{d}	0.925± 0.0024^{f}	0.925± 0.0024^{f}	0.925± 0.0024^{f}
	Glyphosate	0.930± 0.0032^{b}	0.901± 0.0045^{c}	0.801± 0.0032^{f}	0.905± 0.003^{g}	0.852± 0.0020^{i}	0.721± 0.0041^{h}	0.862± 0.00461^{i}	0.724± 0.0034^{j}	0.690± 0.0005^{j}	0.832± 0.0030^{j}	0.632± 0.0040^{k}	0.514± 0.0025^{m}
	Propyl ester of Glyphosate	0.954± 0.002^{b}	0.921± 0.0136^{b}	0.852± 0.003^{e}	0.923± 0.0025^{f}	0.897± 0.0052^{e}	0.829± 0.0080^{c}	0.899± 0.0045^{e}	0.756± 0.0055^{g}	0.700± 0.002^{g}	0.856± 0.0025^{d}	0.664± 0.0055^{g}	0.593± 0.0026^{f}
	Cu-Propyl ester of Glyphosate complex	0.946± 0.0032^{b}	0.934± 0.005^{e}	0.843± 0.001^{c}	0.917± 0.0058^{f}	0.883± 0.002^{c}	0.813± 0.0037^{d}	0.897± 0.004^{c}	0.749± 0.0025^{f}	0.710± 0.002^{f}	0.852± 0.0005^{g}	0.651± 0.0056^{f}	0.562± 0.002^{h}

	Ni-Propyl ester of Glyphosate complex	0.929± 0.0035^{b}	0.894± 0.0105^{k}	0.792± 0.0035^{f}	0.901± 0.001^{c}	0.845± 0.0162^{j}	0.709± 0.002^{m}	0.851± 0.011^{i}	0.716± 0.0032^{k}	0.683± 0.0020^{l}	0.822± 0.0035^{k}	0.628± 0.0030^{h}	0.509± 0.004^{m}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

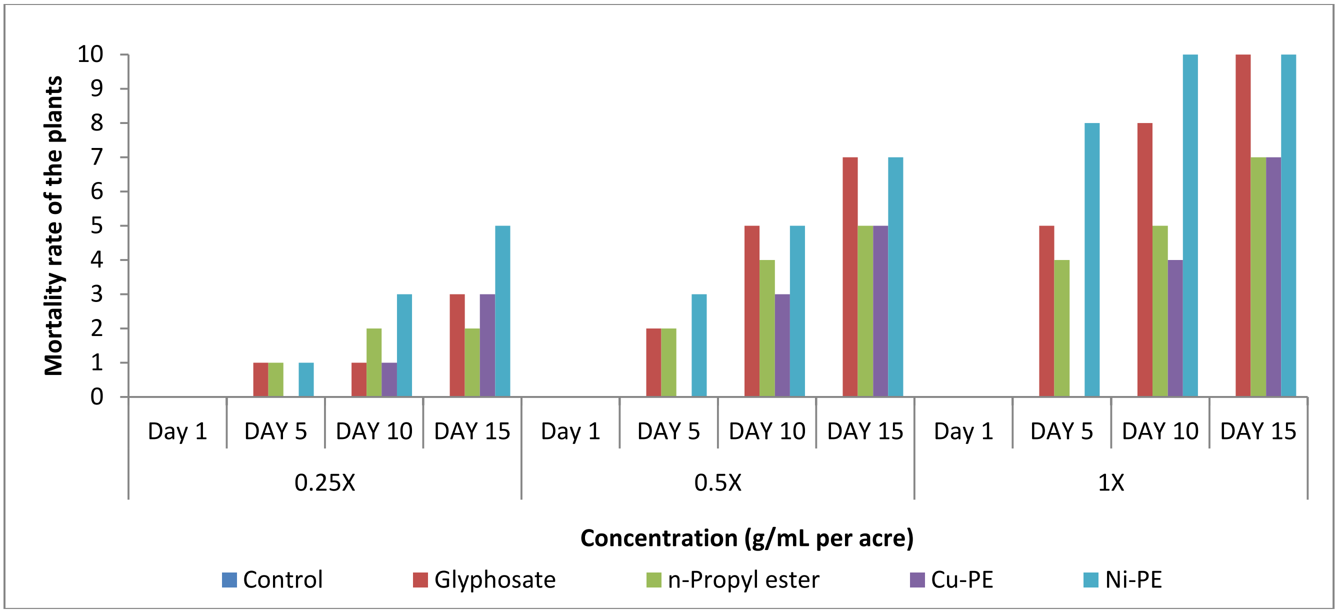


Figure 6.77 Lethal effect of exposure of Metal-Propyl ester of glyphosate complexes on *Cyperus rotundus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). Cu-PE= Copper-Propyl ester of glyphosate complex, Ni-PE = Nickel- Propyl ester of glyphosate complex

6.3.3 Herbicidal Activity Of Synthesized glyphosate derivatives on *Triticum aestivum* (Wheat grass):

6.3.3.1 Ester derivatives of Glyphosate:

Ethyl 2-{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride (**Ethyl ester of glyphosate**) has shown better herbicidal activity than glyphosate on the wheat plants (*Triticum aestivum*). However, the compounds Methyl 2- {[(dimethoxyphosphoryl)methyl] amino}acetate hydrochloride (**Methyl ester of glyphosate**),Isopropyl 2- ({[diisopropoxyphosphoryl] methyl} amino) acetate hydrochloride (**Isopropyl ester of glyphosate**) and Butyl 2-{[(dibutoxy phosphoryl) methyl] amino}acetate hydrochloride (**Butyl ester of glyphosate**) manifested herbicidal effects only on the recommended doses after fifth day of their application. Significant reduction of chlorophyll content in the leaves of *Triticum aestivum* was observed in all the cases after 15 days. Use of these ester derivatives of glyphosate on the leaves of *Triticum aestivum* had a linear effect in decreasing the 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll'. In comparison to control (water treatment) and glyphosate, ethyl ester of glyphosate at all the three test concentrations 0.25X. 0.5 X and 1X had shown much enhanced effect in decreasing the chlorophyll content (**Table 6.24**).

Apart from chlorophyll content determination in the leaves of *Triticum aestivum*, herbicidal activity was also measured on the basis of % of plants killed after their exposure to the ester derivatives of glyphosate. **Figure 6.78** shows the number of plants that become dead after the application of different concentrations of the synthesized ester derivatives of glyphosate. At 0.25X in comparison to glyphosate, ethyl ester of glyphosate has resulted in the death of maximum number of plants (3 after 5 days, 6 after 10 days and 8 after 15 days of exposure). However with increase in the concentration (0.5X), the number of dead plants has increased (4 after 5days, 8 after 10 days and 10 after 15 days). At recommended dose (1X) ethyl ester of glyphosate act as a potent herbicide and killed all the plants after even after 10 days exposure.

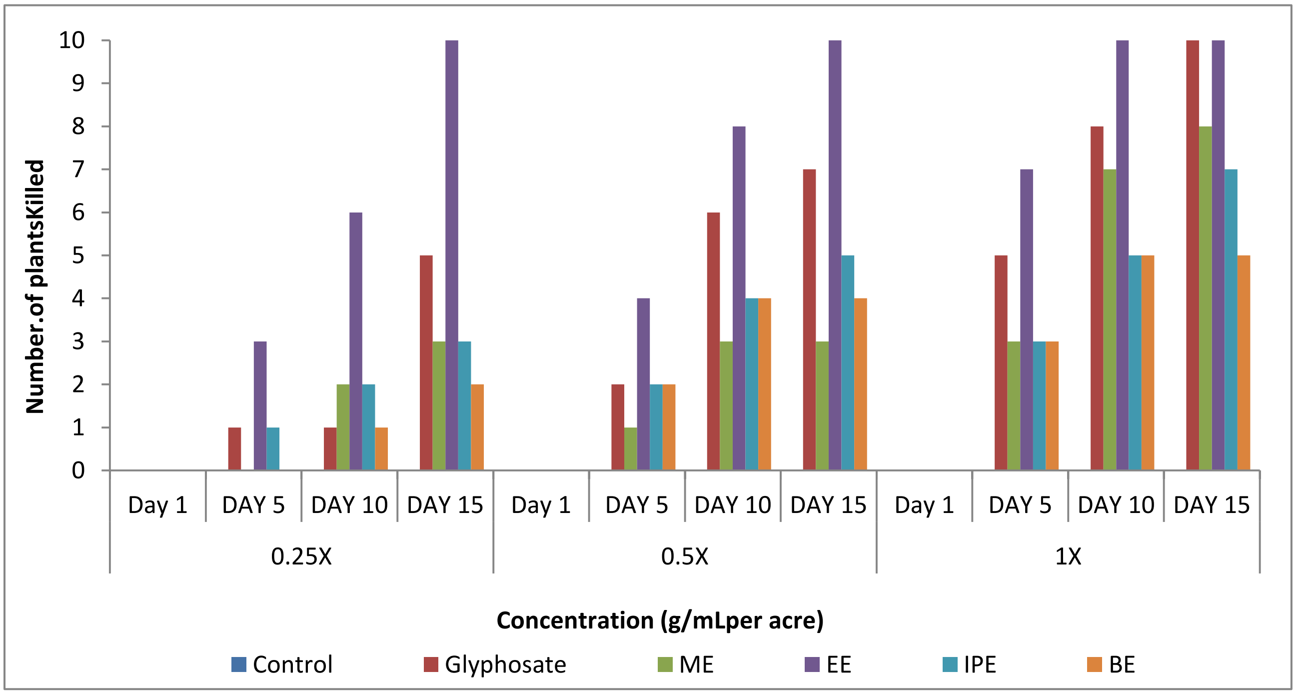


Figure 6.78 Lethal effect of exposure of Esters derivatives of glyphosate on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) .Where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). ME-Methyl ester of glyphosate; EE- Ethyl ester of glyphosate; IPE-Isopropyl ester of glyphosate; BE- Butyl ester of glyphosate

Table 6.23. Effect of synthesized ester derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyl'l content (µg/gFW) in the leaves of *Triticum aestivum* :

Days →		1			5			10			15		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
Chlorophyll a	**Compound**												
	Control	1.289± 0.0015^{a}	1.289± 0.0015 ^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.00058^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.00058^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	Methyl(((dimethylphosphoryl)methyl)amino)acetate hydrochloride	1.287± 0.0010^{b}	1.270± 0.0015^{c}	1.252± 0.0015^{c}	1.239± 0.0011^{c}	1.221± 0.0026^{d}	1.210± 0.0010^{d}	1.205± 0.0005^{e}	1.198± 0.0015^{e}	1.113± 0.0015^{e}	1.131± 0.0010^{j}	1.106± 0.0035^{i}	1.095± 0.001^{l}
	Ethyl 2-(((diethoxyphosphoryl) methyl)amino)acetate hydrochloride	1.231± 0.0005^{f}	1.218± 0.00153^{f}	1.200± 0.0005^{f}	1.198± 0.0005^{g}	1.183± 0.00265^{g}	1.171± 0.001^{g}	1.093± 0.001^{l}	1.053± 0.0015^{l}	1.025± 0.001^{m}	0.905± 0.00153^{o}	0.802± 0.0005^{n}	0.607± 0.0041^{o}
	Isopropyl 2-(((diisopropoxyphosphoryl) methyl)amino)acetate hydrochloride	1.271± 0.001^{c}	1.249^{a}± 0.0016^{d}	1.229± 0.001^{d}	1.254± 0.0005^{d}	1.236± 0.001^{e}	1.222± 0.0025^{e}	1.130± 0.000^{j}	1.108± 0.002^{i}	1.098± 0.002^{l}	1.072± 0.0005^{m}	1.063± 0.001^{j}	1.043± 0.001^{m}
	Butyl 2-(((dibutoxyphosphoryl) methyl)amino)acetate hydrochloride	1.289± 0.0004^{a}	1.252± 0.0015^{d}	1.241± 0.001^{d}	1.254± 0.0004^{d}	1.241± 0.001^{e}	1.225± 0.002^{e}	1.155± 0.001^{i}	1.137± 0.0025^{h}	1.111± 0.001^{j}	1.014± 0.001^{n}	1.009± 0.001^{m}	1.098± 0.001^{n}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.597± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.159± 0.001^{m}	0.053± 0.001^{n}
	Methyl(((dimethylphosphoryl)methyl)amino)acetate hydrochloride	0.623± 0.002^{a}	0.617± 0.0005^{b}	0.623± 0.0026^{a}	0.608± 0.002^{b}	0.594± 0.0015^{d}	0.574± 0.0034^{f}	0.564± 0.0015^{e}	0.565± 0.0015^{f}	0.552± 0.0015^{g}	0.546± 0.003^{i}	0.532± 0.0020^{i}	0.504± 0.0026^{j}
	Ethyl 2-(((diethoxyphosphoryl) methyl)amino)acetate hydrochloride	0.612± 0.0015^{b}	0.615± 0.0015^{b}	0.613± 0.0015^{b}	0.603± 0.003^{c}	0.543± 0.001^{h}	0.537± 0.0030^{i}	0.527± 0.004^{f}	0.501± 0.001^{j}	0.326± 0.002^{o}	0.221± 0.001^{j}	0.086± 0.001^{l}	0.007± 0.002^{p}
	Isopropyl 2-(((diisopropoxyphosphoryl) methyl) amino) acetate hydrochloride	0.621± 0.001^{c}	0.620^{a}± 0.0016^{d}	0.616± 0.001^{d}	0.614± 0.0005^{d}	0.606± 0.001^{e}	0.603± 0.0025^{e}	0.530± 0.000^{j}	0.508± 0.002^{i}	0.498± 0.002^{l}	0.472± 0.0005^{m}	0.363± 0.001^{j}	0.243± 0.001^{m}
	Butyl 2-(((dibutoxyphosphoryl) methyl)amino)acetate hydrochloride	0.622± 0.0004^{a}	0.612± 0.0015^{d}	0.614± 0.001^{d}	0.604± 0.0004^{d}	0.600± 0.001^{e}	0.615± 0.002^{e}	0.555± 0.001^{i}	0.537± 0.0025^{h}	0.511± 0.001^{j}	0.414± 0.001^{n}	0.209± 0.001^{m}	0.198± 0.001^{n}

Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	Methyl(((dimethylphosphoryl)methyl)amino) acetate hydrochloride	1.910± 0.0049^{a}	1.889± 0.0025^{d}	1.877± 0.0030^{c}	1.847± 0.0060^{d}	1.815± 0.0015^{h}	1.784± 0.0035^{a}	1.768± 0.0030^{i}	1.757± 0.0015^{i}	1.665± 0.0051^{i}	1.657± 0.0060^{l}	1.638± 0.0015^{j}	1.599± 0.0025^{l}
	Ethyl 2-(((diethoxyphosphoryl) methyl)amino)acetate hydrochloride	1.843± 0.0036^{c}	1.833± 0.0025^{g}	1.813± 0.0081^{e}	1.892± 0.0026^{d}	1.726 0.0030^{j}	1.688± 0.0043^{j}	1.670± 0.002^{m}	1.554± 0.0010^{m}	1.351± 0.0017^{n}	1.126± 0.0035^{n}	0.888± 0.0037^{o}	0.614± 0.0015^{o}
	Isopropyl 2-(((diisopropoxyphosphoryl) methyl) amino) acetate hydrochloride	1.942± 0.0032^{c}	1.898± 0.0015^{a}	1.858± 0.0030^{c}	1.908± 0.0043^{d}	1.872± 0.0010^{f}	1.844± 0.0005^{d}	1.660± 0.0037^{e}	1.609± 0.0020^{h}	1.596 0.0030^{f}	1.544± 0.0015^{h}	1.426± 0.0010^{l}	1.286± 0.0010^{h}
	Butyl 2-(((dibutoxyphosphoryl) methyl)amino)acetate hydrochloride	1.911± 0.0020^{c}	1.904± 0.0026^{d}	1.882± 0.0047^{c}	1.858± 0.0030^{d}	1.841± 0.0030^{e}	1.840± 0.0025^{d}	1.710± 0.0037^{e}	1.674± 0.0032^{h}	1.622± 0.0070^{c}	1.428± 0.0015^{g}	1.218± 0.0015^{i}	1.296± 0.0030^{h}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.2 Amide Derivatives Of Glyphosate:

Amide derivatives of glyphosate have also shown the herbicidal effects on the common wheat plants (*Triticum aestivum*). Significant reduction in the photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) have been observed in all cases with respect to time and concentration in comparison to control. (**Table 6.25**). Out of the three different synthesized amide derivatives, **Isopropyl amide derivative of glyphosate** [({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid] is more effective in decreasing the 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' content in the plant at all the three concentrations. However with increase in concentration of isopropyl amide derivative i.e. at 1X concentration, further noticeable decrease in the chlorophyll pigments have been noticed. Other two amide derivatives also follow the same trend of reduction in chlorophyll content with increase in concentration of the derivative with respect to time. After 15 days of application, a momentous change in the amount of photosynthetic pigments have been observed in comparison to control. But no significant result was obtained in comparison to glyphosate.

Along with chlorophyll content determination, a significant increase in the number of plants killed after their exposure to the synthesized amide derivatives of glyphosate was noticed. This increase was manifested with increase in the concentration of the derivative and time. At 0.25X, in case **of methyl amide of glyphosate** only 2 plants were killed even after 15 days. At 0.5X, not much change was noticed even after 15 days of treatment. But at 1X, half of the plant population (5 out of 10) were dead. **Isopropyl amide of glyphosate** also followed the similar trend at all the three test concentrations However **butyl amide of glyphosate** killed the minimum number of plants (only 3 after 15 days at 1X) and does not show good herbicidal activity (**Figure 6.79**).

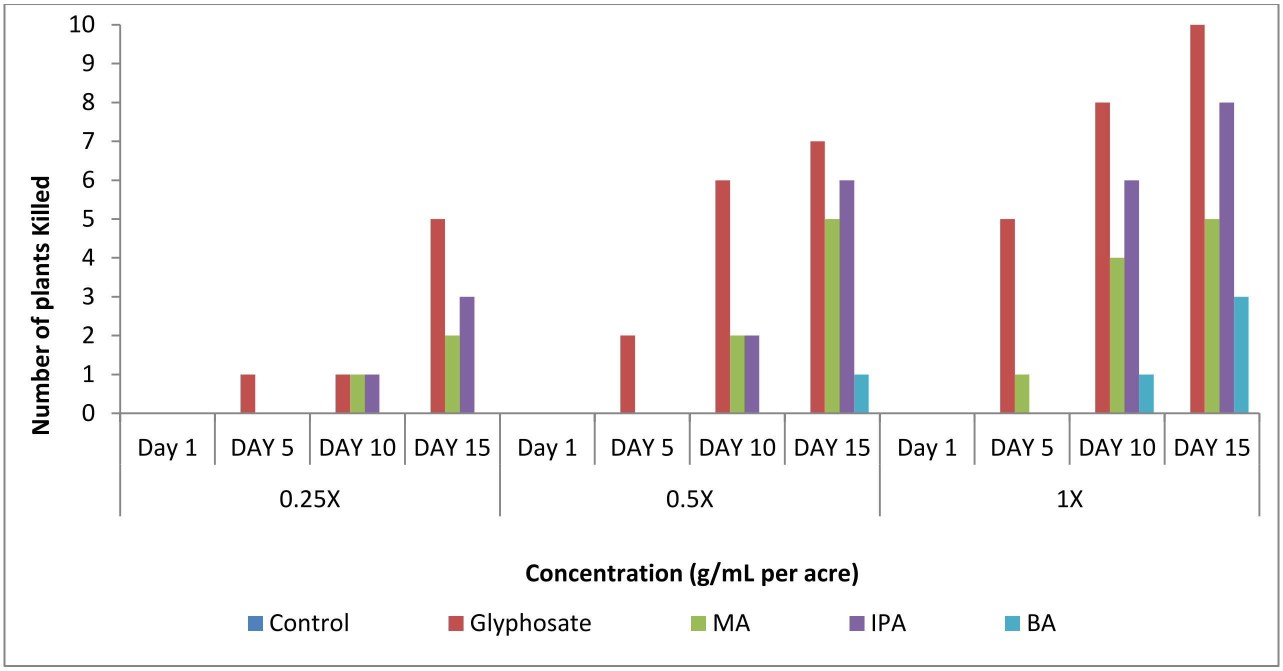


Figure 6.79 Lethal effect of exposure of Amide derivatives of glyphosate on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

MA = ({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid; IPA = ({[2-oxo-2-(propan-2-ylamino) ethyl] amino} methyl)phosphonic acid ;BA = ({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid

Table 6.24 Effect of synthesized amide derivatives of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll' content (µg/gFW) in the leaves of *Triticum aestivum*.

	Days →	**1**			**5**			**10**			**15**		
	Concentration →	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	1.289± 0.0015^{a}	1.289± 0.0015 ^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.00058^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.00058^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	1.285± 0.0016^{b}	1.267± 0.0021^{c}	1.247± 0.0012^{e}	1.257± 0.0045^{d}	1.244± 0.0016^{e}	1.227± 0.0016^{e}	1.239± 0.0063^{d}	1.217± 0.0038^{f}	1.209± 0.0016^{g}	1.231± 0.00163^{d}	1.158± 0.0283^{h}	1.144± 0.0012^{h}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid	1.266± 0.0048^{e}	1.240± 0.0024^{f}	1.225± 0.0016^{g}	1.248± 0.0057^{d}	1.210± 0.0016^{f}	1.185± 0.0021^{g}	1.156± 0.0050^{e}	1.134± 0.0044^{g}	1.123± 0.0012^{h}	1.112± 0.0049^{g}	1.072± 0.0012^{h}	1.042± 0.0012^{h}
	({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid	1.280± 0.0016^{c}	1.243± 0.0020^{e}	1.253± 0.0020^{f}	1.251± 0.0028^{c}	1.216± 0.0016^{f}	1.276± 0.0020^{g}	1.227± 0.0050^{e}	1.210± 0.002^{f}	1.271± 0.0012^{h}	1.211± 0.0020^{f}	1.183± 0.0020^{g}	1.160± 0.0012^{h}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.623± 0.0008^{a}	0.622± 0.0012^{b}	0.621± 0.0026^{b}	0.607± 0.0009^{c}	0.600± 0.0026^{c}	0.590± 0.0012^{c}	0.598± 0.0012^{d}	0.575± 0.0026^{f}	0.556± 0.0032^{d}	0.452± 0.008^{g}	0.362± 0.0024^{f}	0.357± 0.0024^{g}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid	0.623± 0.0016^{d}	0.620± 0.0024^{b}	0.615± 0.0009^{c}	0.613± 0.0021^{g}	0.597± 0.0020^{d}	0.580± 0.0016^{d}	0.590± 0.0004^{i}	0.580± 0.0020^{d}	0.572± 0.0016^{d}	0.518± 0.0012^{b}	0.464± 0.0032^{f}	0.345± 0.0028^{h}
	({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid	0.624± 0.0008^{a}	0.621± 0.0032^{a}	0.619± 0.0016^{c}	0.606± 0.0021^{c}	0.599± 0.0012^{d}	0.596± 0.0016^{d}	0.599± 0.0020^{d}	0.584± 0.0024^{e}	0.580± 0.0021^{e}	0.573± 0.0020^{f}	0.469± 0.0024^{j}	0.455± 0.0024^{g}
Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	({[2-(methylamino)-2-oxoethyl]amino}methyl)phosphonic acid	1.908± 0.0024^{a}	1.889± 0.0016^{b}	1.868± 0.0020^{c}	1.864± 0.0021^{c}	1.844± 0.0016^{c}	1.817± 0.0020^{d}	1.803± 0.0028^{f}	1.789± 0.0012^{d}	1.758.± 0.0021^{d}	1.734± 0.00249^{d}	1.708± 0.0024^{e}	1.694± 0.0012^{f}
	({[2-oxo-2-(propan-2-ylamino)ethyl]amino}methyl)phosphonic acid	1.889± 0.0024^{c}	1.860± 0.0016^{c}	1.840± 0.0024^{d}	1.861± 0.0012^{c}	1.807± 0.0026^{d}	1.765± 0.0012^{d}	1.755± 0.0008^{d}	1.732± 0.0021^{e}	1.709± 0.0016^{f}	1.689± 0.0016^{e}	1.676± 0.0020^{f}	1.658± 0.0024^{f}

	({[2-(butylamino)-2-oxoethyl]amino}methyl)phosphonic acid	1.904± 0.0024^{b}	1.864± 0.0024^{b}	1.872± 0.0012^{c}	1.857± 0.0020^{b}	1.832± 0.0008^{b}	1.826± 0.0024^{d}	1.803± 0.0012^{d}	1.796± 0.0024^{d}	1.776± 0.0047^{e}	1.753± 0.0008^{h}	1.743± 0.0021^{e}	1.721± 0.002^{g}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.3 Boc-protected glyphosate derivative, Boc-protected guanidine derivative of glyphosate and its de-protected analogue:

Boc-Protected glyphosate derivative also showed similar kind of results of herbicidal activity as shown by amide derivatives of glyphosate. As compared to control (water treatment), the plants treated with Boc-protected glyphosate have shown momentous reduction in photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll). A considerable decrease in the amount of 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' has been observed at all test concentrations with respect to time (**Table 6.26**). Boc-protected guanidine derivative of glyphosate and its de-protected analogue (guanidine derivative of glyphosate) have also shown herbicidal properties on the wheat plants. A regular decrease in the chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) was found in both the cases at all the test concentrations. In comparison to control (water treatment) all of these newly synthesized derivatives are effective herbicides. But the **guanidine derivative of glyphosate** (de-protected guanidine derivative of glyphosate) is more effective in showing its herbicidal effects in comparison to the Boc-protected guanidine derivative of glyphosate.

Herbicidal activity of the synthesized Boc-protected glyphosate, Boc-protected guanidine derivative of glyphosate and guanidine derivative of glyphosate on *Triticum aestivum* was also established on the basis of the total number of plants killed after their exposure to these derivatives. It was found that in comparison to glyphosate no considerable decrease in the number of plants was found in all these derivatives. Mortality rate of *Triticum aestivum* with respect to increase in concentration of Boc- protected glyphosate and time was perceived by using 10 plants in each pot. It was observed that Boc-protected glyphosate did not show any significant effect on the mortality of the plants. This compound has shown its herbicidal activity only at higher concentration (0.5X and 1X) . Foliar spray of Boc-protected glyphosate at 0.5X has resulted in death of 3 plants after 15 days, whereas glyphosate has shown significant reduction in the number of plants even after 10th day (5 plants were dead out of 10). At 1X concentration of Boc-protected glyphosate, half of the plants (5 out of 10) has become dead (In case of glyphosate at 1X concentration, 8 out of 10 plants were dead). However at higher concentration (1X) guanidine derivative of glyphosate was effective in killing the plants (4 after 10 days and 6 after 15 days). But these results are not noteworthy with respect to glyphosate. Use of Boc-protected guanidine glyphosate and guanidine derivative of glyphosate on the wheat plants, at all the three test concentrations has effectively decreased the content of photosynthetic pigments in the plant but the number of deaths caused in the plant was less (Boc-protected guanidine glyphosate killed 3 plants at 1X after 10 and 15 days and guanidine derivative of glyphosate killed 4 plants after 10 and 6 plants after 15 days) and was not crucial (**Figure 6.80**).

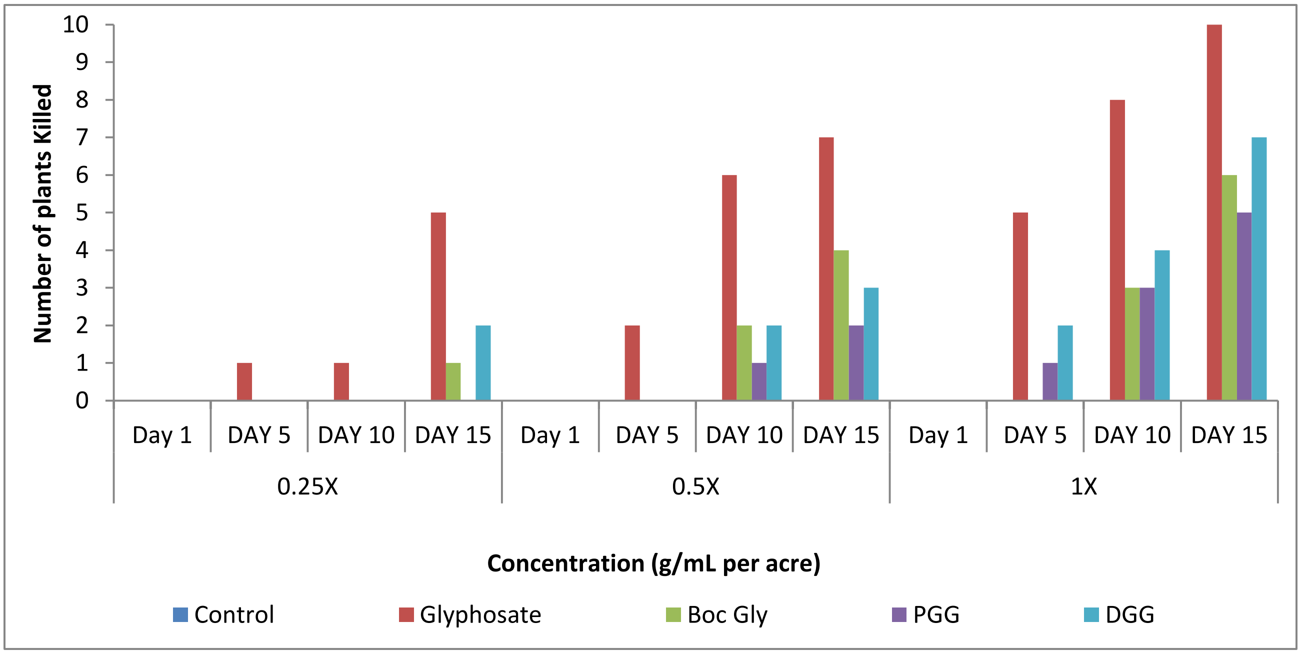


Figure 6.80 Lethal effect of exposure of Boc- Protected glyphosate, Boc-protected guanidine glyphosate and De-protected guanidine glyphosate on *Triticum aestivum* after 15 days Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Boc-Protected Gly= (2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid; PGG (Boc-protected guanidine gly) = 2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid ; DGG (De-protected guanidine gly) = 2-(1-(Phosphonomethyl)guanidino) ethanoic acid

Table 6.25 Effect of of Boc-protected glyphosate derivative, Boc-protected guanidine derivative of glyphosate and its deprotected analogue (guanidine derivative of glyphosate) on the chlorophyll content a (μg/gFW), chlorophyll content b(μg/gFW) and total chlorophyll content (μg/gFW) in the leaves of *Triticum aestivum*.

	Days →	**1**			**5**			**10**			**15**		
	Concentration →	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	1.289± 0.0015^{a}	1.289± 0.0015^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.00058^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	1.275± 0.0026^{c}	1.255± 0.0016^{f}	1.233± 0.0025^{d}	1.243± 0.0020^{f}	1.235± 0.0036^{d}	1.224± 0.003^{d}	1.229± 0.0017^{d}	1.203± 0.00081^{e}	1.192± 0.002^{f}	1.216± 0.00251^{d}	1.194± 0.0037^{f}	1.163± 0.0020^{e}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	1.284±0.0021^{c}	1.250± 0.0015^{d}	1.240± 0.0045^{e}	1.247± 0.0015^{d}	1.236± 0.002^{d}	1.224± 0.0026^{d}	1.202± 0.0025^{g}	1.185± 0.0036^{h}	1.170± 0.0025^{h}	1.161± 0.0020^{h}	1.154± 0.0015^{h}	1.142± 0.0032^{i}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	1.272± 0.0032^{d}	1.235± 0.002^{d}	1.232± 0.0017^{e}	1.236± 0.0015^{e}	1.225± 0.002^{d}	1.219± 0.005^{d}	1.188± 0.00152^{h}	1.174± 0.0041^{h}	1.162± 0.002^{h}	1.161± 0.002^{h}	1.152± 0.00015^{i}	1.133± 0.002^{e}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	0.624± 0.0012^{a}	0.621± 0.0012^{a}	0.620± 0.0012^{b}	0.614± 0.001^{c}	0.603± 0.0012^{d}	0.594± 0.0018^{e}	0.595± 0.0008^{d}	0.584± 0.0016^{e}	0.575± 0.002^{f}	0.485± 0.0012^{e}	0.470± 0.002^{h}	0.463± 0.0021^{i}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl)guanidino) ethanoic acid	0.623± 0.003^{e}	0.623± 0.007^{a}	0.618± 0.0043^{e}	0.615± 0.0011^{a}	0.616± 0.002^{e}	0.603± 0.0025^{e}	0.605± 0.0025^{e}	0.589± 0.005^{g}	0.583± 0.0028^{g}	0.496± 0.0036^{j}	0.465± 0.0035^{i}	0.454± 0.002^{h}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	0.621± 0.002^{f}	0.617± 0.0025^{b}	0.613± 0.0058^{e}	0.611± 0.0072^{b}	0.599± 0.0030^{e}	0.584± 0.0064^{j}	0.594± 0.0025^{j}	0.571± 0.007^{i}	0.560± 0.0052^{i}	0.481± 0.0035^{g}	0.451± 0.0032^{h}	0.433± 0.001^{h}
Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	(2-((tert-butoxycarbonyl) (phosphonomethyl)amino) ethanoic acid	1.889± 0.0021^{h}	1.876± 0.002^{h}	1.851± 0.0021^{d}	1.860± 0.0043^{b}	1.849± 0.0032^{c}	1.818± 0.002^{f}	1.824± 0.0016^{f}	1.787± 0.0024^{d}	1.767± 0.0029^{f}	1.701± 0.0016^{g}	1.664± 0.0016^{f}	1.626± 0.00205^{e}
	2-(2.,3-bis(tert-butoxycarbonyl)-1-	1.908±	1.873±	1.858±	1.862±	1.852±	1.827±	1.807±	1.774±	1.753±	1.657±	1.619±	1.596±

	(phosphonomethyl)guanidino) ethanoic acid	0.0015^{b}	0.0036^{b}	0.003^{e}	0.0035^{c}	0.0003^{e}	0.0032^{c}	0.0025^{e}	0.0025^{g}	0.00624^{g}	0.0025^{d}	0.0036^{g}	0.0026^{f}
	2-(1-(Phosphonomethyl)guanidino) ethanoic acid	1.893± 0.0058^{b}	1.852± 0.005^{e}	1.845± 0.001^{c}	1.847± 0.0035^{f}	1.844± 0.004^{c}	1.803± 0.0028^{d}	1.782± 0.003^{c}	1.745± 0.0235^{f}	1.722± 0.002^{f}	1.642± 0.004^{g}	1.603± 0.0036^{f}	1.566± 0.00020^{h}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability (p< 0.01). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.4 Thioxylated ester derivatives of glyphosate:

Thioxylated ester derivatives of glyphosate have shown excellent herbicidal effects on wheat plants after 15 days treatment on three test concentrations (0.25X, 0.5X and 1X). Among these thioxylated esters of glyphosate, **thioxylated ethyl ester of glyphosate** (*O*-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate) have shown best herbicidal effects. It has significantly decreased the chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) and killed the plant. Thioxylated ethyl ester of glyphosate behave as a potent herbicide and is extremely effective in decreasing the content of photosynthetic pigments of plant in comparison to glyphosate. Plants treated with thioxylated ethyl ester derivative of glyphosate at 1X have shown tremendous decrease in 'chlorophyll a' in contrast to glyphosate. Other thioxylated ester derivatives of glyphosate are also effectual in killing the weed. They have also reduced the content of photosynthetic pigments in the plant at all the test concentrations. Major diminution in 'chlorophyll a', 'chlorophyll b' and 'total chlorophyll' was found in the plants treated with 0.25X of **thioxylated isopropyl ester of glyphosate** (O-propan-2-yl({[bis(propan-2-yloxy)phosphorothioyl] methyl}amino)ethanethioate). However this decrease became much pronounced with increase in the concentration of the thioxylated isopropyl ester of glyphosate **Table 6.27**.

Synthesized Thioxylated ester derivatives of glyphosate also act as potent herbicides and competently killed the plant. Thioxylated ethyl ester of glyphosate is adequate in killing the plant in contrast to glyphosate. Even at the lowest concentration(0.25X) half of the plants were exterminated by this compound after 10 days of the treatment. The number of plants killed by the thioxylated ethyl ester of glyphosate was increased with increase in the concentration of the compound. At 0.5X, 6 plants were killed after 5 days and 10 plants were killed after 15 days. The rate at which the plants become dead after their exposure to this compound was greatly increased with increase in concentration and time. However at 1X all the plants (10) were dead even after 10 days exposure. Similar results were obtained in case of Thioxylated isopropyl ester of glyphosate. It has also shown its herbicidal effects even at the lowest concentration of 0.25X. It effectively killed 7 out of 10 plants after 15 days **(Figure 6.81)**. At 1X more than half of the plants were killed by this compound after 10 days exposure. All these findings showed that thioxylated ester derivatives of glyphosate are potent herbicides and effectively killed the plant.

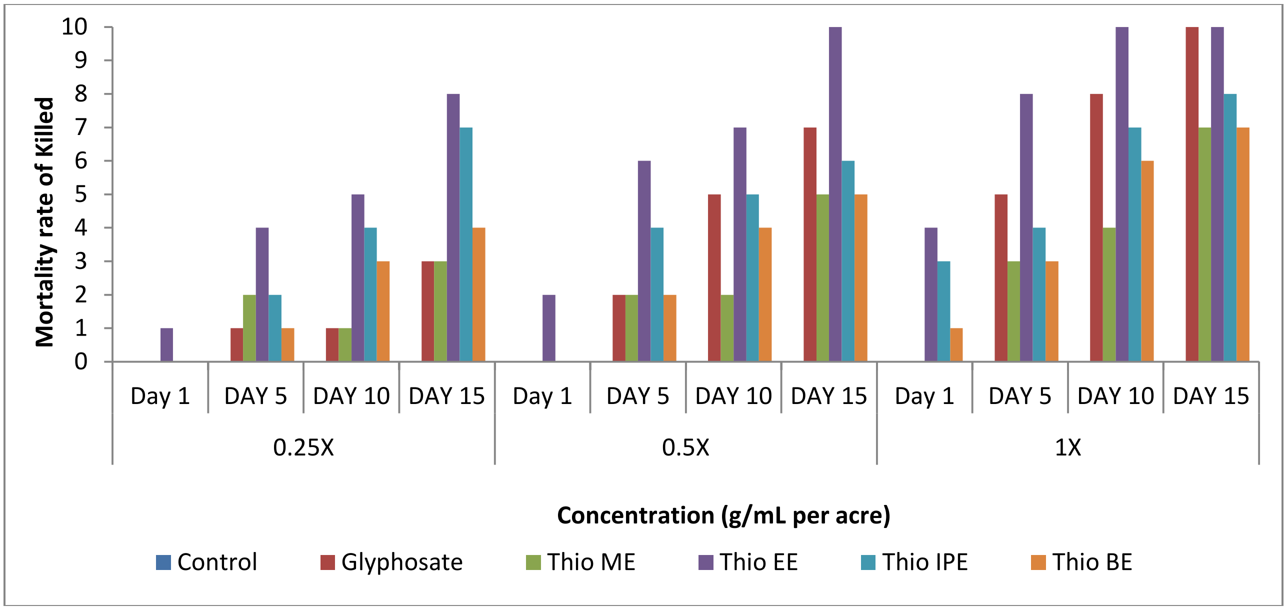


Figure 6.81 Lethal effect of exposure of Thioxylated ester derivatives of glyphosate on *Parthenium hysterophorus* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Where, Thio ME = O-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate; ThioEE = O-ethyl {[(diethoxyphosphorothioyl)methyl]amino}ethanethioate; ThioIPE =O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl] methyl} amino) ethanethioate; Thio BE = O-butyl {[(dibutoxyphosphorothioyl) methyl] amino} ethanethioate.

Table 6.26 Effect Thioxylated Ester derivatives of glyphosate on the chlorophyll content a (μg/gFW), chlorophyll content b(μg/gFW) and total chlorophyll content (μg/gFW) in the leaves of *Triticum aestivum*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	1.289± 0.0015^{a}	1.289± 0.0015 ^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.00058^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	O-methyl{[(dimethoxyphosphorothioyl)methyl] amino}ethanethioate	1.271± 0.0025^{c}	1.260± 0.0020^{c}	1.239± 0.003^{d}	1.281± 0.0020^{b}	1.232± 0.0015^{d}	1.254± 0.036^{g}	1.251± 0.0040^{c}	1.205± 0.0028^{b}	1.152± 0.0015^{g}	1.141± 0.0032^{g}	1.104± 0.0025^{h}	1.098± 0.0026^{i}
	O-ethyl{[(diethoxyphosphorothioyl)methyl] amino}ethanethioate	1.198± 0.0025^{f}	1.185± 0.0041^{f}	1.165± 0.002^{f}	1.184± 0.0015^{f}	1.164± 0.0011^{f}	1.130± 0.0015^{g}	1.051± 0.0040^{i}	1.039± 0.0035^{i}	1.023± 0.0012^{i}	0.904± 0.00050j	0.520± 0.0025^{j}	0.116± 0.00016^{j}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl]methyl} amino)ethanethioate	1.263± 0.0032^{c}	1.252± 0.0015^{e}	1.220± 0.0035^{d}	1.235± 0.0041^{d}	1.226± 0.0036^{d}	1.199± 0.001^{f}	1.139± 0.0030^{g}	1.116± 0.0015^{h}	1.102± 0.002^{h}	0.956± 0.0025^{i}	0.849± 0.004^{i}	0.226± 0.003^{i}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino}ethanethioate	1.267± 0.0020^{c}	1.254± 0.0015^{e}	1.234± 0.0003^{d}	1.242± 0.0020^{e}	1.233± 0.0025^{d}	1.195± 0.0036^{f}	1.153± 0.0030^{g}	1.145± 0.057^{g}	1.084± 0.0025^{i}	1.070± 0.0030^{i}	1.056± 0.0015^{i}	1.034± 0.0025^{i}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	O-methyl{[(dimethoxyphosphorothioyl)methyl amino} ethanethioate	0.621± 0.0083^{a}	0.600± 0.00624^{c}	0.599± 0.0068^{d}	0.596± 0.0026^{d}	0.584± 0.0032^{e}	0.565± 0.002^{f}	0.581± 0.0068^{e}	0.566± 0.0025^{f}	0.429± 0.002^{i}	0.362± 0.00073^{j}	0.295± 0.0026^{m}	0.175± 0.00326^{l}
	O-ethyl{[(diethoxyphosphorothioyl)methyl] amino} ethanethioate	0.603± 0.0047^{c}	0.594± 0.0045^{d}	0.571± 0.0035^{e}	0.579± 0.007^{e}	0.463± 0.0035^{f}	0.445± 0.002^{h}	0.363± 0.0015^{f}	0.218± 0.0025^{i}	0.192± 0.0050^{j}	0.0937± 0.00041^{j}	0.0427± 0.0017^{l}	0.020± 0.0036^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl] methyl} amino)ethanethioate	0.616± 0.003^{b}	0.604± 0.0032^{c}	0.593± 0.0015^{d}	0.597± 0.0037^{d}	0.477± 0.0026^{g}	0.453± 0.0025^{f}	0.474± 0.0015^{g}	0.358± 0.0073^{f}	0.235± 0.0015^{h}	0.195± 0.0003^{j}	0.0468± 0.00055^{l}	0.042± 0.0016^{m}
	O-butyl {[(dibutoxyphosphorothioyl)methyl] amino} ethanethioate	0.621± 0.0040^{b}	0.605± 0.002^{c}	0.597± 0.0026^{d}	0.593± 0.0040^{d}	0.583± 0.0015^{e}	0.563± 0.005^{f}	0.585± 0.0032^{e}	0.553± 0.0047^{f}	0.535± 0.0015^{h}	0.400± 0.00026^{n}	0.392± 0.00072^{j}	0.373± 0.0010^{o}
Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}

	O-methyl {[(dimethoxyphosphorothioyl) methyl] amino} ethanethioate	1.892± 0.004^{c}	1.860± 0.0171^{c}	1.838± 0.003^{e}	1.877± 0.0132^{g}	1.816± 0.0206^{g}	1.800± 0.0035^{i}	1.832± 0.0079^{i}	1.771± 0.0124^{j}	1.581± 0.0066^{j}	1.503± 0.0058^{j}	1.399± 0.0015^{k}	1.273± 0.002^{l}
	O-ethyl {[(diethoxyphosphorothioyl) methyl] amino} ethanethioate	1.819± 0.0096^{e}	1.779± 0.0291^{g}	1.736± 0.0032^{g}	1.732± 0.0075^{h}	1.627± 0.003^{h}	1.575± 0.0077^{n}	1.414± 0.0015^{j}	1.257± 0.0121^{j}	1.215± 0.0020^{m}	0.997± 0.0036^{k}	0.563± 0.0055^{l}	0.136± 0.003^{m}
	O-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl] methyl} amino)ethanethioate	1.879± 0.0035^{c}	1.856± 0.0045^{e}	1.813± 0.004^{e}	1.836± 0.0030^{g}	1.703± 0.0189^{g}	1.652± 0.0025^{h}	1.613± 0.006^{j}	1.474± 0.0102^{j}	1.337± 0.0049^{k}	1.151± 0.00814^{k}	0.895± 0.0015^{k}	0.268± 0.0121^{m}
	O-butyl{[(dibutoxyphosphorothioyl)methyl] amino} ethanethioate	1.888± 0.0176^{c}	1.859± 0.0025^{e}	1.831± 0.0062^{e}	1.835± 0.0537^{g}	1.816± 0.0015^{g}	1.758± 0.0052^{h}	1.738± 0.0168^{j}	1.698± 0.0132^{j}	1.619± 0.0032^{k}	1.470± 0.0090^{k}	1.448± 0.0055^{k}	1.407± 0.0075^{l}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.5 Metal Complexes of Glyphosate: Five different metal complexes of glyphosate were synthesized and their herbicidal activity was checked in context of decrease in the chlorophyll content in the leaves of *Triticum aestivum* on exposure to these derivatives. Also the lethal effect of these derivatives on the plants was measured by counting the number of plants killed with respect to time and concentration. As compared to control all the metal complexes have shown decrease in photosynthetic pigments (Chlorophyll a, Chlorophyll b and total Chlorophyll) but no considerable decrease was found in comparison to glyphosate. No significant trend in the decrease of chlorophyll content (chlorophyll a, chlorophyll b, total chlorophyll) was observed in all the cases at all the three test concentrations (0.25X, 0.5X and 1X) (**Table 6.28**).

Herbicidal effect of the synthesized metal complexes of glyphosate was also evaluated by inscribing the number of plants killed by the synthesized metal complexes. Maximum number of plants were killed only at the recommended dose (1X) by the **Co-glyphosate complex** (5 out of 10) (**Figure 6.82**). Other derivatives didn't show any significant reduction in the number of plants upon their exposure to these derivatives. No major difference in the results with respect to each other and control were obtained at the lower concentrations of these derivatives.

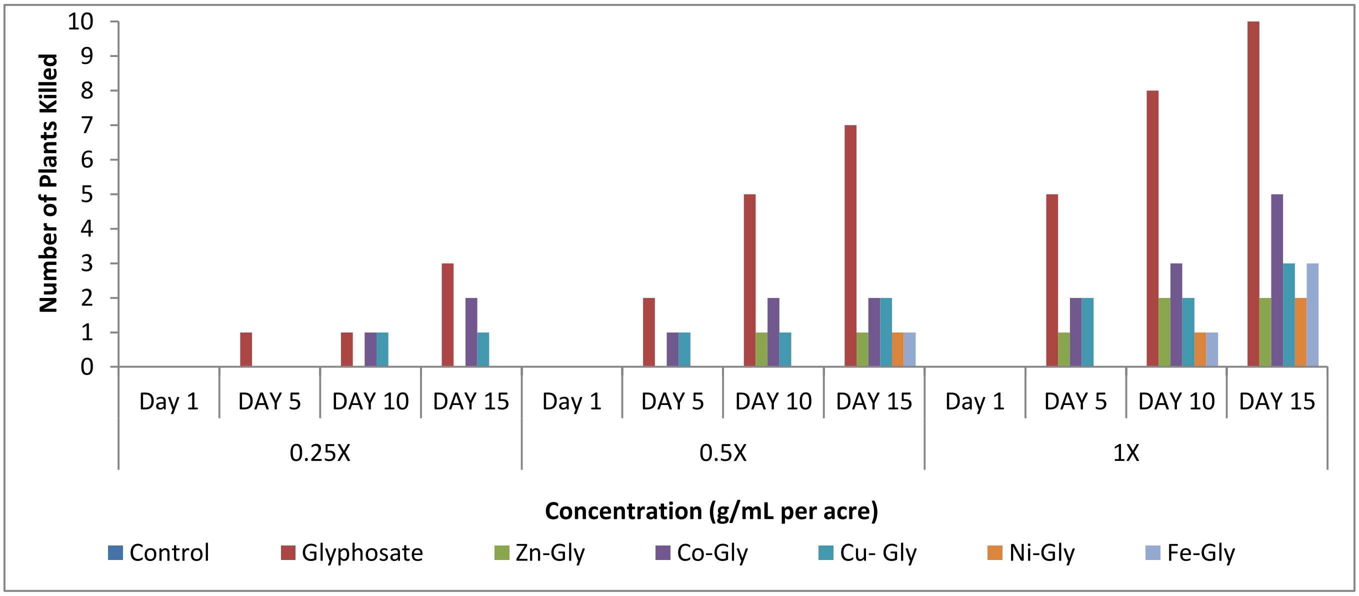


Figure 6.82 Lethal effect of exposure of Metal complexes of glyphosate on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Zn-Gly = Zinc-Glyphosate complex, Co-Gly = Cobalt-Glyphosate complex, Cu-Gly= Copper-Glyphosate complex, Ni-Gly= Nickel-Glyphosate complex, Fe-Gly= Iron-Glyphosate complex

Table 6.27 Effect of Metal complexes of glyphosate on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b'(µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Triticum aestivum*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	1.289± 0.0015^{a}	1.289± 0.0015 ^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.00058^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	Zn-Glyphosate complex	1.283± 0.0055^{b}	1.274± 0.0051^{c}	1.270± 0.0030^{d}	1.264± 0.0005^{f}	1.263± 0.0026^{d}	1.193± 0.0017^{g}	1.220± 0.0032^{c}	1.251± 0.0041^{b}	1.172± 0.0056^{g}	1.206± 0.004^{g}	1.182± 0.0025^{h}	1.144± 0.0025^{i}
	Co-Glyphosate complex	1.287± 0.0050^{f}	1.275± 0.0049^{f}	1.251± 0.0037^{f}	1.266± 0.0089^{f}	1.265± 0.0120^{f}	1.232± 0.0025^{g}	1.233± 0.0073^{i}	1.234± 0.0061^{i}	1.211± 0.0023^{i}	1.200± 0.0035^{j}	1.149± 0.0075^{j}	1.140± 0.0030^{j}
	Cu-Glyphosate complex	1.294± 0.007^{c}	1.272± 0.0066^{e}	1.249± 0.0015^{d}	1.257± 0.002^{d}	1.267± 0.0166^{d}	1.230± 0.002^{f}	1.221± 0.0056^{g}	1.243± 0.0015^{h}	1.224± 0.003^{h}	1.208± 0.0282^{i}	1.147± 0.0056^{i}	1.121± 0.0052^{i}
	Ni-Glyphosate complex	1.292± 0.0020^{c}	1.270± 0.0049^{e}	1.243± 0.001^{d}	1.269± 0.0020^{e}	1.265± 0.0134^{d}	1.226± 0.001^{f}	1.224± 0.0036^{g}	1.252± 0.0036^{g}	1.203± 0.0026^{i}	1.219± 0.004^{i}	1.141± 0.0030^{i}	1.131± 0.0043^{i}
	Fe-Glyphosate complex	1.285± 0.0026^{b}	1.271± 0.0037^{c}	1.235± 0.0045^{j}	1.260± 0.0055^{c}	1.250± 0.0026^{c}	1.210± 0.0030^{k}	1.213± 0.0055^{g}	1.228± 0.0133^{i}	1.143± 0.0017^{j}	1.176± 0.0025^{g}	1.132± 0.0052^{l}	1.110± 0.0032^{m}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	Zn-Glyphosate complex	0.624± 0.0045^{k}	0.622± 0.0032^{c}	0.619± 0.0015^{d}	0.609± 0.0043^{d}	0.610± 0.0017^{e}	0.589± 0.003^{f}	0.591± 0.077^{e}	0.562± 0.0026^{f}	0.548± 0.0035^{i}	0.442± 0.0015^{j}	0.404± 0.0015^{m}	0.394± 0.0081^{l}
	Co-Glyphosate complex	0.623± 0.0026^{c}	0.620± 0.0030^{d}	0.618± 0.0030^{e}	0.610± 0.0015^{e}	0.615± 0.0025^{f}	0.562± 0.0152^{h}	0.592± 0.068^{f}	0.575± 0.0026^{i}	0.557± 0.0014^{j}	0.451± 0.0026^{j}	0.412± 0.0026^{l}	0.397± 0.0035^{m}
	Cu-Glyphosate complex	0.624± 0.0020^{b}	0.622± 0.0037^{c}	0.617± 0.002^{d}	0.615± 0.0055^{d}	0.609± 0.001^{g}	0.595± 0.0005^{f}	0.593± 0.003^{g}	0.578± 0.0045^{f}	0.574± 0.0045^{h}	0.469± 0.0025^{j}	0.451± 0.0035^{l}	0.330± 0.0011^{m}
	Ni-Glyphosate complex	0.623± 0.003^{b}	0.621± 0.0015^{c}	0.619± 0.0015^{d}	0.617± 0.0051^{d}	0.615± 0.002^{e}	0.571± 0.007^{f}	0.596± 0.0015^{e}	0.569± 0.0028^{f}	0.544± 0.0020^{h}	0.451± 0.0051^{n}	0.415.± 0.003^{j}	0.396± 0.0009^{o}
	Fe-Glyphosate complex	0.623±	0.620±	0.615±	0.607±	0.599±	0.560±	0.590±	0.561±	0.541±	0.424±	0.400±	0.226±

		0.0025^{k}	0.0011^{k}	0.0015^{d}	0.0025^{d}	0.0045	0.007^{m}	0.0017^{e}	0.0023^{h}	0.0026^{l}	0.004^{h}	0.0070^{k}	0.0004^{n}
Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	Zn-Glyphosate complex	1.907 ± 0.0015^{c}	1.896± 0.002^{c}	1.889± 0.0030^{e}	1.873± 0.003^{g}	1.870± 0.0030^{g}	1.782± 0.0020^{i}	1.811± 0.0035^{i}	1.813± 0.0020^{j}	1.720± 0.004^{j}	1.648± 0.0035^{j}	1.586± 0.0035^{k}	1.538± 0.0020^{l}
	Co-Glyphosate complex	1.910± 0.003^{e}	1.895± 0.006^{g}	1.869± 0.0025^{g}	1.876± 0.0015^{h}	1.880± 0.002^{h}	1.794± 0.002^{n}	1.825± 0.0014^{j}	1.809± 0.0035^{j}	1.768± 0.001^{m}	1.651± 0.0011^{k}	1.561± 0.0015^{m}	1.537± 0.0035^{m}
	Cu-Glyphosate complex	1.917± 0.002^{c}	1.892± 0.0030^{e}	1.866± 0.003^{e}	1.872± 0.0005^{g}	1.876± 0.0026^{g}	1.825± 0.0026^{h}	1.814± 0.0045^{j}	1.821± 0.0075^{j}	1.798± 0.002^{k}	1.677± 0.00092^{k}	1.598± 0.003^{k}	1.451± 0.0032^{m}
	Ni-Glyphosate complex	1.915± 0.0015^{c}	1.891± 0.002^{e}	1.862± 0.002^{e}	1.886± 0.0078^{g}	1.880± 0.0036^{g}	1.797± 0.001^{h}	1.820± 0.0020^{j}	1.821± 0.0065^{j}	1.747± 0.002^{k}	1.670± 0.004^{k}	1.556± 0.0025^{k}	1.527± 0.0075^{l}
	Fe-Glyphosate complex	1.908± 0.0152^{d}	1.891± 0.005^{h}	1.850± 0.0026^{l}	1.867± 0.007^{k}	1.849± 0.0026^{n}	1.770± 0.0030^{l}	1.803± 0.0026^{l}	17.89± 0.0036^{m}	1.684± 0.0005^{f}	1.600± 0.0035^{m}	1.532± 0.0050^{k}	1.336± 0.0051^{o}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.6 Metal Complexes of Methyl Ester Glyphosate: A series of five different metal complexes of methyl ester derivative of glyphosate were synthesized. Their herbicidal effect was checked by determining the chlorophyll content left in the wheat (*Triticum aestivum*) after their exposure to these derivatives. The mortality rate of plant with respect to time and concentration was also evaluated. All the synthesized derivatives showed moderate to good herbicidal effects on the plant. Out of these derivatives, **Ni-methyl ester derivative of glyphosate complex** (Ni-ME) has shown preeminent herbicidal activity against the grass (**Table 6.29)**

Detrimental effects of the synthesized metal complexes of methyl ester derivatives of glyphosate on the weed were also scrutinized from the mortality rate of the plants. After 15 days treatment it has been found that Ni-ME complex has killed maximum number of weed plants as compared to glyphosate. Even at 0.5X it has killed 9 out of 10 plants whereas glyphosate killed only7 plants. Even at 1X, all plants were killed after 10 days in case of Ni-ME complex whereas glyphosate killed only 8 plants (**Figure 6.83**). From the comparison between the herbicidal effects of both these herbicides, it has been found that Ni-ME complex was more effective in decreasing the chlorophyll content and killing maximum number of weed plants as compared to the glyphosate.

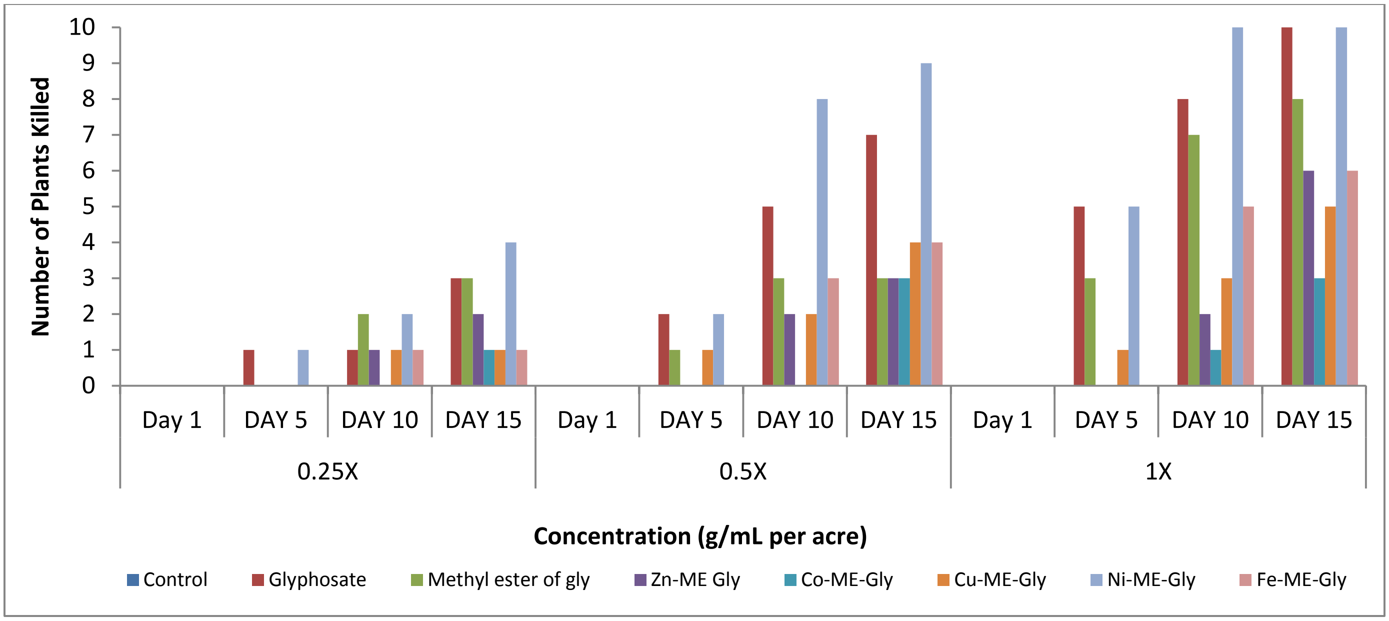


Figure 6.83 Lethal effect of exposure of Metal-Methyl ester of glyphosate complexes on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). Zn-ME Gly = Zinc-Methyl ester of glyphosate complex, Co-ME Gly = Cobalt-Methyl ester of glyphosate complex, Cu-ME Gly= Copper- Methyl ester of glyphosate complex, Ni-ME Gly= Nickel- Methyl ester of glyphosate complex, Fe- ME Gly= Iron- Methyl ester of glyphosate complex

Table 6.28 Effect of Metal-methyl ester of glyphosate complexes on the 'chlorophyll content a' (μg/gFW), 'chlorophyll content b'(μg/gFW) and 'total chlorophyll content' (μg/gFW) in the leaves of *Triticum aestivum*.

Days →		**1**			**5**			**10**			**15**		
Concentration →		**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	1.289± 0.0015^{a}	1.289± 0.0015^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.00058^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	Zn- Methyl ester of Glyphosate complex	1.282± 0.0050^{b}	1.254± 0.0015^{c}	1.229± 0.0032^{d}	1.274± 0.0023^{f}	1.240± 0.0030^{d}	1.201± 0.001^{g}	1.243± 0.00208^{c}	1.231± 0.0035^{b}	1.183± 0.0026^{g}	1.220± 0.0321^{g}	1.223± 0.0081^{h}	1.162± 0.0040^{i}
	Co- Methyl ester of Glyphosate complex	1.287± 0.0075^{f}	1.257± 0.0030^{f}	1.229± 0.0030^{f}	1.279± 0.0026^{f}	1.249± 0.0015^{f}	1.209± 0.0017^{g}	1.247± 0.0036^{i}	1.232± 0.0014^{i}	1.187± 0.0045^{i}	1.224± 0.002^{j}	1.220± 0.0003^{j}	1.169± 0.0015^{j}
	Cu- Methyl ester of Glyphosate complex	1.284± 0.0025^{c}	1.254± 0.002^{e}	1.232± 0.0037^{d}	1.275± 0.0035^{d}	1.251± 0.0005^{d}	1.199± 0.0034^{f}	1.241± 0.0025^{g}	1.230± 0.0045^{h}	1.175± 0.0028^{h}	1.227± 0.0032^{i}	1.216± 0.0011^{i}	1.157± 0.0026^{i}
	Ni- Methyl ester of Glyphosate complex	1.270± 0.0051^{c}	1.250± 0.00152^{e}	1.225± 0.001^{d}	1.234± 0.0023^{e}	1.222± 0.0078^{d}	1.174± 0.0028^{f}	1.213± 0.0035^{g}	1.132± 0.002^{g}	1.093± 0.003^{i}	1.202± 0.002^{i}	1.110± 0.0009^{i}	1.034± 0.0035^{i}
	Fe- Methyl ester of Glyphosate complex	1.285± 0.0051^{b}	1.255± 0.0015^{c}	1.238± 0.0025^{j}	1.275± 0.0036^{c}	1.235± 0.0070^{c}	1.207± 0.003^{k}	1.242± 0.002^{g}	1.220± 0.0026^{i}	1.189± 0.0026^{j}	1.221± 0.0025^{g}	1.190± 0.0004^{l}	1.149± 0.003^{m}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	Zn- Methyl ester of Glyphosate complex	0.623± 0.005^{k}	0.622± 0.0061^{c}	0.618± 0.0032^{d}	0.615± 0.0026^{d}	0.604± 0.0027^{e}	0.595± 0.003^{f}	0.598± 0.0015^{e}	0.576± 0.0043^{f}	0.546± 0.0041^{i}	0.474± 0.0036^{j}	0.405± 0.005^{m}	0.311± 0.0025^{l}
	Co- Methyl ester of Glyphosate complex	0.623± 0.0075^{c}	0.620± 0.0075^{d}	0.619± 0.0025^{e}	0.616± 0.0023^{e}	0.609± 0.0026^{f}	0.592± 0.005^{h}	0.597± 0.0032^{f}	0.579± 0.0035^{i}	0.540± 0.0032^{j}	0.470± 0.0025^{j}	0.400± 0.0025^{l}	0.314± 0.0036^{m}
	Cu- Methyl ester of Glyphosate complex	0.622± 0.0025^{b}	0.621± 0.0026^{c}	0.615± 0.002^{d}	0.609± 0.0026^{d}	0.607± 0.002^{g}	0.590± 0.007^{m}	0.595± 0.002^{g}	0.584± 0.002^{f}	0.547± 0.0076^{h}	0.472± 0.001^{j}	0.410± 0.007^{l}	0.317± 0.0017^{m}
	Ni- Methyl ester of Glyphosate complex	0.623± 0.0051^{b}	0.622± 0.0052^{c}	0.614± 0.0037^{d}	0.606± 0.0035^{d}	0.599± 0.003^{e}	0.575± 0.005^{f}	0.552± 0.002^{e}	0.502± 0.001^{f}	0.505± 0.0015^{h}	0.375± 0.037^{n}	0.309.± 0.047^{j}	0.199± 0.005^{o}
	Fe- Methyl ester of Glyphosate complex	0.624± 0.0051^{k}	0.623± 0.0052^{k}	0.616± 0.0096^{d}	0.610± 0.0035^{d}	0.611± 0.035^{k}	0.582± 0.0025^{j}	0.589± 0.0027^{e}	0.570± 0.002^{h}	0.534± 0.0055^{l}	0.465± 0.0036^{h}	0.498± 0.003^{k}	0.303± 0.0015^{n}

Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	Zn- Methyl ester of Glyphosate complex	1.905 ± 0.0025^{c}	1.876± 0.003^{c}	1.847± 0.0077^{e}	1.889± 0.004^{g}	1.844± 0.004^{g}	1.796± 0.0011^{i}	1.841± 0.0025^{i}	1.807± 0.002^{j}	1.729± 0.004^{j}	1.694± 0.0031^{j}	1.628± 0.0040^{k}	1.473± 0.0025^{l}
	Co- Methyl ester of Glyphosate complex	1.911± 0.007^{e}	1.877± 0.0027^{g}	1.848± 0.007^{g}	1.895± 0.0037^{h}	1.858± 0.005^{h}	1.801± 0.0095^{n}	1.844± 0.0037^{j}	1.811± 0.0023^{j}	1.727± 0.010^{m}	1.694± 0.0039^{k}	1.620± 0.0032^{m}	1.483± 0.0052^{m}
	Cu- Methyl ester of Glyphosate complex	1.906± 0.0074^{c}	1.875± 0.001^{e}	1.847± 0.0026^{e}	1.884± 0.0024^{g}	1.858± 0.0036^{g}	1.789± 0.0545^{h}	1.836± 0.0035^{j}	1.814± 0.0026^{j}	1.722± 0.002^{k}	1.699± 0.0052^{k}	1.626± 0.0040^{k}	1.474± 0.0057^{m}
	Ni- Methyl ester of Glyphosate complex	1.893± 0.0064^{c}	1.872± 0.005^{e}	1.839± 0.004^{e}	1.840± 0.0058^{g}	1.821± 0.0015^{g}	1.749± 0.0015^{h}	1.765± 0.0049^{j}	1.634± 0.0035^{j}	1.598± 0.002^{k}	1.577± 0.004^{k}	1.419± 0.0035^{k}	1.233± 0.0043^{l}
	Fe- Methyl ester of Glyphosate complex	1.909± 0.0032^{d}	1.878± 0.009^{h}	1.854± 0.0026^{l}	1.885± 0.0014^{k}	1.846± 0.0035^{n}	1,789± 0.0058^{l}	1.831± 0.0036^{l}	1.790± 0.0075^{m}	1.723± 0.0002^{f}	1.686± 0.002^{m}	1.688± 0.0036^{k}	1.452± 0.0251^{o}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.7 Metal Complexes of Ethyl ester of glyphosate:

Herbicidal effects of the synthesized metal complexes of ethyl ester derivative of glyphosate was evaluated on the wheat grass (*Triticum aestivum*). Decrease in the content of photosynthetic pigments was recorded with respect to concentration of the derivative and time.

In comparison to control (water treatment) all the five metal complexes have shown diminution in the chlorophyll content. However this decrease in the chlorophyll content of treated plants was not comparable to glyphosate. Ethyl ester derivative of glyphosate has caused tremendous reductionin the chlorophyll content of treated plants at all the test concentrations (0.25X, 0.5X and 1X).Out of all the five metal complexes of ethyl ester derivative of glyphosate, **Ni-ethyl ester derivative** (Ni-EE gly) has momentously decreased the amount of 'Chl a,'Chl b' and 'Chl a+b' at all the three concentrations. Apart from Ni-EE gly, other metal complexes have also shown reduction in the chlorophyll content of the weed but their results are less significant as compared to glyphosate and Ni-EE gly **(Table 6.30).**

In spite of reduction in the photosynthetic pigments, herbicidal activity of the metal complexes of ethyl ester derivative of glyphosate was also assessed based on the number of plants killed after 15 days. In comparison to glyphosate, ethyl ester of glyphosate has killed maximum number of plants . However the **Ni-ethyl ester derivative of glyphosate** has also shown analogous results. It has killed 6 out of 10 plants at 0.5X after 10 days of treatment. However at the same concentration after 15 days, 7 plants were killed. At the highest concentration (1X) 9 plants out of 10 were killed by the complex **(Figure 6.84).** These results showed that apart from ethyl ester derivative of glyphosate, Ni-EE gly has also shown good herbicidal effects on the wheat plants.

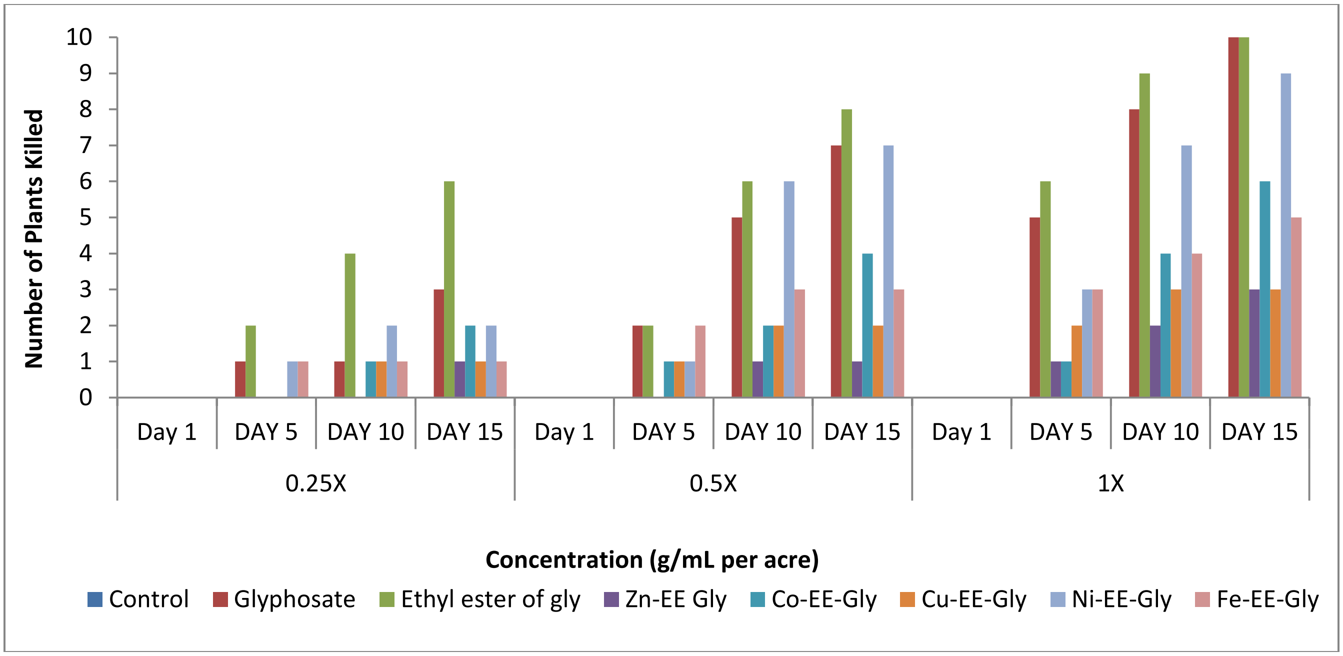


Figure 6.84 Lethal effect of exposure of Metal-Ethyl ester of glyphosate complexes on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).
Zn-EE Gly = Zinc-Ethyl ester of glyphosate complex, Co-EE Gly = Cobalt- Ethyl ester of glyphosate complex, Cu-EE Gly= Copper- Ethyl ester of glyphosate complex, Ni-EE Gly= Nickel- Ethyl ester of glyphosate complex, Fe- EE Gly= Iron- Ethyl ester of glyphosate complex

Table 6.29 Effect of Metal-Ethyl ester of glyphosate complexes on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b' (µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Triticum aestivum*.

	Days →	**1**			**5**			**10**			**15**		
	Concentration →	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
Chlorophyll a	**Compound**												
	Control	1.289± 0.0015^{a}	1.289± 0.0015 ^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.0005^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	Zn- Ethyl ester of Glyphosate complex	1.288± 0.0036^{c}	1.269± 0.0035^{c}	1.233± 0.0073^{d}	1.247± 0.0041^{b}	1.202± 0.004^{d}	1.184± 0.0094^{j}	1.175± 0.0025^{c}	1.135± 0.003^{b}	1.124± 0.0040^{g}	1.109± 0.0036^{g}	1.096± 0.0045^{h}	1.044± 0.0055^{i}
	Co- Ethyl ester of Glyphosate complex	1.289± 0.0032^{f}	1.272± 0.0020^{f}	1.237± 0.0028^{f}	1.248± 0.003 ^{f}	1.208± 0.0032^{f}	1.183± 0.019^{g}	1.173± 0.0035^{i}	1.135± 0.0035^{i}	1.124± 0.0030^{i}	1.077± 0.0087j	1.069± 0.0035^{j}	1.057± 0.003^{j}
	Cu- Ethyl ester of Glyphosate complex	1.284± 0.0086^{c}	1.262± 0.0085^{e}	1.231± 0.0096^{d}	1.245± 0.0035^{d}	1.209± 0.0026^{d}	1.182± 0.0015^{f}	1.172± 0.0041^{g}	1.129± 0.0058^{h}	1.120± 0.010^{h}	1.078± 0.0036^{i}	1.064± 0.002^{i}	1.050± 0.01069^{i}
	Ni- Ethyl ester of Glyphosate complex	1.281± 0.0035^{c}	1.254± 0.030^{e}	1.229± 0.0049^{d}	1.240± 0.0015^{e}	1.199± 0.004^{d}	1.157± 0.0546^{f}	1.141± 0.0041^{g}	1.124± 0.002^{g}	1.119± 0.0045^{i}	1.073± 0.0065^{i}	1.062± 0.004^{i}	1.038± 0.002^{i}
	Fe- Ethyl ester of Glyphosate complex	1.285± 0.0043^{e}	1.268± 0.0036^{c}	1.234± 0.0037^{g}	1.249± 0.0035^{b}	1.205± 0.0041^{d}	1.190± 0.0485^{k}	1.184± 0.0065^{i}	1.145± 0.0234^{g}	1.122± 0.002	1.079± 0.0035^{i}	1.069± 0.0041^{j}	1.055± 0.003^{i}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	1.218± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	Zn- Ethyl ester of Glyphosate complex	0.632± 0.002^{a}	0.624± 0.0026^{c}	0.620± 0.0075^{d}	0.610± 0.0078^{d}	0.600± 0.0025^{e}	0.589± 0.005^{f}	0.590± 0.0026^{e}	0.564± 0.0061^{f}	0.494± 0.0040^{i}	0.457± 0.0023^{j}	0.314± 0.003^{m}	0.201± 0.0040^{l}
	Co- Ethyl ester of Glyphosate complex	0.646± 0.003^{c}	0.629± 0.0041^{d}	0.628± 0.0020^{e}	0.619± 0.0036^{e}	0.599± 0.0025^{f}	0.584± 0.0026^{h}	0.590± 0.0032^{f}	0.569± 0.0025^{i}	0.499± 0.0025^{j}	0.440± 0.0041^{j}	0.417± 0.0041^{l}	0.285± 0.0015^{m}
	Cu- Ethyl l ester of Glyphosate complex	0.629± 0.001^{b}	0.625± 0.0045^{c}	0.624± 0.0036^{d}	0.619± 0.0025^{d}	0.599± 0.004^{g}	0.596± 0.0083^{f}	0.589± 0.0058^{g}	0.568± 0.0036^{f}	0.501± 0.0020^{h}	0.432± 0.0035^{j}	0.409± 0.0062^{l}	0.272± 0.002^{m}
	Ni- Ethyl ester of Glyphosate complex	0.628± 0.002^{b}	0.620± 0.003^{c}	0.625± 0.0035^{d}	0.607± 0.0020^{d}	0.597± 0.0025^{e}	0.560± 0.0026^{f}	0.588± 0.0025^{e}	0.565± 0.0035^{f}	0.484± 0.0017^{h}	0.415± 0.005^{n}	0.404± 0.0007^{j}	0.191± 0.003^{o}
	Fe- Ethyl ester of Glyphosate complex	0.631± 0.0037^{c}	0.628± 0.0035^{d}	0.622± 0.0055^{k}	0.612± 0.0066^{d}	0.602± 0.0056^{h}	0.575± 0.0035^{f}	0.595± 0.005^{i}	0.564± 0.003	0.582± 0.0052^{l}	0.420± 0.0041^{m}	0.407± 0.0015^{j}	0.198± 0.004^{0}

Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	Zn- Ethyl ester of Glyphosate complex	1.920± 0.002^{c}	1.893± 0.0032^{c}	1.853± 0.0045^{e}	1.857± 0.003^{g}	1.802± 0.0197^{g}	1.773± 0.0094^{i}	1.765± 0.0020^{i}	1.699± 0.004^{j}	1.618± 0.0040^{j}	1.566± 0.0036^{j}	1.410± 0.0045^{k}	1.245± 0.0055^{l}
	Co- Ethyl l ester of Glyphosate complex	1.935± 0.0086^{e}	1.901± 0.0234^{g}	1.865± 0.049^{g}	1.867± 0.003^{h}	1.807± 0.0047^{h}	1.767± 0.0192^{n}	1.763± 0.0037^{j}	1.704± 0.0035^{j}	1.623± 0.003^{m}	1.534± 0.0087^{k}	1.486± 0.0032^{l}	1.342± 0.003^{m}
	Cu- Ethyl ester of Glyphosate complex	1.913± 0.0035^{c}	1.887± 0.0020^{e}	1.855± 0.0028^{e}	1.847± 0.0015^{g}	1.808± 0.0072^{g}	1.778± 0.0015^{h}	1.761± 0.0035^{j}	1.698± 0.0058^{j}	1.621± 0.0102^{k}	1.510± 0.0036^{k}	1.473± 0.0026^{k}	1.322± 0.0106^{m}
	Ni- Ethyl ester of Glyphosate complex	1.909± 0.0043^{c}	1.874± 0.0102^{e}	1.854± 0.0096^{e}	1.847± 0.0035^{g}	1.796± 0.003^{g}	1.717± 0.0546^{h}	1.729± 0.004^{j}	1.689± 0.002^{j}	1.603± 0.0030^{k}	1.488± 0.0065^{k}	1.466± 0.004^{k}	1.229± 0.002^{l}
	Fe- Ethyl ester of Glyphosate complex	1.916± 0.0062^{c}	1.896± 0.0085	1.856± 0.004	1.861± 0.0065^{h}	1.807± 0.003^{h}	1.765± 0.0485	1.779± 0.0065^{k}	1.709± 0.0036	1.704± 0.0045	1.499± 0.0035^{l}	1.469± 0.0041^{k}	1.253± 0.0015^{k}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p< 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.8 Metal Complexes of Isopropyl ester derivative of glyphosate:

Herbicidal effect of five different metal complexes of isopropyl ester derivative of glyphosate was scrutinized on the wheat grass. Plants were treated with three different concentrations (0.25X, 0.5X and 1X) of the derivatives and chlorophyll content from their leaves was evaluated. None of the derivative have shown better results as compared to glyphosate. Little decrease in the chlorophyll pigments has been noticed in all cases **(Table 6.31).** In comparison to control, only a slight decrease in 'chlorophyll a' was observed at all the test concentrations even after 15 days. Amongst all the derivatives, **Fe-Isopropyl ester derivative of glyphosate** (Fe-IPE gly), at 0.25X has shown reduction in 'chlorophyll conten't in comparison to control. **(Figure 6.85)**. Not significant decrease in the photosynthetic pigments was observed in other metal complexes of iso propyl ester derivative of glyphosate.

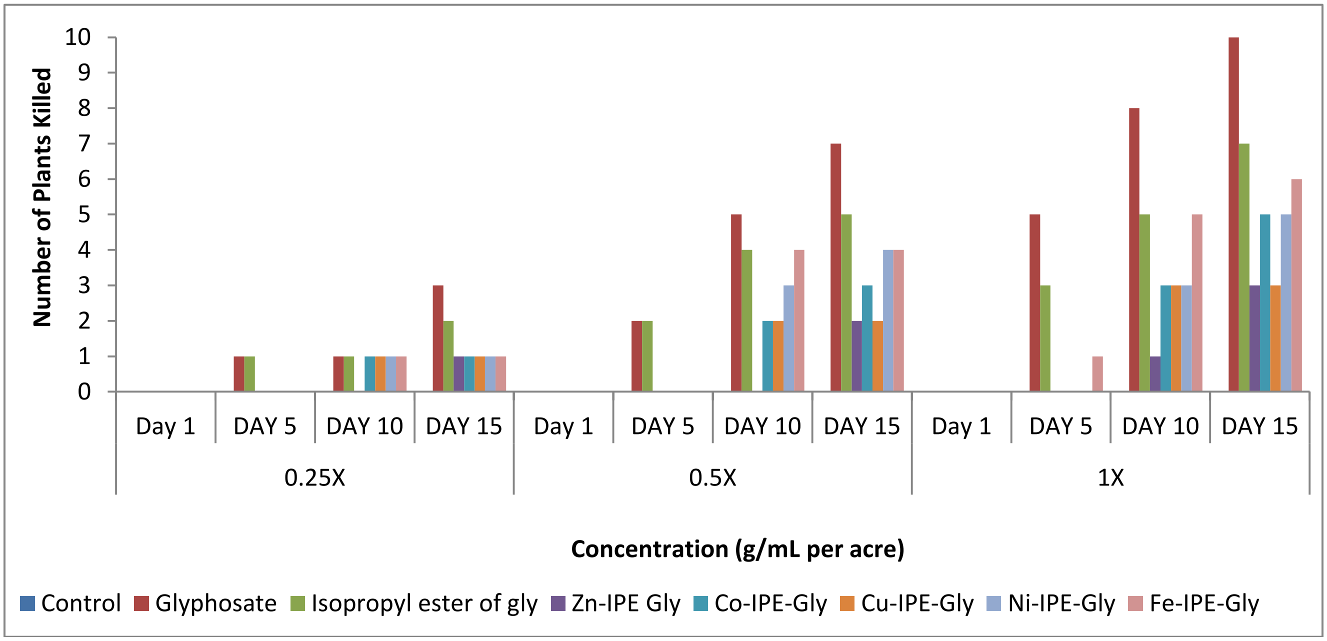


Figure 6.85 Lethal effect of exposure of Metal-Isopropyl ester of glyphosate complexes on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose).

Zn-IPE Gly = Zinc-Isopropyl ester of glyphosate complex, Co-IPE Gly = Cobalt-Isopropyl ester of glyphosate complex, Cu-IPE Gly= Copper-Isopropyl ester of glyphosate complex, Ni-IPE Gly= Nickel-Isopropyl ester of glyphosate complex, Fe-IPE Gly= Iron-Isopropyl ester of glyphosate complex

Table 6.30 Effect of Metal-Isopropyl ester of glyphosate complexes on the 'chlorophyll content a' (µg/gFW), 'chlorophyll content b' (µg/gFW) and 'total chlorophyll content' (µg/gFW) in the leaves of *Triticum aestivum*.

	Days →	**1**			**5**			**10**			**15**		
	Concentration →	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**	**0.25X**	**0.5X**	**1X**
	Compound												
Chlorophyll a	**Control**	1.289± 0.0015^{a}	1.289± 0.0015^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.0005^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	Zn-Isopropyl ester of Glyphosate complex	1.285± 0.002^{c}	1.255± 0.0034^{c}	1.227± 0.0037^{d}	1.277± 0.0015^{b}	1.239± 0.002^{d}	1.215± 0.001^{j}	1.253± 0.0025^{c}	1.204± 0.005^{b}	1.179± 0.002^{g}	1.180± 0.0032^{g}	1.154± 0.0030^{h}	1.113± 0.0025^{i}
	Co-Isopropyl ester of Glyphosate complex	1.289± 0.0005^{f}	1.272± 0.005^{f}	1.231± 0.0015^{f}	1.270± 0.0035^{f}	1.253± 0.002^{f}	1.218± 0.015^{g}	1.255± 0.0058^{i}	1.201± 0.0025^{i}	1.182± 0.004^{i}	1.179± 0.0045^{j}	1.159± 0.0056^{j}	1.124± 0.002^{j}
	Cu-Isopropyl ester of Glyphosate complex	1.287± 0.003^{c}	1.284± 0.0105^{e}	1.234± 0.0043^{d}	1.261± 0.0047^{d}	1.252± 0.0052^{d}	1.212± 0.002^{f}	1.254± 0.001^{g}	1.207± 0.0032^{h}	1.195± 0.002^{h}	1.177± 0.004^{i}	1.158± 0.0055^{i}	1.114± 0.002^{i}
	Ni-Isopropyl ester of Glyphosate complex	1.289± 0.0035^{c}	1.264± 0.003^{e}	1.255± 0.0052^{d}	1.269± 0.016^{e}	1.242± 0.0026^{d}	1.204± 0.002^{f}	1.244± 0.0037^{g}	1.205± 0.002^{g}	1.183± 0.0045^{i}	1.180± 0.0110^{i}	1.157± 0.001^{i}	1.103± 0.037^{i}
	Fe-Isopropyl ester of Glyphosate complex	1.274± 0.0030^{e}	1.252± 0.0085^{c}	1.225± 0.0035^{g}	1.255± 0.004^{b}	1.235± 0.0026^{d}	1.196± 0.003^{k}	1.242± 0.0036^{i}	1.200± 0.0045^{g}	1.174± 0.008	1.176± 0.0041^{i}	1.155± 0.0031^{j}	1.089± 0.002^{i}
Chlorophyll b	**Control**	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	Zn-Isopropyl ester of Glyphosate complex	0.624 0.003^{a}	0.620± 0.0025^{c}	0.617± 0.0025^{d}	0.616± 0.0078^{d}	0.600± 0.0025^{e}	0.571± 0.0015^{f}	0.597± 0.0026^{e}	0.564± 0.0025^{f}	0.532± 0.0020^{i}	0.475± 0.002^{j}	0.400± 0.005^{m}	0.289± 0.0040^{l}
	Co-Isopropyl ester of Glyphosate complex	0.623± 0.003^{c}	0.621± 0.0030^{d}	0.619± 0.0025^{e}	0.612± 0.0036^{e}	0.605± 0.002^{f}	0.582± 0.002^{h}	0.599± 0.0032^{f}	0.568± 0.002^{i}	0.515± 0.0072^{j}	0.462± 0.002^{j}	0.425± 0.003^{l}	0.291± 0.0036^{m}
	Cu-Isopropyl ester of Glyphosate complex	0.624± 0.001^{b}	0.622± 0.001^{c}	0.620± 0.001^{d}	0.611± 0.0025^{d}	0.601± 0.0026^{g}	0.595± 0.002^{f}	0.599± 0.0058^{g}	0.569± 0.0026^{f}	0.501± 0.0030^{h}	0.440± 0.004^{j}	0.417± 0.0045^{l}	0.275± 0.002^{m}
	Ni-Isopropyl ester of Glyphosate complex	0.623± 0.002^{b}	0.620± 0.009^{c}	0.618± 0.0020^{d}	0.615± 0.0020^{d}	0.611± 0.0011^{e}	0.581± 0.001^{f}	0.595± 0.0025^{e}	0.582± 0.004^{f}	0.499± 0.002^{h}	0.431± 0.0035^{n}	0.414± 0.003^{j}	0.252± 0.003^{o}
	Fe-Isopropyl ester of Glyphosate complex	0.623± 0.0037^{c}	0.620± 0.0047^{d}	0.615± 0.0035^{k}	0.609± 0.0066^{d}	0.599± 0.0056^{h}	0.563± 0.002^{f}	0.589± 0.005^{i}	0.571± 0.0011^{i}	0.481± 0.00152^{l}	0.420± 0.0041^{m}	0.401± 0.001^{j}	0.121± 0.007^{0}

Total Chlorophyll	**Control**	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	Zn- Isopropyl ester of Glyphosate complex	1.909± 0.002^{c}	1.875± 0.0045^{c}	1.844± 0.0036^{e}	1.893± 0.004^{g}	1.839± 0.0274^{g}	1.786± 0.0045^{i}	1.850± 0.0043^{i}	1.768± 0.003^{j}	1.711± 0.0020^{j}	1.655± 0.0030^{j}	1.554± 0.0040^{k}	1.402± 0.0034^{l}
	Co-Isopropyl ester of Glyphosate complex	1.912± 0.002^{e}	1.893± 0.0251^{g}	1.848± 0.0068^{g}	1.882± 0.0017^{h}	1.858± 0.003^{h}	1.800± 0.007^{n}	1.854± 0.002^{j}	1.770± 0.008^{j}	1.697± 0.0020^{m}	1.641± 0.0015^{k}	1.584± 0.0032^{l}	1.415± 0.0026^{m}
	Cu-Isopropyl ester of Glyphosate complex	1.911± 0.0036^{c}	1.906± 0.0026^{e}	1.854± 0.0045^{e}	1.872± 0.0037^{g}	1.853± 0.0020^{g}	1.807± 0.0041^{h}	1.853± 0.0037^{j}	1.775± 0.0040^{j}	1.696± 0.0035^{k}	1.617± 0.003^{k}	1.583± 0.0030^{k}	1.389± 0.0023^{m}
	Ni- Isopropyl ester of Glyphosate complex	1.912± 0.007^{c}	1.884± 0.0264^{e}	1.873± 0.0045^{e}	1.884± 0.0025^{g}	1.853± 0.002^{g}	1.785± 0.0025^{h}	1.839± 0.004^{j}	1.787± 0.0055^{j}	1.682± 0.0051^{k}	1.600± 0.003^{k}	1.571± 0.002^{k}	1.355± 0.004^{l}
	Fe-Isopropyl ester of Glyphosate complex	1.897± 0.0020^{c}	1.872± 0.001	1.840± 0.0051	1.864± 0.005^{h}	1.834± 0.004^{h}	1.759± 0.0035	1.831± 0.0060^{k}	1.771± 0.001	1.655± 0.0025	1.596± 0.001^{l}	1.556± 0.0032^{k}	1.210± 0.0005^{k}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3).). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves.

6.3.3.9 Metal Complexes of n-Propyl ester derivative of glyphosate:

Herbicidal effects of the metal complexes of n-Propyl ester derivative of glyphosate was also studied on wheat grass. Reduction in the chlorophyll content was recorded in the treated plants for 15 days and was compared with control and glyphosate treatment. A remarkable decline of photosynthetic pigments was recorded in the plants treated with the derivatives. Also a notable decrease in the number of weed plants (plants killed) was recorded after their treatment with the derivatives at different concentrations. After 15 days, **Ni-Propyl ester derivative of glyphosate** (Ni-PE) has effectively decreased the 'chlorophyll content' in the treated plants. However, Cu complex of n-propyl ester derivative of glyphosate (Cu-PE) has also caused noteworthy decrease in chlorophyll content but its effect is less pronounced than glyphosate (**Table 6.32**).

Thus, its noteworthy to mention here that Ni-propyl ester derivative of glyphosate (Ni-PE) had good herbicidal properties and effectively killed the weed. It effectively killed all the plants (10 out of 10) at the recommended dose after 10 days of its application **(Figure 6.86)**.

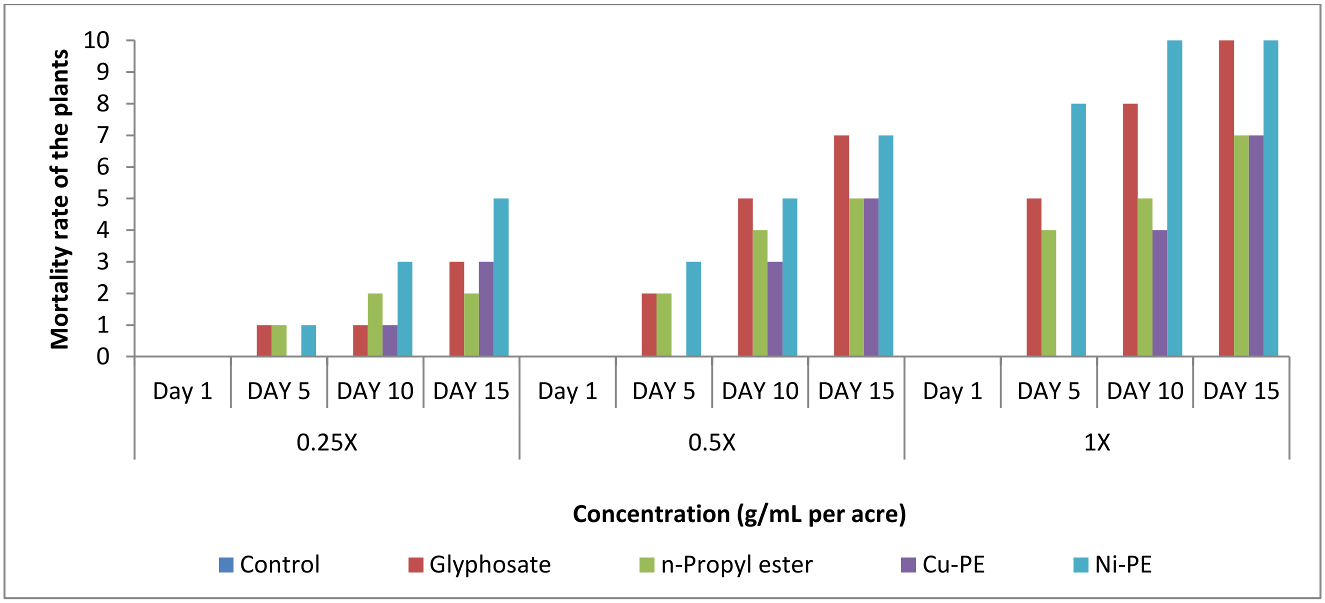


Figure 6.86 Lethal effect of exposure of Metal-Propyl ester of glyphosate complexes on *Triticum aestivum* after 15 days. Values are mean of 3 (n=3) where 0.25X = One fourth of recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose). Cu-PE= Copper-Propyl ester of glyphosate complex, Ni-PE = Nickel- Propyl ester of glyphosate complex

Table 6.31. Effect of Metal-Propyl ester of glyphosate complexes on the chlorophyll content a (µg/gFW), chlorophyll content b(µg/gFW) and total chlorophyll content (µg/gFW) in the leaves of *Triticum aestivum*.

Days ⟶		1			5			10			15		
Concentration ⟶		0.25X	0.5X	1X	0.25X	0.5X	1X	0.25X	0.5X	1X	0.25X	0.5X	1X
	Compound												
Chlorophyll a	Control	1.289± 0.0015^{a}	1.289± 0.0015 ^{a}	1.289± 0.0015^{a}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.287± 0.002^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.286± 0.001^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}	1.284± 0.0015^{b}
	Glyphosate	1.265± 0.001^{c}	1.234± 0.002^{e}	1.223± 0.0015^{e}	1.239± 0.0005^{e}	1.142± 0.002^{h}	1.134± 0.0020^{h}	1.129± 0.001^{h}	1.126± 0.0035^{h}	1.117± 0.0020^{i}	1.066± 0.0005^{m}	1.059± 0.001^{j}	1.032± 0.0020^{m}
	Propyl ester of Glyphosate	1.285± 0.0041^{c}	1.258± 0.0075^{d}	1.231± 0.0032^{h}	1.265± 0.0251^{d}	1.239± 0.005^{f}	1.210± 0.0041^{h}	1.202± 0.0061^{g}	1.210± 0.0036^{h}	1.186± 0.0025^{h}	1.176± 0.0030^{e}	1.195± 0.002^{h}	1.115± 0.0036^{i}
	Cu-Propyl ester of Glyphosate complex	1.298± 0.0045^{a}	1.260± 0.002^{j}	1.235± 0.0086^{h}	1.282± 0.004^{e}	1.242± 0.0026^{j}	1.213± 0.003^{h}	1.217± 0.0025^{d}	1.221± 0.0026^{h}	1.192± 0.003^{h}	1.179± 0.0041^{h}	1.190± 0.003^{i}	1.110± 0.0087^{p}
	Ni-Propyl ester of Glyphosate complex	1.264± 0.003^{d}	1.225± 0.0036^{l}	1.197± 0.0035^{h}	1.224± 0.0030^{e}	1.137± 0.0026^{h}	1.128± 0.0035^{k}	1.119± 0.0035^{j}	1.109± 0.00173^{j}	1.092± 0.0035^{l}	1.057± 0.0062^{j}	1.027± 0.0040^{n}	1.008± 0.0036^{o}
Chlorophyll b	Control	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.624± 0.0015^{a}	0.618± 0.0015^{b}	0.618± 0.0015^{c}	0.618± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.604± 0.0015^{c}	0.597± 0.0015^{d}	0.597± 0.0015^{d}	0.597± 0.0015^{d}
	Glyphosate	0.622± 0.0015^{a}	0.619± 0.0015^{b}	0.613± 0.0015^{c}	0.605± 0.0040^{c}	0.596± 0.0015^{d}	0.557± 0.0015^{d}	0.583± 0.002^{e}	0.559± 0.0026^{g}	0.473± 0.0026^{f}	0.373± 0.003^{l}	0.359± 0.001^{m}	0.053± 0.001^{n}
	Propyl ester of Glyphosate	0.624± 0.003^{e}	0.619± 0.0049^{h}	0.615± 0.0096^{i}	0.616± 0.0047^{g}	0.605± 0.0014^{f}	0.589± 0.054^{g}	0.599± 0.0032^{h}	0.529± 0.0030^{g}	0.556± 0.0055^{g}	0.535± 0.0045^{j}	0.511± 0.0055^{i}	0.508± 0.003^{h}
	Cu-Propyl ester of Glyphosate complex	0.623± 0.023^{f}	0.620± 0.0028^{h}	0.624± 0.0049^{j}	0.611± 0.003^{b}	0.598± 0.0192^{h}	0.531± 0.0485^{g}	0.595± 0.002^{h}	0.530± 0.0030^{g}	0.519± 0.003^{p}	0.521± 0.004^{g}	0.509± 0.003^{h}	0.497± 0.0035^{q}
	Ni-Propyl ester of Glyphosate complex	0.617± 0.0020^{b}	0.603± 0.0096^{e}	0.599± 0.0037^{k}	0.579± 0.002^{h}	0.572± 0.0015^{i}	0.548± 0.0015^{n}	0.530± 0.004^{i}	0.505± 0.0045^{l}	0.420± 0.0106^{m}	0.361± 0.0058^{n}	0.302± 0.0106^{f}	0.042± 0.0005^{r}
Total Chlorophyll	Control	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.913± 0.0010^{a}	1.895± 0.0025^{b}	1.895± 0.0010^{b}	1.895± 0.0025^{b}	1.890± 0.0015^{d}	1.890± 0.0015^{e}	1.890± 0.0015^{c}	1.881± 0.0030^{d}	1.881± 0.0030^{f}	1.881± 0.0030^{c}
	Glyphosate	1.887± 0.0010^{b}	1.858± 0.0015^{c}	1.827± 0.0011^{c}	1.847± 0.0040^{f}	1.838± 0.0020^{j}	1.730± 0.0015^{g}	1.712± 0.0096^{j}	1.685± 0.0025^{n}	1.590± 0.0026^{j}	1.439± 0.0130^{m}	1.218± 0.0015^{p}	1.085± 0.0020^{m}
	Propyl ester of Glyphosate	1.909± 0.002^{b}	1.877± 0.0136^{b}	1.846± 0.003^{e}	1.881± 0.0025^{f}	1.844± 0.0052^{e}	1.799± 0.0080^{c}	1.801± 0.0045^{e}	1.739± 0.0055^{g}	1.742± 0.002^{g}	1.711± 0.0025^{d}	1.706± 0.0055^{g}	1.623± 0.0026^{f}
	Cu-Propyl ester of Glyphosate complex	1.921± 0.0032^{b}	1.880± 0.005^{e}	1.859± 0.001^{c}	1.893± 0.0058^{f}	1.840± 0.002^{c}	1.744± 0.0037^{d}	1.812± 0.004^{c}	1.751± 0.0025^{f}	1.711± 0.002^{f}	1.700± 0.0005^{g}	1.699± 0.0056^{f}	1.607± 0.002^{h}

	Ni-Propyl ester of Glyphosate complex	1.881± 0.0035^{b}	1.828± 0.0105^{k}	1.796± 0.0035^{f}	1.803± 0.001^{c}	1.709± 0.0162^{j}	1.676± 0.002^{m}	1.649± 0.011^{i}	1.614± 0.0032^{k}	1.512± 0.0020^{l}	1.418± 0.0035^{k}	1.329± 0.0030^{h}	1.050± 0.004^{m}

Where 0.25X= One fourth of the recommended dose, 0.5X= Half of recommended dose and 1X= Recommended dose (441g/L per acre) .Values are mean of ± SD (n=3). The Scott-Knott Test for probability was applied. All of the data is significant at the level of 1% probability ($p < 0.01$). The averages followed by the same letter do not differ statistically between themselves

6.4 ANTIMICROBIAL ACTIVITY OF SYNTHESISED GLYPHOSATE DERIVATIVES:

The antimicrobial activity of different synthesized derivatives of glyphosate has been carried out using Kirby-Bauer disc diffusion method[152]. All the derivatives were checked for their antimicrobial activities. Two plant growth-promoting rhizo bacteria (PGPR) *Pseudomonas fulva* (Gram negative) (Accession Number- MF 782684), *Pseudomonas putida* (Gram negative) (Accession Number- MF 782681) and two fungal species *Aspergillus fumigatus* (NCIM-902), *Candida albicans* (MTCC-183) were used to assess the antibacterial and antifungal activity of the synthesized derivatives. Very few derivatives have exhibited antibacterial and antifungal activity against these microbes.

6.4.1 Antibacterial Activity: Antibacterial activity of the 36 synthesized derivatives of glyphosate was analyzed against plant growth-promoting rhizo bacteria (PGPR) *Pseudomonas fulva* (Gram negative) (Accession Number- MF 782684) and *Pseudomonas putida* (Gram negative) (Accession Number- MF 782681). Muller Hinton Agar Media (MHA) was used for the in-vitro studies. Three different concentrations (250ppm,500ppm and 1000ppm) of the derivatives were used and were compared with control(water).

6.4.1.1 *Pseudomonas fulva* (Accession Number- MF 782684)**:** Out of all the synthesized derivatives of glyphosate, only **n-Propyl ester of glyphosate** has shown the zone of inhibition only at the highest concentration of 1000ppm. A small zone of inhibition (1mm) was noticed only at 1000ppm.These results indicate that this derivative can inhibit the growth of the gram negative bacteria (*Pseudomonas fulva*) only at highest concentration **(Figure 6.91 (F))**. Rest of the synthesized derivatives of glyphosate remains ineffective against the bacterium. They do not show any sign of toxicity against this plant growth-promoting rhizo bacteria.

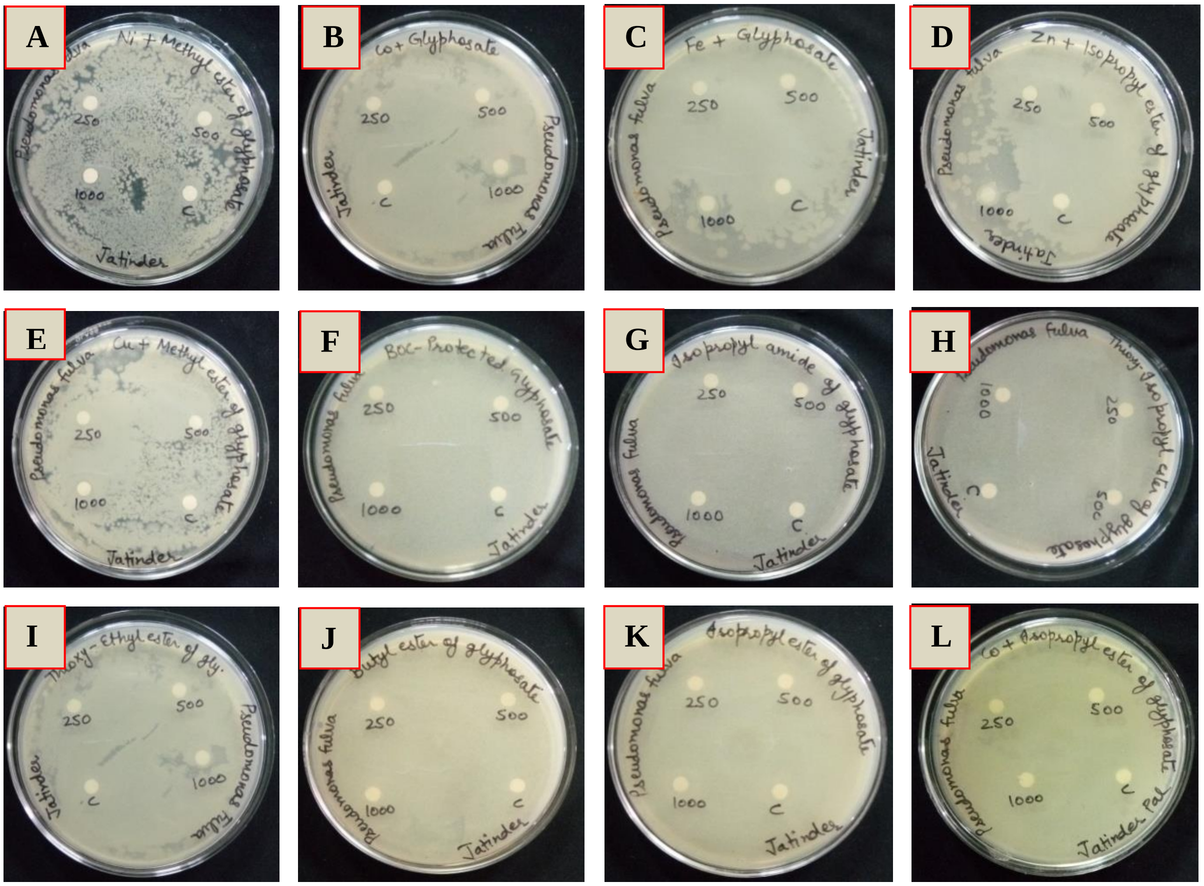


Figure: 6.87 Antibacterial activity shown by *Pseudomonas fulva* against some of the synthesized derivatives of glyphosate (where: **A**: Ni-Methyl Ester derivative of glyphosate complex, **B**: Co-glyphosate complex, **C**: Fe-glyphosate complex, **D**: Zn-Isopropyl Ester derivative of glyphosate complex, **E**: Cu-Methyl Ester derivative of glyphosate complex, **F**:Boc-Protected glyphosate, **G**: Isopropyl amide of glyphosate, **H**: Thioxylated isopropyl ester of glyphosate, **I:** Thioxylated ethyl ester of glyphosate, **J:** Butyl ester of glyphosate, **K**: Isopropyl ester of glyphosate, **L**: Co-Isopropyl Ester derivative of glyphosate complex)

6.4.1.2 *Pseudomonas putida* (Accession Number- MF 782681) **:** Among the different synthesized derivatives of glyphosate, only **Cu-Propyl ester of glyphosate** (Cu-PE) has shown antibacterial activity against this microbe. Very small zone of inhibition (0.5 mm) was noticed only at the highest concentration of 1000ppm. This reveals that this derivative(Cu-PE) can restrain the growth of the gram negative bacteria (*Pseudomonas putida*) only at highest concentration **(Figure 6.91(G))**. Whereas other synthesized derivatives of glyphosate remains ineffectual against the microbes. They do not show any sign of toxicity against this plant growth-promoting rhizo bacteria.

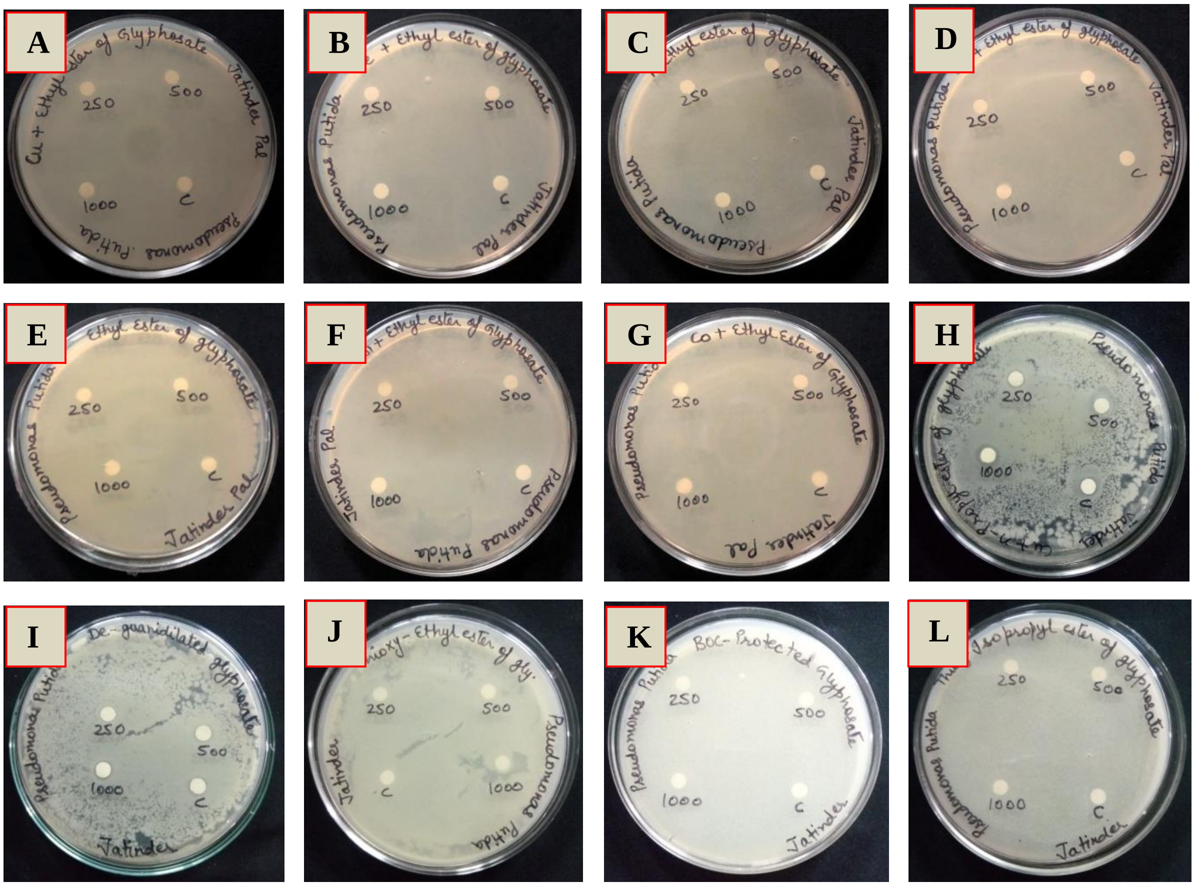


Figure : 6.88 Antibacterial activity shown by *Pseudomonas putida* against some of the glyphosate derivatives (where: **A**: Cu-Ethyl ester derivative of glyphosate complex, **B**:Fe-Ethyl ester derivative of glyphosate, **C**: Methyl ester derivative of glyphosate, **D**:Zn-Ethyl ester derivative of glyphosate, **E**: Ethyl ester derivatives of glyphosate, **F**: Ni-Ethyl ester derivative of glyphosate, **G**: Co-Ethyl ester derivative of glyphosate, **H**: Cu-Propyl ester derivative of glyphosate, **I:** Guanidilated glyphosate, **J:**Thioxylated Ethyl ester derivative of glyphosate, **K**: Boc-Protected glyphosate, **L**: Thioxylated isopropyl ester derivative of glyphosate)

6.4.2 Antifungal Activity: 36 synthesized derivatives of glyphosate were scrutinized for antifungal activity against test fungi *Aspergillus fumigatus* (NCIM-902) and *Candida albicans* (). Potato Dextrose Agar (PDA) was used as a media for the in-vitro studies. Paper discs dipped with three different concentrations (250 ppm, 500ppm and 1000ppm) of the derivatives were inoculated in the media plates. Results were examined after 48 hours of incubation.

6.4.2.1 ***Aspergillus fumigatus*** (NCIM-902): None of the synthesized derivatives of glyphosate has shown any activity against the fungal species *Aspergillus fumigatus*. All of them remain inactive and no zone of inhibition is reported in any case. These results claimed that the newly synthesized derivatives are non-toxic to this saprophytic fungi.

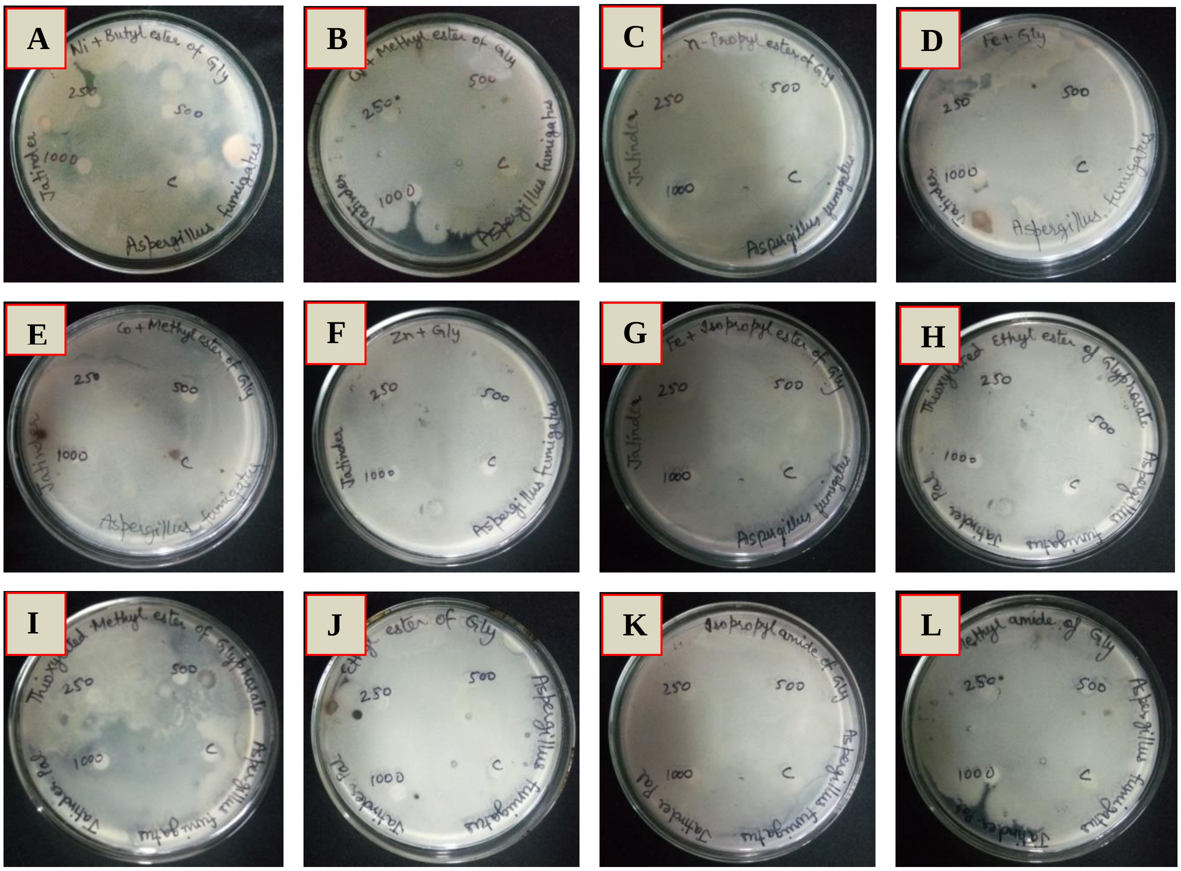


Figure: 6.89 Antifungal activity shown by *Aspergillus fumigatus* against some of the glyphosate derivatives (where: **A**: Ni-Butyl ester derivative of glyphosate, **B**:Cu-Methyl ester derivative of glyphosate, **C**: n-Propyl ester derivative of glyphosate, **D**: Fe-Glyphosate complex, **E**: Co-Methyl ester derivative of glyphosate, **F**: Zn-Glyphosate complex, **G**: Fe-Isopropyl ester derivative of glyphosate, **H**: Thioxylated Ethyl ester derivative of glyphosate, **I:** Thioxylated Methyl ester derivative of glyphosate, **J:** Ethyl ester derivative of glyphosate, **K**: Isopropyl amide derivative of glyphosate, **L**: Methyl amide derivative of glyphosate)

6.4.2.2 ***Candida albicans*** (MTCC-183)-): Four out of 36 synthesized derivatives of glyphosate have shown antifungal activity against *Candida albicans*. **Cu-Isopropyl ester of glyphosate (Figure 6.91(B)** has shown anti fungal effects by showing the zone of inhibition of 3mm at the highest concentration of 1000ppm. **Zn-Methyl ester of glyphosate (Figure 6.91(C)** has also shown the zone of inhibition at all the three test concentrations (250ppm, 500ppm and 1000ppm). Similar results were shown by **Boc-protected guanidilated derivative of glyphosate (Figure 6.91(D)**. Also the **thioxylated ethyl ester of glyphosate (Figure 6.91(E)** has also shown the zone of inhibition of 2.5mm at the highest concentration of 1000ppm. From all these results it can be concluded that the anti fungal effects of the different synthesized derivatives of glyphosate decreases with increase in dilution. However rest of the derivatives of glyphosate has not shown any effect against this fungal species.

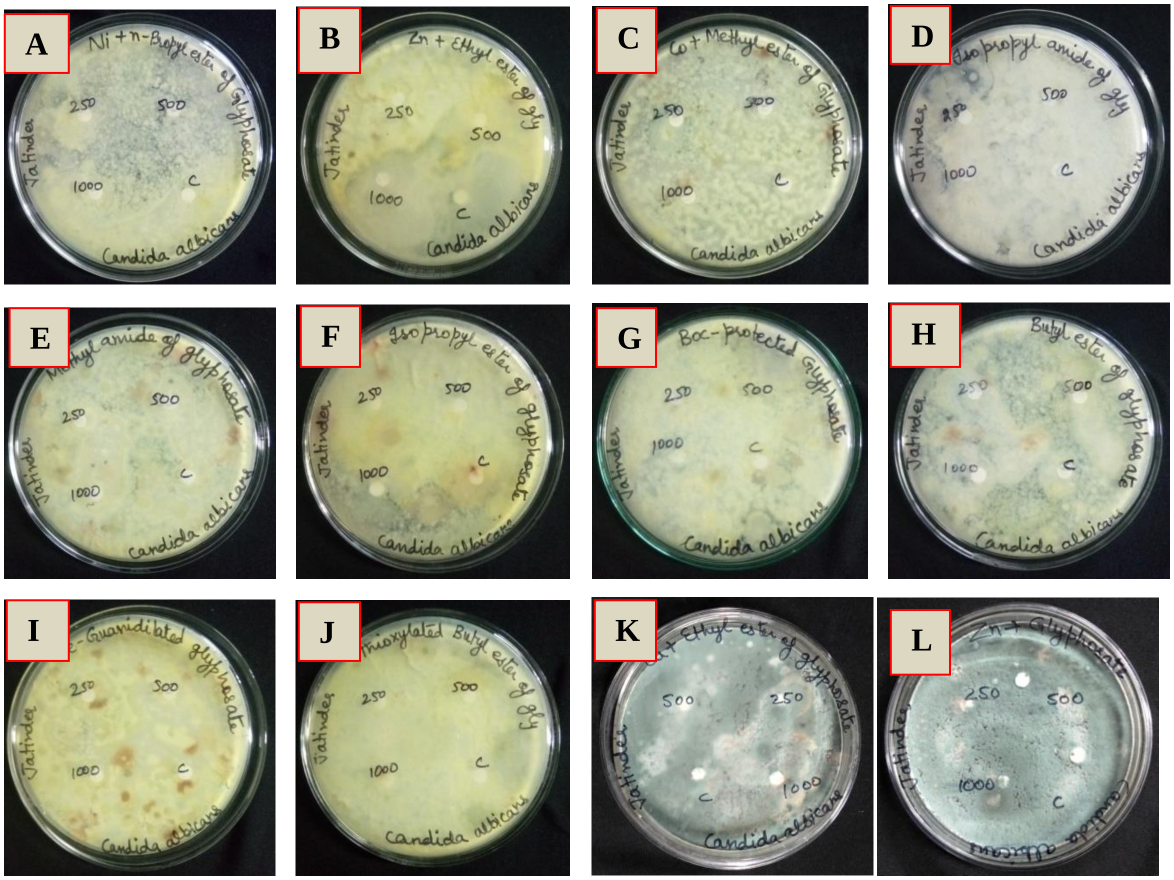


Figure: 6.90 Antifungal activity shown by *Candida albicans* against some of the glyphosate derivatives (where: **A**: Ni-Butyl ester derivative of glyphosate, **B**: Zn-Ethyl ester derivative of glyphosate, **C**: Co-Methyl ester derivative of glyphosate, **D**: Isopropyl amide derivative of glyphosate, **E**: Methylamide derivative of glyphosate, **F**: Isopropyl ester derivative of glyphosate, **G**: Boc-protected glyphosate, **H**: Butyl ester derivative of glyphosate, **I:** Guanidilated glyphosate, **J:** Thioxylated Butyl ester derivative of glyphosate, **K**: Cu-Ethyl ester derivative of glyphosate, **L**: Zn-Glyphosate complex)

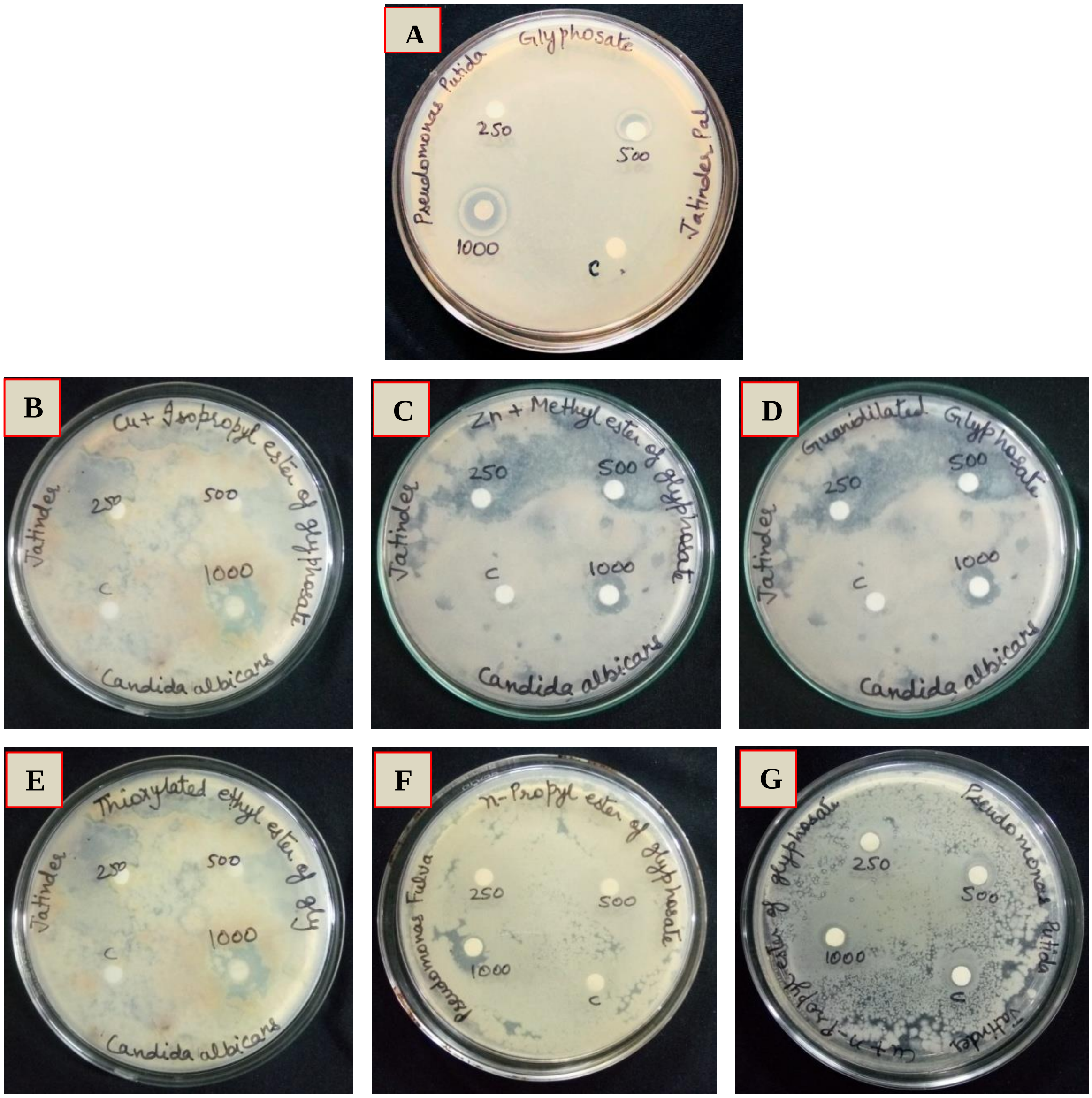


Figure: 6.91 Antimicrobial (antibacterial and antifungal) activity shown by some of the glyphosate derivatives (Where: **A**: Glyphosate, **B**: Cu-Isopropyl ester of glyphosate, **C**: Zn-Methyl ester derivative of glyphosate, **D**: Guanidilated glyphosate, **E**: Thioxylated Ethyl ester derivative of glyphosate, **F**: n-Propyl ester derivative of glyphosate, **G**: Cu-Propyl ester derivative of glyphosate)

Glyphosate inhibit the shikimate pathway of the microbes and thus suppresses their growth. Thus, glyphosate can act as a broad-spectrum antimicrobial agent which can show its effectiveness against bacterial and fungal species. Apart from this glyphosate has also shown a major decrease in the radial growth of all ectomycorrhizal fungal species like *Cenococcum geophilum* Fr., *Pisolithus tinctorius* (Pers.) Coker and Couch and *Hebeloma longicaudum* (Pers.). The growth of these ectomycorrhizal fungal species was completely inhibited when glyphosate was used at higher concentration $\geq$ 5000 ppm[101] . Chakravarty et al. evaluated the harmful outcomes of glyphosate on the common mycorrhizal fungal species like *Hebeloma crustuliniforme*, *Laccaria laccata*, *Thelephora americana*, *T. terrestris* and *Suillus tomentosus*. It has been found that glyphosate reduced the growth of these fungal microorganisms at concentrations above 10 ppm[102]. Glyphosate shows negative impact on the growth of certain rhizospheric microbial communities. Bacteria like fluorescent *pseudomonads*, Mn-transforming bacteria, and indoleacetic acid-producing bacteria present in the rhizosphere soils of soybean were treated with glyphosate. Glyphosate increased the profusion of *Fusarium spp*. while it reduced the profusion of fluorescent *pseudomonads*, Mn-reducing bacteria and indole acetic acid-producing rhizobacteria[103].

Another research group also studied the consequences of glyphosate on soil rhizosphere-associated colonies and found that the application of glyphosate increases the relative abundance of *proteobacteria* (particularly gammaproteobacteria). But the excessive use of glyphosate on glyphosate- resistant crops like corn and soybean decreases the relative abundance of *Acidobacteria*. Since *Acidobacteria* are also implicated in biogeochemical processes, the decline in the profusion of these bacteria results in considerable alterations in nutrient condition of the rhizosphere and would affect plant growth[104].

Since glyphosate act as a potent antimicrobial agent against various microorganisms present in the ecosystem. Only a few synthesized derivatives of glyphosate have shown anti microbial effects against the mentioned microbes even at the highest test concentration (1000ppm). Thus, it has been found that these derivatives are non-effective against the microbes present in the soil. and doesn't cause any harm to them,

6.5 MICROBIAL DEGRADATION OF SYNTHESIZED GLYPHOSATE DERIVATIVES:

Microbial degradation of the two synthesized glyphosate derivatives was carried out using *Actinomyces sp.* (Gram positive) (Accession Number-KJ 854403.1). Only two derivatives, Ethyl ester of glyphosate and thioxylated ethyl ester of glyphosate were taken to carried out their degradation studies. These two synthesized derivatives were used because both of these have shown excellent herbicidal activity. They have significantly reduced the chlorophyll content and have killed maximum number of weed plants. Their herbicidal effects were better than the glyphosate itself.

Degraded products formed after the degradation of the ethyl ester of glyphosate and thioxylated ethyl ester of glyphosate were analyzed using ESI-MS. Degradation using the bacterial species were analyzed after 3 and 7 days of time interval.

From the ESI-MS of ethyl ester derivative of glyphosate, three molecular ion peaks were obtained. Molecular ion peak with maximum abundance(100%) has been found at 254 (m/z) which corresponds to the molecular mass of ethyl ester of glyphosate. Two other peaks were also found at 97 (m/z) Methyl-phosphonic acid and 79 (m/z) Metaphosphoric acid **(Figure 6.93)**. After 3 days , the *Actinomyces sp*. decomposes ~ 40% of ethyl ester derivative of glyphosate into methyl phosphonic acid; m/z 97(75%), and metaphosphoric acid; m/z 79(60%). However, after 7 days of degradation, ethyl ester derivative of glyphosate was degraded to 60% and the degraded products with100% relative abundance of methyl phosphonic acid; m/z 97, and 75% of metaphosphoric acid; m/z 79 were obtained. These results showed that *Actinomyces sp* are capable of degrading the ethyl ester derivative of glyphosate.

Similar results were obtained in case of thioxylated ethyl ester derivative of glyphosate. After 3 days, *Actionomyces sp*. degrades 40% of the thioxylated ethyl ester derivative of glyphosate into methyl phosphonic acid; m/z 97(60)% and metaphosphoric acid; m/z 79(60%). However after 7 days, the degradation was increased to 60% with methylphosphonic acid (100%) and metaphosphoric acid (60%) **(Figure 6.94)**.

These results were in agreement with the degradation of glyphosate with *Actinomyces sp*. After 3 and 7 days relative abundance of methyl phosphoric acid;m/z 97 was found to be 55% and 85% respectively and that of metaphosphoric acid was 40% and 65% respectively **(Figure 6.92)**.

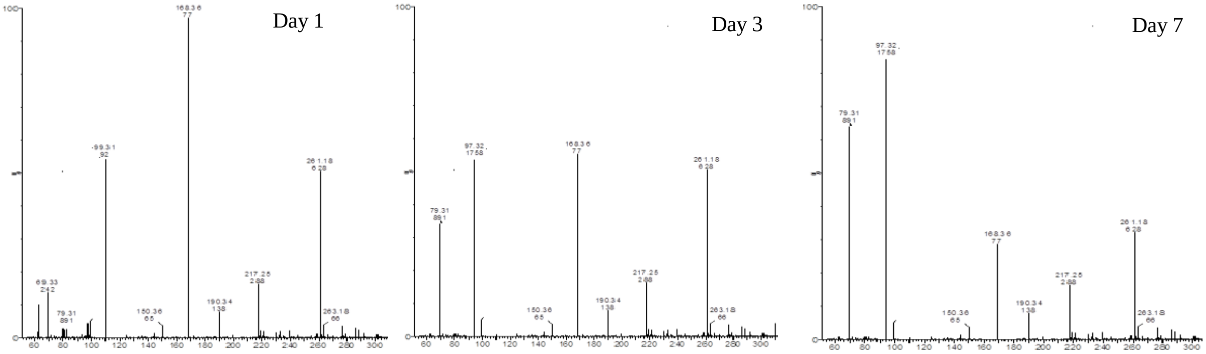


Figure: 6.92 Mass spectrum of glyphosate degradation for day1,3 and 7 by *Actinomyces* sp.

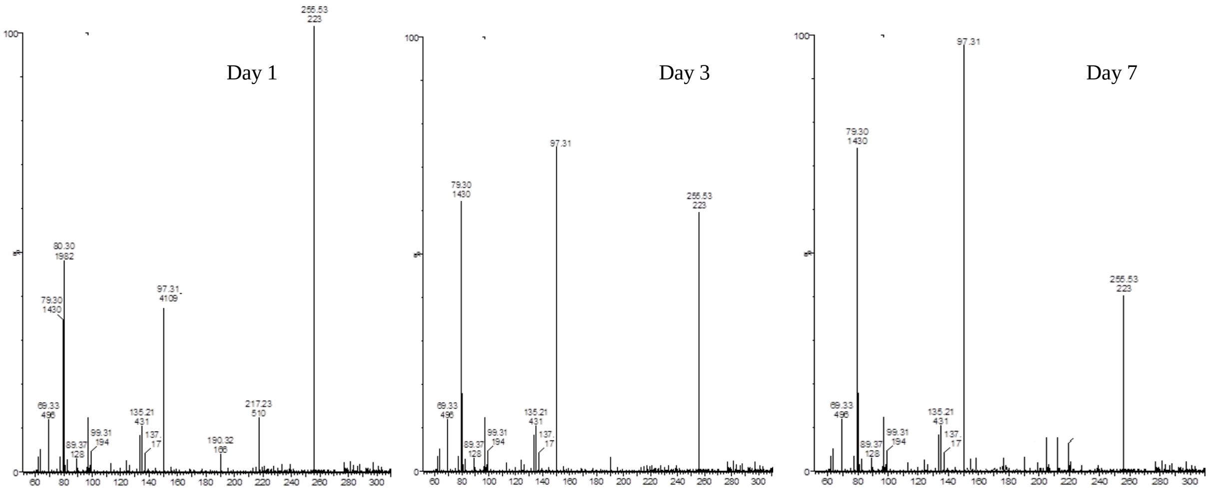


Figure:6.93 Mass spectrum of Ethyl ester derivative of glyphosate degradation for day1,3 and 7 by *Actinomyces* sp.

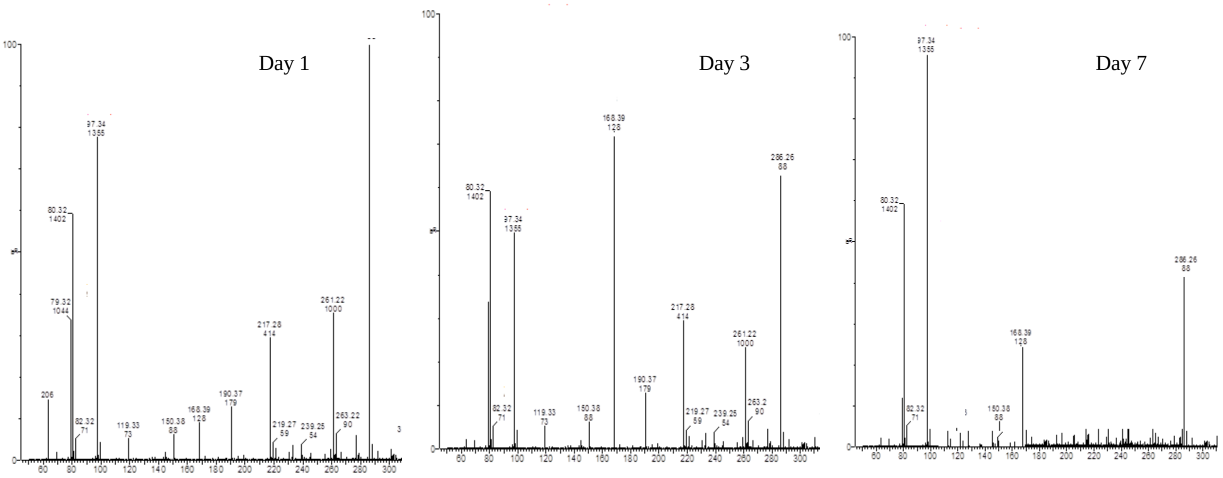


Figure:6.94 Mass spectrum of Thioxylated ethyl ester derivative of glyphosate degradation for day1,3 and 7 by *Actinomyces* sp.

6.6 INSECTICIDAL ACTIVITY OF SYNTHESIZED GLYPHOSATE DERIVATIVES:

Insecticidal activity of the 36 synthesized derivatives of glyphosate was checked on *Drosophilla melanogaster* (Commonly known as Fruit fly). Three different concentrations (2.5ppm, 5ppm and 10ppm) of glyphosate and its synthesized derivatives were mixed into the corn media and the flies were allowed to fed on them. The activity was monitored for 24 hours, 48 hours and 72 hours.

Figure 6.95 (a-c) Insecticidal activity of synthesized derivatives of glyphosate on *Drosophilla melanogaster* at different concentrations (2.5ppm, 5 ppm and 10ppm)

Figure 6.96 Test organism (*Drosophila melanogaster*) used for insecticidal activity of glyphosate and its derivatives (Where A: Female species of *D. melanogaster*, B: Male species of *D. melanogaster*

Insecticidal activity on the fruit fly was evaluated on the basis of mortality of flies, caused due to exposure of synthesized derivatives in comparison to control and glyphosate. Very few derivatives of glyphosate have shown their detrimental effects on these insects. Most of the synthesized derivatives remain ineffectual against *Drosophila melanogaster* at all the three test concentrations. Butyl amide of glyphosate, Methyl amide of glyphosate, Fe-Methyl ester of glyphosate complex, Zn-Ethyl ester of glyphosate complex and Co- Methyl ester of glyphosate complex do not cause any mortality in the culture tubes at all the concentrations. Some of the synthesized derivatives (Methyl ester of glyphosate, Ethyl ester of glyphosate, Isopropyl ester of glyphosate, Thioxylated isopropyl ester of glyphsate, Thioxylated butyl ester of glyphosate, Cu-Propyl ester of glyphosate complex, Cu-Isopropyl ester of glyphosate complex, Fe-Isopropyl ester of glyphosate complex, Co-Isopropyl ester of glyphosate complex, Zn-Isopropyl ester of glyphosate complex, Cu-Methyl ester of glyphosate complex, Zn-Methyl ester of glyphosate complex Cu- Ethyl ester of glyphosate, Zn-Ethyl ester of glyphosate, Co-glyphosate complex and Zn-glyphosate complex) have shown negligible toxicity and killed only 1 out of 10 flies **(Table 6.32)**. However few of the synthesized derivatives (Ni-Propyl ester of glyphosate complex, Ni-Isopropyl ester of glyphosate complex, Fe-Ethyl ester of glyphosate complex, Ni-Ethyl ester of glyphosate complex, Ni-methyl ester of glyphosate complex, Co-Methyl ester of glyphosate complex, Fe-glyphosate complex, Thioxylated ethyl ester of glyphosate, Thioxylated methyl ester of glyphosate, Isopropyl amide of glyphosate and Boc-protected guanidilated glyphosate have killed only 1 or 2 flies at the highest concentration (10ppm) after 72 hours only. Out of 36, only two synthesized derivatives namely Boc-protected glyphosate and guanidilated glyphosate have shown minor toxicity. At the highest concentration (10ppm) only 1 fly was killed after 24 hours, 3 were killed after 48 hours and 5 out of 10 flies were killed after 72 hours. Similar results were shown by guanidilated glyphosate. At 10ppm 1 fly was killed after 24 hours, 2 were killed after 48 hours and 3 flies were killed after 72 hours. At lower doses (2.5ppm and 5ppm) no noteworthy decrease in the number of flies was noticed.

These results have shown that the synthesized derivatives of glyphosate remain non-toxic to *Drosophilla melanogaster* and do not showed any insecticidal activity against these insects. Similar studies were carried by different research groups, de Aguiar et al. examined the outcomes of glyphosate containing herbicides (Roundup) in *Drosophila melanogaster* concerning their antioxidant defence system, oxidative stress and acetylcholinesterase activity. Flies (1 to 3 days old) were fed with four concentrations (1mg/L, 2mg/L, 5mg/L and 10mg/L) of herbicide dose mixed in the diet for 24 hours and 96 hours. Herbicide exposure to the flies leads to the reduction in the reactive oxygen species (ROS) level in flies exposed to 96 hours. Antioxidant capacity against peroxyl radicals (ACAP) and gene expression of the antioxidant defence system show significant rise after 24 hours exposure. However lipid peroxidation level does not show any considerable change at both exposure periods. Also the activity of enzyme the acetylcholinesterase was not affected by Roundup exposure. Thus it was concluded that exposure of flies to Roundup caused an early propulsion of the antioxidant defence system in the flies and prevent the harm caused by reactive oxygen species[116].

Kaya et al. analysed the genotoxicity effects of four herbicides (propanil, maleic hydeazine, glyphosate and 2,4,5-tri chlorophenoxy acetic acid) in the wing spot test of *Drosophila melanogaster*. 3-day old larvae were chronically fed to these herbicides. Standard (ST) cross and high-bioactivation (HB) cross having the flare-3 and multiple wing hair markers were used for the test. Glyphosate resulted in the weak but momentous increase in the prevalence of small single spots only in standard cross indicating that it is genotoxic in these strains. No response was noticed in high-bioactivation (HB) cross[117.]

Table: 6.32 Effect of synthesized derivatives of glyphosate (Mortality rate) on *Drosophila melanogaster* with respect to concentration and time

Concentration →	**2.5ppm**			**5 ppm**			**10 ppm**		
Time →	**24 hours**	**48 hours**	**72 hours**	**24 hours**	**48 hours**	**72 hours**	**24 hours**	**48 hours**	**72 hours**
Control	10	10	10	10	10	10	10	10	10
Glyphosate	10	10	10	10	10	9	9	8	7
Methyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Butyl Ester of Glyphosate	10	10	10	10	10	10	10	10	10
Methyl Amide of Glyphosate	10	10	10	10	10	10	10	10	10
Isopropyl Amide of Glyphosate	10	10	10	10	10	10	10	9	8
Butyl Amide of Glyphosate	10	10	10	10	10	10	10	10	10
Boc-Protected Glyphosate	10	10	9	10	9	8	9	7	5
Boc-Protected Guanidilated Glyphosate	10	10	10	10	10	10	10	9	8
Guanidilated Glyphosate	10	10	9	10	9	8	9	8	7
Thioxylated Methyl Ester of Glyphosate	10	10	10	10	10	10	10	9	8
Thioxylated Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	9	9
Thioxylated Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Thioxylated Butyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Zn- Glyphosate Complex	10	10	10	10	10	10	10	10	9
Co- Glyphosate Complex	10	10	10	10	10	10	10	10	9
Cu- Glyphosate Complex	10	10	10	10	10	10	10	10	9
Ni- Glyphosate Complex	10	10	10	10	10	10	10	10	9
Fe- Glyphosate Complex	10	10	10	10	10	9	10	9	8
Zn- Methyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Co- Methyl Ester of Glyphosate	10	10	10	10	10	9	10	9	8
Cu- Methyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Ni- Methyl Ester of Glyphosate	10	10	10	10	10	10	10	9	9
Fe- Methyl Ester of Glyphosate	10	10	10	10	10	10	10	10	10
Zn- Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	10	10

Co- Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	10	10
Cu- Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Ni- Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	9	8
Fe- Ethyl Ester of Glyphosate	10	10	10	10	10	10	10	9	8
Zn- Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Co- Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Cu- Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Ni- Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	9	7
Fe- Isopropyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Cu- Propyl Ester of Glyphosate	10	10	10	10	10	10	10	10	9
Ni- Propyl Ester of Glyphosate	10	10	10	10	9	9	10	9	8

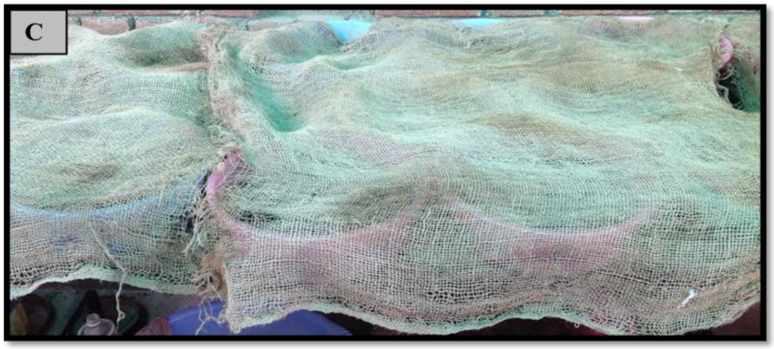
6.7 TOXICITY EVALUATION OF SYNTHESIZED GLYPHOSATE DERIVATIVES:

Toxicity evaluation of the synthesized derivatives of glyphosate was done on Earthworms (*Esenia fetida*). Mortality of earthworms was taken as the criterion to analyze toxicological effects of the synthesized derivatives of glyphosate. Toxicological study on the earthworms was done in accordance with OECD recommendations. Three variable concentrations (10 mg/kg, 20 mg/kg and 40 mg/kg) of the derivatives were mixed with the soil and 10 mature earthworms having fully developed clitellum were added in it for toxicity tests.

Majority of the synthesized derivatives of glyphosate have neither shown any sign of toxicity nor killed any earthworm. They remain ineffective in the presence of these derivatives. Very few derivatives of glyphosate (Ni-methyl ester of glyphosate complex, Co-methyl ester of glyphosate complex, Ni-ethyl ester of glyphosate complex, Fe-ethyl ester of glyphosate complex, Ni-Isopropyl ester of glyphosate complex, Fe-glyphosate complex, Isopropyl amide of glyphosate, Boc-protected guanidilated glyphosate, Thioxylated ethyl ester of glyphosate and Thioxylated methyl ester of glyphosate) have killed only 1earthworm out of 10 only at the highest concentration (40mg) after 14 days of treatment. Alike results were also shown by Ni-propyl ester of glyphosate (1 earthworm was killed out of 10 at 40mg/kg after 14 days of application). However Boc-protected glyphosate and guanidilated glyphosate have shown similar results as that of glyphosate, 3 out of 2 earthworms were killed in all the three cases at the highest concentration of 40mg/kg after 14 days of exposure **(Table 6.33)**.

These results were in confirmation with experiment conducted by Correia et al. He performed laboratory tests on *Eisenia fetida* to investigate the toxicological effects produced by glyphosate on it. Five concentrations of glyphosate (1, 10, 100, 500 and 1000 mg) were used as test concentrations. The experiment was carried out for 56 days. No mortality was observed in the soils treated with glyphosate at any of these concentrations. However, steady and considerable decrease in the mean body weight was found at all the test concentrations. Glyphosate revealed severe harmful effects on the procreation and development of earthworms in the range of test concentrations. No cocoons or young worms were detected in the soil treated with the herbicide. Apart from this, significant anatomical changes were also observed after 30 days of the experiment. Morphological abnormalities like the elevation of body, curling and coiling were observed in all the specimens treated with the maximumt test concentration of soil treated with glyphosate[54].

Another research group used glyphosate-based herbicide Groundclear (containing 5% of isopropylamine salt of glyphosate), to examine its acute toxicity on *Eisenia fetida*. Earthworms were treated with five different concentrations of the herbicide; however, the worms exposed to the recommended dose for 24–48 h show very little mortality. But they show avoidance behavior against the herbicide. The presence of herbicide in the soil also affects the locomotor activity of the worms. Thus, the use of herbicide may not directly cause any harm to them, but it can cause severe long-term effects[127].



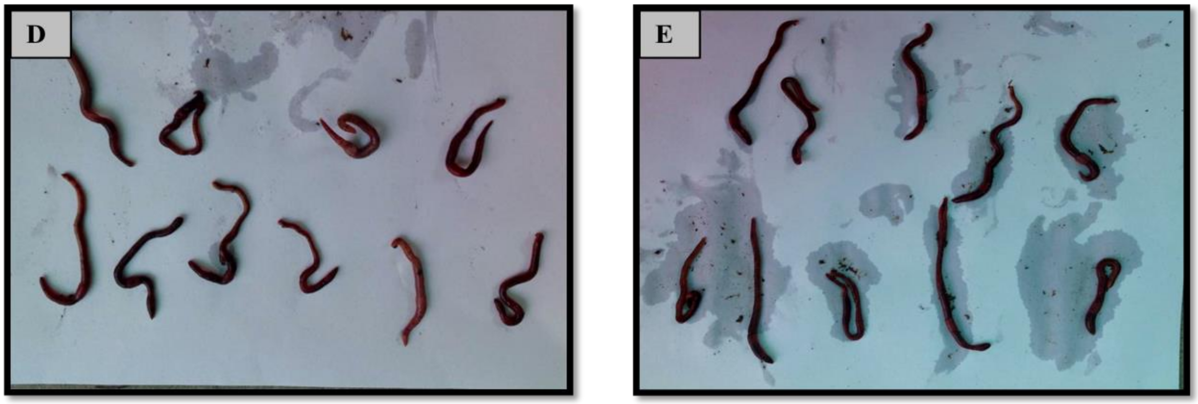


Figure: 6.97 Toxicity evaluation of glyphosate and its derivatives with earthworm species *Eisenia fetida*. (A: experimental setup for toxicity evaluation. B-C: Earthworm species used for toxicity evaluation.)

Table: 6.33 Effect of synthesized derivatives of glyphosate (Mortality rate) on Earthworms (*Esenia fetida*) with respect to concentration and time:

CONCENTRATION	**10mg/kg**		**20mg/kg**		**40mg/kg**	
TIME	**7days**	**14 days**	**7 days**	**14 days**	**7days**	**14 days**
Control	10	10	10	10	10	10
Glyphosate	10	10	10	10	9	8
Methyl Ester of Glyphosate	10	10	10	10	10	10
Ethyl Ester of Glyphosate	10	10	10	10	10	10
Isopropyl Ester of Glyphosate	10	10	10	10	10	10
Butyl Ester of Glyphosate	10	10	10	10	10	10
Methyl Amide of Glyphosate	10	10	10	10	10	10
Isopropyl Amide of Glyphosate	10	10	10	10	10	9
Butyl Amide of Glyphosate	10	10	10	10	10	10
Boc-Protected Glyphosate	10	10	10	9	9	7
Boc-Protected Guanidilated Glyphosate	10	10	10	10	10	9
Guanidilated Glyphosate	10	10	10	9	9	8
Thioxylated Methyl Ester of Glyphosate	10	10	10	10	10	9
Thioxylated Ethyl Ester of Glyphosate	10	10	10	10	10	9
Thioxylated Isopropyl Ester of Glyphosate	10	10	10	10	10	10
Thioxylated Butyl Ester of Glyphosate	10	10	10	10	10	10
Zn- Glyphosate Complex	10	10	10	10	10	10
Co- Glyphosate Complex	10	10	10	10	10	10
Cu- Glyphosate Complex	10	10	10	10	10	10
Ni- Glyphosate Complex	10	10	10	10	10	10
Fe- Glyphosate Complex	10	10	10	10	10	9
Zn- Methyl Ester of Glyphosate	10	10	10	10	10	10
Co- Methyl Ester of Glyphosate	10	10	10	10	10	9
Cu- Methyl Ester of Glyphosate	10	10	10	10	10	10
Ni- Methyl Ester of Glyphosate	10	10	10	10	10	9
Fe- Methyl Ester of Glyphosate	10	10	10	10	10	10
Zn- Ethyl Ester of Glyphosate	10	10	10	10	10	10

Co- Ethyl Ester of Glyphosate	10	10	10	10	10	10
Cu- Ethyl Ester of Glyphosate	10	10	10	10	10	10
Ni- Ethyl Ester of Glyphosate	10	10	10	10	10	9
Fe- Ethyl Ester of Glyphosate	10	10	10	10	10	9
Zn- Isopropyl Ester of Glyphosate	10	10	10	10	10	10
Co- Isopropyl Ester of Glyphosate	10	10	10	10	10	10
Cu- Isopropyl Ester of Glyphosate	10	10	10	10	10	10
Ni- Isopropyl Ester of Glyphosate	10	10	10	10	10	9
Fe- Isopropyl Ester of Glyphosate	10	10	10	10	10	10
Cu- Propyl Ester of Glyphosate	10	10	10	10	10	10
Ni- Propyl Ester of Glyphosate	10	10	10	9	10	9

CHAPTER 7

SUMMARY AND CONCLUSION

Structural modifications in the glyphosate molecule were done and 36 different glyphosate derivatives were synthesized and their herbicidal, antimicrobial, insecticidal and toxicological studies were conducted. Apart from these activities microbial degradation of the synthesized glyphosate derivatives were also carried out.

Seven different type of glyphosate derivatives were synthesized and their characterization was done using FTIR, ^{1}HNMR and ESI-MS. A series of ester derivatives of glyphosate were synthesized by using thionyl chloride and corresponding alcohol. It is an effective and efficient method used for the synthesis of alkyl substituted ester derivatives of glyphosate. Four different ester derivatives (Methyl 2-{[(dimethoxyphosphoryl) methyl] amino} acetate hydrochloride, Ethyl 2{[(diethoxyphosphoryl) methyl] amino}acetate hydrochloride, Isopropyl 2({[diiso propox--yphosphoryl] methyl}amino) acetate hydrochloride, Butyl 2-{[(dibutoxyphosphoryl) methyl] amino}acetate hydrochloride) were synthesized by using different alcohols. Second type of glyphosate derivatives are the amide derivatives of glyphosate, they were synthesized by condensation of glyphosate with required amine separately using mixed anhydride method of coupling with Isobutyl chloroformate as a coupling reagent in presence of inert solvent. Three amide derivatives were synthesized namely ({[2-(methylamino)-2-oxo ethyl] amino} methyl) phosphonic acid, ({[2-oxo-2-(propan-2-yl amino) ethyl] amino} methyl) phosphonic acid, ({[2-(butyl amino)-2-oxoethyl]amino}methyl)phosphonic acid.

Another derivative of glyphosate was synthesized by Boc-Protection of glyphosate in which di-tert-butyl dicarbonate was used as a protecting group. The derivative prepared was (2-((tert-butoxycarbonyl)(phosphono methyl) amino) ethanoic acid. Another derivative 2-(2,3-bis(tert-butoxycarbonyl)-1-(phosphonomethyl) guanidino) ethanoic acid was synthesized by treating glyphosate with guanidinylating reagent (1,3 -Bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudo- -urea) in dioxane as a solvent. Next derivative of glyphosate synthesized was 2-(1-phosphonomethyl) guanidino)ethanoic acid. It was synthesized by acidolytic cleavage of Boc groups using trifluoroaceticacid (TFA). A new series of thioxylated ester derivatives of glyphosate were prepared, by refluxing the ester derivatives of glyphosate with phosphorus pentasulphide in dioxane as a solvent. Four thioxylated ester derivatives of glyphosate were synthesized (*O*-methyl {[(dimethoxyphosphorothioyl)methyl]amino}ethanethioate, *O*-ethyl {[(diethoxy phosphorothioyl) methyl]amino}ethanethioate, *O*-propan-2-yl({[bis(propan-2-yloxy) phosphorothioyl) methyl]amino}ethanethioate and *O*-butyl {[(dibutoxy phosphorothioyl) methyl]amino}ethanethioate). Apart from these, metal complexes of glyphosate and ester derivatives of glyphosate (25 total) were also synthesized. Five essential metal ions Zn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+} and Fe^{3+} were used to prepare these metal complexes. The formation of different glyphosate derivatives was confirmed from spectroscopic studies.

Post-emergent herbicidal effects of synthesized glyphosate derivatives were checked against two common weeds *Parthenium hysterophorus* and *Cyperus rotundus* and Wheat grass (*Triticum aestivum*). Analysis of the Chlorophyll content was used as an important parameter to evaluate the detrimental and herbicidal effects of these synthesized derivatives on them. Three different concentrations i.e [1X (recommended dose 441g/L per acre), 0.5X (half of recommended dose) and 0.25X (one-fourth of recommended dose)] of glyphosate and the synthesized derivatives were applied on the plants. A significant decrease in the chlorophyll was observed in all the three test concentrations after their exposure to the synthesized glyphosate derivatives. Among the 36 synthesized glyphosate derivatives, Ethyl 2{[(diethoxyphosphoryl) methyl] amino}acetate hydrochloride (Ethyl ester of glyphosate) and *O*-ethyl{[(diethoxyphosphorothioyl) methyl]amino} ethanethioate (Thioxylated ethyl ester of glyphosate) have shown best herbicidal effects on the weed. Ethyl 2{[(diethoxyphosphoryl)methyl]amino}acetate hydrochloride have shown better herbicidal activity than the glyphosate even on one fourth of the recommended dose after first day of its application. In comparison to control (water treatment) and glyphosate it had shown much enhanced effect in decreasing the chlorophyll content(at all the three test concentrations. Thioxylated ethyl ester of glyphosate behave as a potent herbicide and is extremely effective in decreasing the content of photosynthetic pigments of plants in comparison to glyphosate. Plants treated with thioxylated ethyl ester derivative of glyphosate at 1X have shown tremendous decrease in chl a, chl b) and chl a+b in contrast to glyphosate.

The antimicrobial activity of different synthesized glyphosate derivatives has been carried out using Kirby-Bauer disc diffusion method. All the derivatives were checked for their microbicidal effects on microbes. Two plant growth-promoting rhizo bacteria (PGPR) *Pseudomonas fulva* (Gram negative) (Accession Number- MF 782684), *Pseudomonas putida* (Gram negative) (Accession Number- MF 782681) and two fungal species *Aspergillus fumigatus* (NCIM-902), *Candida albicans* (MTCC-183) were used to assess the microbicidal effects of the synthesized derivatives. Very few derivatives have shown antibacterial and antifungal activity against these microbes. Out of all the synthesized derivatives of glyphosate, only n-Propyl ester derivative of glyphosate has shown the zone of inhibition only at the highest concentration of 1000ppm against gram negative bacteria (*Pseudomonas fulva*). A small zone of inhibition (1mm) was noticed only at 1000ppm. Cu complexed with n-propyl ester derivative of glyphosate (Cu-PE) has shown antibacterial activity against *Pseudomonas putida*. Very small zone of inhibition (0.5 mm) was noticed only at the highest concentration of 1000ppm. None of the synthesized glyphosate derivative has shown any activity against the fungal species *Aspergillus fumigatus*. Four out of 36 synthesized derivatives of glyphosate have shown antifungal activity against *Candida albicans*. Cu-Isopropyl ester derivative of glyphosate has shown anti fungal effects by showing the zone of inhibition of 3mm at the highest concentration of 1000ppm. Zn-Methyl ester derivative of glyphosate has also shown the zone of inhibition at all the three test concentrations (250ppm, 500ppm and 1000ppm). Similar results were shown by Boc-protected guanidilated derivative of glyphosate. Also the thioxylated ethyl ester derivative of glyphosate has also shown the zone of inhibition of 2.5mm at the highest concentration of 1000ppm.

Microbial degradation of glyphosate derivatives was done using bacterial species *Actinomyces.* sp. Ethyl ester derivative of glyphosate and Thioxylated ethyl ester derivative of glyphosate were degraded by this bacteria and methylphosphonic acid and metaphosphonic acid were formed as the degraded products. After 3 days , the *Actinomyces sp*. decomposes ~ 40% of ethyl ester derivative of glyphosate into methyl phosphonic acid; m/z 97(75%), and metaphosphoric acid; m/z 79(60%). However, after 7 days of degradation, ethyl ester derivative of glyphosate was degraded to 60% and the degraded products with 100% relative abundance of methyl phosphonic acid; m/z 97, and 75% of metaphosphoric acid; m/z 79 were obtained. After 3 days, *Actionomyces sp*. degrades 40% of the thioxylated ethyl ester derivative of glyphosate into methyl phosphonic acid; m/z 97(60)% and metaphosphoric acid; m/z 79(60%). However after 7 days, the degradation was increased to 60% with methylphosphonic acid (100%) and metaphosphoric acid (60%).

Insecticidal activity on the fruit fly was evaluated on the basis of mortality of flies, caused due to exposure of synthesized derivatives in comparison to control and glyphosate. Very few derivatives of glyphosate have shown their detrimental effects on these insects. Most of the synthesized derivatives remain ineffectual against *Drosophila melanogaster* at all the three test concentrations. Out of 36, only two synthesized derivatives namely Boc-protected glyphosate and guanidilated glyphosate have shown minor toxicity. At the highest concentration (10ppm) only 1 fly was killed after 24 hours, 3 were killed after 48 hours and 5 out of 10 flies were killed after 72 hours. Similar results were shown by guanidilated glyphosate. At 10ppm 1 fly was killed after 24 hours, 2 were killed after 48 hours and 3 flies were killed after 72 hours. At lower doses (2.5ppm and 5ppm) no significant reduction in the number of flies was observed. These results have shown that the most of the synthesized derivatives of glyphosate remain non-toxic to *Drosophilla melanogaster* and do not showed any insecticidal activity against these insects.

Toxicity evaluation of the synthesized derivatives of glyphosate was done on Earthworms (*Esenia fetida*). Mortality of earthworms was taken as the criterion to analyze toxicological effects of the synthesized derivatives of glyphosate. Toxicological study on the earthworms was done in accordance with OECD recommendations. Three different concentrations (10 mg/kg, 20 mg/kg and 40 mg/kg) of the derivatives were mixed with the soil and 10 mature earthworms having well developed clitellum were added in it for toxicity tests. Majority of the synthesized derivatives of glyphosate have neither shown any sign of toxicity nor killed any earthworm. They remain ineffective in the presence of these derivatives. However, Boc-protected glyphosate and guanidilated glyphosate have shown similar results as that of glyphosate, 3 out of 2 earthworms were killed in all the three cases at the highest concentration of 40mg/kg after 14 days of exposure.

From all these results it can be concluded that the synthesized derivatives of glyphosate act as potent herbicides, out of which ethyl ester of glyphosate and thioxylated ethyl ester of glyphosate have shown better results than glyphosate. All these derivatives remain non-toxic to microbes, insects and earthworms and can be easily degraded by soil microbes. So these newly synthesized glyphosate derivatives may be a solution to the problems caused by glyphosate.

CHAPTER 8

BIBLIOGRAPHY

1. Taylor, E.L., Holley, A.G. and Kirk, M., 2007. Pesticide development: a brief look at the history. *Athens, GA: Southern Regional Extension Forestry*.

2. Bus, J.S. and Hammond, L.E., 2007. Regulatory progress, toxicology, and public concerns with 2, 4-D: where do we stand after two decades? *Crop Prot., 26*(3), pp.266-269.

3. Boye, B., Dieng, M.M. and Brillas, E., 2003. Anodic oxidation, electro-Fenton and photoelectro-Fenton treatments of 2, 4, 5-trichlorophenoxyacetic acid. *J. Electroanal. Chem., 557*, pp.135-146.

4. Duke, S.O. and Powles, S.B., 2008. Glyphosate: a once-in-a-century herbicide. *Pest Manage. Sci.*, *64*(4), pp.319-325.

5. Young, B.G., 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. *Weed Technol., 20*(2), pp.301-307.

6. Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R. and Vandenberg, L.N., 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environ. Health*, *15*(1), pp.19.

7. Bonny, S., 2008. Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. *Agron. Sustain. Dev., 28*(1), pp.21-32.

8. Heap, I., 2014. Global perspective of herbicide-resistant weeds. *Pest Manage. Sci.*, *70*(9), pp.1306-1315.

9. Steinmann, H.H., Dickeduisberg, M. and Theuvsen, L., 2012. Uses and benefits of glyphosate in German arable farming. *Crop Prot.*, *42*, pp.164-169.

10. Nedelkoska, T.V. and Low, G.C., 2004. High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate. *Anal. Chim. Acta.*, *511*(1), pp.145-153.

11. May, M.J., 2003. Economic consequences for UK farmers of growing GM herbicide tolerant sugar beet. *Ann Appl Biol.*, *142*(1), pp.41-48.

12. Phipps, R.H. and Park, J.R., 2002. Environmental benefits of genetically modified crops: global and European perspectives on their ability to reduce pesticide use. *J. Animal Feed Sci.*, *11*(1), pp.1-18.

13. Woodburn, A.T., 2000. Glyphosate: production, pricing and use worldwide. *Pest Manage Sci: formerly Pesticide Science*, *56*(4), pp.309-312.

14. Dill, G.M., CaJacob, C.A. and Padgette, S.R., 2008. Glyphosate-resistant crops: adoption, use and future considerations. *Pest Manage Sci: formerly Pesticide Science*, *64*(4), pp.326-331.

15. Gianessi, L.P., 2005. Economic and herbicide use impacts of glyphosate-resistant crops. *Pest Manage Sci: formerly Pesticide Science*, *61*(3), pp.241-245.

16. Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.*, *28*(1), p.3.

17. Gill, J.P.K., Sethi, N. and Mohan, A., 2017. Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. *Environ. Chem. Lett.*, *15*(1), pp.85-100.

18. Chamberlain, K., Evans, A.A. and Bromilow, R.H., 1996. 1-Octanol/Water Partition Coefficient (Kow) and pKa for Ionisable Pesticides Measured by a pH-Metric Method. *Pestic Sci.*, *47*(3), pp.265-271.

19. Shoval, S. and Yariv, S., 1979. The interaction between roundup (glyphosate) and montmorillonite. Part I, Infrared study of the sorption of glyphosate by montmorillonite. *Clays Clay Miner*, *27*(1), pp.19-28.

20. Contardo-Jara, V., Klingelmann, E. and Wiegand, C., 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in Lumbriculus variegatus and its effects on biotransformation and antioxidant enzymes. *Environ Pollut.*, *157*(1), pp.57-63.

21. Jia, D., Wang, L., Li, C. and Wang, X., 2012. Solid–liquid phase equilibrium of glyphosate in selected solvents. Fluid Phase Equilibr, *327*, pp.1-8.

22. Fu, W., Guo, Q. and Wang, J., 2010. Solubility of glyphosate in ethanol+ water, 1-propanol+ water, and 2-propanol+ water from (293 to 333) K. *J. Chem. Eng. Data*, *55*(9), pp.3915-3917.

23. Knuuttila, P.E.K.K.A. and Knuuttila, H.I.L.K.K.A., 1979. The crystal and molecular structure of N-(phosphonomethyl) glycine (glyphosate). *Acta Chem. Scand, 33,* pp.623-626.

24. Shaner, D., 2006. An overview of glyphosate mode of action: Why is it such a great herbicide. *North Centr. Weed Sci. Soc. Proc. 61, 94.*

25. Herrmann, K.M. and Weaver, L.M., 1999. The shikimate pathway. *Annu. Rev. Plant Biol.*, *50*(1), pp.473-503.

26. Rueppel, M.L., Brightwell, B.B., Schaefer, J. and Marvel, J.T., 1977. Metabolism and degradation of glyphosate in soil and water. *J. Agric. Food Chem.*, *25*(3), pp.517-528.

27. Anton, F.A., Cuadra, L.M., Gutierrez, P., Laborda, E. and Laborda, P., 1993. Degradationsl behavior of the pesticides glyphosate and diflubenzuron in water. *Bull Environ Contam Toxicol*, *51*(6), pp.881-888.

28. Zaranyika, M.F. and Nyandoro, M.G., 1993. Degradation of glyphosate in the aquatic environment: An enzymic kinetic model that takes into account microbial degradation of both free and colloidal (or sediment) particle adsorbed glyphosate. *J. Agric. Food Chem.*, *41*(5), pp.838-842.

29. Assalin, M.R., De Moraes, S.G., Queiroz, S.C., Ferracini, V.L. and Duran, N., 2009. Studies on degradation of glyphosate by several oxidative chemical processes: Ozonation, photolysis and heterogeneous photocatalysis. *J. Environ. Sci. Health Part B*, *45*(1), pp.89-94.

30. Barrett, K.A. and McBride, M., 2005. Oxidative degradation of glyphosate and aminomethylphosphonate by manganese oxide. *Environ. Sci. Technol*., *39*(23), pp.9223-9228.

31. Dollinger, J., Dagès, C. and Voltz, M., 2015. Glyphosate sorption to soils and sediments predicted by pedotransfer functions. *Environ. Chem Letters*, *13*(3), pp.293-307.

32. Duke, S.O., Lydon, J., Koskinen, W.C., Moorman, T.B., Chaney, R.L. and Hammerschmidt, R., 2012. Glyphosate effects on plant mineral nutrition, crop

rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *J. Agric. Food Chem.*, *60*(42), pp.10375-10397.

33. Jacob, G.S., Schaefer, J., Stejskal, E.O. and McKay, R.A., 1985. Solid-state NMR determination of glyphosate metabolism in a Pseudomonas sp. *J. Biol. Chem.*, *260*(10), pp.5899-5905.

34. Moore, J.K., Braymer, H.D. and Larson, A.D., 1983. Isolation of a Pseudomonas sp. which utilizes the phosphonate herbicide glyphosate. *Appl. Environ. Microbiol.*, *46*(2), pp.316-320.

35. Dick, R.E. and Quinn, J.P., 1995. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. *Appl. Microbiol. Biotechnol.*, *43*(3), pp.545-550.

36. Liu, C.M., McLean, P.A., Sookdeo, C.C. and Cannon, F.C., 1991. Degradation of the herbicide glyphosate by members of the family Rhizobiaceae. *Appl. Environ. Microbiol.*, *57*(6), pp.1799-1804.

37. McAuliffe, K.S., Hallas, L.E. and Kulpa, C.F., 1990. Glyphosate degradation byAgrobacterium radiobacter isolated from activated sludge. J *Ind Microbiol Biotechnol.*, *6*(3), pp.219-221.

38. Pipke, R., Amrhein, N., Jacob, G.S., Schaefer, J. and Kishore, G.M., 1987.
Metabolism of glyphosate in an Arthrobacter sp. GLP-1. *Eur J Biochem.*, *165*(2), pp.267-273.

39. Pipke, R. and Amrhein, N., 1988. Degradation of the phosphonate herbicide glyphosate by Arthrobacter atrocyaneus ATCC 13752. *Appl. Environ. Microbiol.*, *54*(5), pp.1293-1296.

40. Obojska, A., Lejczak, B. and Kubrak, M., 1999. Degradation of phosphonates by streptomycete isolates. *Appl. Microbiol. Biotechnol*, *51*(6), pp.872-876.

41. Lane, M., Lorenz, N., Saxena, J., Ramsier, C., & Dick, R. P. (2012). The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. *Pedobiologia*, *55*(6), 335-342.

42. Lund-Hoie, K. and Friestad, H.O., 1986. Photodegradation of the herbicide glyphosate in water. *Bull Environ Contam Toxicol*., *36*(1), pp.723-729.

43. Carlisle, S.M. and Trevors, J.T., 1988. Glyphosate in the environment. *Water Air Soil Pollut*, *39*(3-4), pp.409-420.

44. Dinelli, G., 2000. Response to glyphosate and electrophoretic variation of Cynodon dactylon (L) Pers populations. *Pest Manag Sci: formerly Pesticide Science*, *56*(4), pp.327-335.

45. Bryson, C.T. and Wills, G.D., 1985. Susceptibility of bermudagrass (Cynodon dactylon) biotypes to several herbicides. *Weed Sci*, *33*(6), pp.848-852.

46. Westwood, J.H. and Weller, S.C., 1997. Cellular mechanisms influence differential glyphosate sensitivity in field bindweed (Convolvulus arvensis) biotypes. *Weed Sci*, pp.2-11.

47. Pratley, J., Urwin, N., Stanton, R., Baines, P., Broster, J., Cullis, K., Schafer, D., Bohn, J. and Krueger, R., 1999. Resistance to glyphosate in Lolium rigidum. I. Bioevaluation. *Weed Sci*, pp.405-411.

48. Powles, S. B., & Preston, C. (2006). Evolved glyphosate resistance in plants: biochemical and genetic basis of resistance. *Weed Technol*, *20*(2), 282-289.

49. Johnson, W.G., Davis, V.M., Kruger, G.R. and Weller, S.C., 2009. Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. *Eur J Agron*, *31*(3), pp.162-172.

50. PadgeUe, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M. and Fraley, R.T., 2018. New Weed Control Opportunities: Development of Soybeans with a Roundup Ready"'Gene. In *Herbicide-resistant crops* (pp. 65-100). CRC Press.

51. Vande Berg, B.J., Hammer, P.E., Chun, B.L., Schouten, L.C., Carr, B., Guo, R., Peters, C., Hinson, T.K., Beilinson, V., Shekita, A. and Deter, R., 2008. Characterization and plant expression of a glyphosate-tolerant enolpyruvylshikimate phosphate synthase. *Pest Manage. Sci: formerly Pesticide Science*, *64*(4), pp.340-345.

52. Pereira, J.L., Antunes, S.C., Castro, B.B., Marques, C.R., Gonçalves, A.M., Gonçalves, F. and Pereira, R., 2009. Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: commercial formulation versus active ingredient. *Ecotoxicology*, *18*(4), pp.455-463.

53. Haney, R.L., Senseman, S.A., Hons, F.M. and Zuberer, D.A., 2000. Effect of glyphosate on soil microbial activity and biomass. *Weed Sci.*, *48*(1), pp.89-93.

54. Correia, F.V. and Moreira, J.C., 2010. Effects of glyphosate and 2, 4-D on earthworms (Eisenia foetida) in laboratory tests. *Bull Environ Contam toxicol.*, *85*(3), pp.264-268.

55. García-Torres, T., Giuffré, L., Romaniuk, R., Ríos, R.P. and Pagano, E.A., 2014. Exposure assessment to glyphosate of two species of annelids. *Bull Environ Contam toxicol.*, *93*(2), pp.209-
214

56. Piola, L., Fuchs, J., Oneto, M.L., Basack, S., Kesten, E. and Casabé, N., 2013. Comparative toxicity of two glyphosate-based formulations to Eisenia andrei under laboratory conditions. *Chemosphere*, *91*(4), pp.545-551.

57. Gaupp-Berghausen, M., Hofer, M., Rewald, B. and Zaller, J.G., 2015. Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Sci. rep.*, *5*, p.12886.

58. Marc, J., Mulner-Lorillon, O. and Bellé, R., 2004. Glyphosate-based pesticides affect cell cycle regulation. *Biol. Cell*, *96*(3), pp.245-249.

59. Burlew, D.A., 2010. *The effects of pesticide-contaminated pollen on larval development of the honey bee, Apis mellifera* (Doctoral dissertation, Evergreen State College).

60. Neskovic, N.K., Poleksic, V., Elezovic, I., Karan, V. and Budimir, M., 1996. Biochemical and histopathological effects of glyphosate on carp, Cyprinus carpio L. *Bull Environl Contam Toxicol*, *56*(2), pp.295-302.

61. Richard, S., Moslemi, S., Sipahutar, H., Benachour, N. and Seralini, G.E., 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environ Health Persp*, *113*(6), p.716.

62. Koller, V.J., Fürhacker, M., Nersesyan, A., Mišík, M., Eisenbauer, M. and Knasmueller, S., 2012. Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch. Toxicol*., *86*(5), pp.805-813.

63. Gill, J.P.K., Sethi, N., Mohan, A., Datta, S. and Girdhar, M., 2017. Glyphosate toxicity for animals. *Environ. Chem Lett*, pp.1-26.

64. Bogdanova, A., Piunova, V., Berger, D., Fedorov, A.V. and Neckers, D.C., 2007. Synthesis and biological activity of photopolymerizable derivatives of glyphosate. *Biomacromolecules, 8*(2), pp.439-447.

65. Subramaniam, V. and Hoggard, P.E., 1988. Metal complexes of glyphosate. J. *Agric. Food Chem*., *36*(6), pp.1326-1329.

66. Abdullah, M.P., Daud, J., Hong, K.S. and Yew, C.H., 1995. Improved method for the determination of glyphosate in water. *J Chromatogr A* , *697*(1-2), pp.363-369.

67. Nedelkoska, T.V. and Low, G.C., 2004. High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethylchloroformate. *Anal. Chim. Acta*, *511*(1), pp.145-153.

68. Carlisle, S.M. and Trevors, J.T., 1988. Glyphosate in the environment. *Water Air Soil Poll*, *39*(3-4), pp.409-420.

69. Kataoka, H., Horii, K. and Makita, M., 1991. Determination of the herbicide glyphosate and its metabolite (aminomethyl) phosphonic acid by gas chromatography with flame photometric detection. *Agric Biol Chem., 55*(1), pp.195-198.

70. Kataoka, H., Ryu, S., Sakiyama, N. and Makita, M., 1996. Simple and rapid determination of the herbicides glyphosate and glufosinate in river water, soil and carrot samples by gas chromatography with flame photometric detection. *J Chromatogr A*, *726*(1-2), pp.253-258.

71. Kudzin, Z.H., Gralak, D.K., Drabowicz, J. and Łuczak, J., 2002. Novel approach for the simultaneous analysis of glyphosate and its metabolites. *J Chromatogr A* , *947*(1), pp.129-141.

72. Sancho, J.V., Hernández, F., López, F.J., Hogendoorn, E.A., Dijkman, E. and van Zoonen, P., 1996. Rapid determination of glufosinate, glyphosate and AMPA in environmental water samples using precolumn fluorogenic labeling and coupled-column liquid chromatography. *J Chromatogr A* , *737*, pp.75-83.

73. Sanchís, J., Kantiani, L., Llorca, M., Rubio, F., Ginebreda, A., Fraile, J., Garrido, T. and Farré, M., 2012. Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Anal. Bioanal. Chem*, *402*(7), pp.2335-2345.

74.Nedelkoska, T.V. and Low, G.C., 2004. High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate. *Anal. Chim. Acta*, *511*(1), pp.145-153.

75. Sun, Y., Wang, C., Wen, Q., Wang, G., Wang, H., Qu, Q. and Hu, X., 2010. Determination of glyphosate and aminomethylphosphonic acid in water by LC using a new labeling reagent, 4-methoxybenzenesulfonyl fluoride. *Chromatographia*, *72*(7-8), pp.679-686.

76. Qian, K., Tang, T., Shi, T., Wang, F., Li, J. and Cao, Y., 2009. Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3, 5-dinitrobenzotrifluoride. *Anal. Chim. Acta*, *635*(2), pp.222-226.

77. Bhaskara, B.L. and Nagaraja, P., 2006. Direct sensitive spectrophotometric determination of glyphosate by using ninhydrin as a chromogenic reagent in formulations and environmental water samples. *Helv. Chim. Acta*, *89*(11), pp.2686-2693.

78. Jan, M.R., Shah, J., Muhammad, M. and Ara, B., 2009. Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method. *J Hazard Mater*., *169*(1-3), pp.742-745.

79. Merás, I.D., Díaz, T.G. and Franco, M.A., 2005. Simultaneous fluorimetric determination of glyphosate and its metabolite, aminomethylphosphonic acid, in water, previous derivatization with NBD-Cl and by partial least squares calibration (PLS). *Talanta*, *65*(1), pp.7-14.

80. Jiang, J. and Lucy, C.A., 2007. Determination of glyphosate using off-line ion exchange preconcentration and capillary electrophoresis-laser induced fluorescence detection. *Talanta*, *72*(1), pp.113-118.

81. Wei, X., Gao, X., Zhao, L., Peng, X., Zhou, L., Wang, J. and Pu, Q., 2013. Fast and interference-free determination of glyphosate and glufosinate residues through electrophoresis in disposable microfluidic chips. *J Chromatogr A*, *1281*, pp.148-154.

82. Hu, J.Y., Chen, C.L. and Li, J.Z., 2008. A simple method for the determination of glyphosate residues in soil by capillary gas chromatography with nitrogen phosphorus. *J Anal Chem*, *63*(4), pp.371-375.

83. Zelenkova, N.F. and Vinokurova, N.G., 2008. Determination of glyphosate and its biodegradation products by chromatographic methods. *J Anal Chem* , *63*(9), pp.871-874.

84. Botero-Coy, A.M., Ibáñez, M., Sancho, J.V. and Hernández, F., 2013. Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. *J Chromatogr A*, *1292*, pp.132-141.

85. Druart, C., Delhomme, O., De Vaufleury, A., Ntcho, E. and Millet, M., 2011. Optimization of extraction procedure and chromatographic separation of glyphosate, glufosinate and aminomethylphosphonic acid in soil. *Anal. Bioanal. Chem*, *399*(4), pp.1725-1732.

86. Lundgren, L.N., 1986. A new method for the determination of glyphosate and (aminomethyl) phosphonic acid residues in soils. *J. Agric. Food Chem.*, *34*(3), pp.535-538.

87. Mao, M.K. and Franz, J.E., 1991. A facile general synthesis of thiocarboxylate S-esters of glyphosate and its derivatives. *Synthesis*, *1991*(11), pp.920-922.

88. Hensley, D.L., Beuerman, D.S.N. and Carpenter, P.L., 1978. The inactivation of glyphosate by various soils and metal salts. *Weed Res*, *18*(5), pp.287-291.

89. Sprankle, P., Meggitt, W.F. and Penner, D., 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. *Weed Sci*, *23*(3), pp.229-234.

90. Vereecken, H., 2005. Mobility and leaching of glyphosate: a review. *Pest Manag Sci*, *61*(12), pp.1139-1151.

91. Pearson, R.G., 1963. Hard and soft acids and bases. *J Am Chem Soc* , *85*(22), pp.3533-3539.

92. Sundaram, A. and Sundaram, K.M.S., 1997. Solubility products of six metal-glyphosate complexes in water and forestry soils, and their influence on glyphosate toxicity to plants. *J. Environ. Sci. Health. Part B, 32*(4), pp.583-598.

93. Glass, R.L., 1984. Metal complex formation by glyphosate. *J. Agric. Food Chem*., *32*(6), pp.1249-1253.

94. Sagatys, D.S., Dahlgren, C., Smith, G., Bott, R.C. and Willis, A.C., 2000. Metal complexes with N-(phosphonomethyl) glycine (glyphosate): The preparation and characterization of the group 2 metal complexes with glyphosate and the crystal structure of barium glyphosate dihydrate *Aust. J. Chem, 53*(2), pp.77-81.

95. Haney, R.L., Senseman, S.A., Hons, F.M. and Zuberer, D.A., 2000. Effect of glyphosate on soil microbial activity and biomass. *Weed Sci*, *48*(1), pp.89-93.

96. Roberts, C.W., Roberts, F., Lyons, R.E., Kirisits, M.J., Mui, E.J., Finnerty, J., Johnson, J.J., Ferguson, D.J., Coggins, J.R., Krell, T. and Coombs, G.H., 2002. The shikimate pathway and its branches in apicomplexan parasites. *J. Infect. Dis, 185*(Supplement_1), pp.S25-S36.

97. Clair, E., Linn, L., Travert, C., Amiel, C., Séralini, G.E. and Panoff, J.M., 2012. Effects of Roundup® and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. *Curr. Microbiol., 64*(5), pp.486-491.

98. Kurenbach, B., Marjoshi, D., Amábile-Cuevas, C.F., Ferguson, G.C., Godsoe, W., Gibson, P. and Heinemann, J.A., 2015. Sublethal exposure to commercial formulations of

the herbicides Dicamba, 2, 4-Dichlorophenoxyacetic acid, and Glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium. *MBio*, *6*(2), pp.e00009-15.

99. Bonnet, J.L., Bonnemoy, F., Dusser, M. and Bohatier, J., 2007. Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria Vibrio fischeri and the ciliate Tetrahymena pyriformis. *Environ Toxicol: An International Journal*, *22*(1), pp.78-91.

100. Richardson, J.T., Frans, R.E. and Talbert, R.E., 1979. Reactions of Euglena gracilis to fluometuron, MSMA, metribuzin, and glyphosate. *Weed Sci*, *27*(6), pp.619-624.

101. Estok, D., Freedman, B. and Boyle, D., 1989. Effects of the herbicides 2, 4-D, glyphosate, hexazinone, and triclopyr on the growth of three species of ectomycorrhizal fungi. *Bull Environ Contam Toxicol.*, *42*(6), pp.835-839.

102. Chakravarty, P. and Sidhu, S.S., 1987. Effect of glyphosate, hexazinone and triclopyr on in vitro growth of five species of ectomycorrhizal fungi. *Eur J Plant Patho 17*(4-5), pp.204-210.

103. Zobiole, L.H.S., Kremer, R.J., Oliveira Jr, R.S. and Constantin, J., 2011. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. *J. Appl. Microbiol.*, *110*(1), pp.118-127.

104. Newman, M.M., Hoilett, N., Lorenz, N., Dick, R.P., Liles, M.R., Ramsier, C. and Kloepper, J.W., 2016. Glyphosate effects on soil rhizosphere-associated bacterial communities.

Sci. Total Environ., *543*, pp.155-160.

105. Shehata, A.A., Schrödl, W., Aldin, A.A., Hafez, H.M. and Krüger, M., 2013. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. *Curr Microbiol*, *66*(4), pp.350-358.

106. Singh, B.K. and Walker, A., 2006. Microbial degradation of organophosphorus compounds. *FEMS Microbiol Rev 30*(3), pp.428-471.

107. Ternan, N.G., Mc Grath, J.W., Mc Mullan, G. and Quinn, J.P., 1998. Organophosphonates: occurrence, synthesis and biodegradation by microorganisms. World *J Microbiol Biotechno, 14*(5), pp.635-647.

108. Peñaloza-Vazquez, A., Mena, G.L., Herrera-Estrella, L. and Bailey, A.M., 1995. Cloning and sequencing of the genes involved in glyphosate utilization by Pseudomonas pseudomallei. *Appl. Environ. Microbio*l., *61*(2), pp.538-543.

109. Pipke, R. and Amrhein, N., 1988. Degradation of the phosphonate herbicide glyphosate by Arthrobacter atrocyaneus ATCC 13752. *Appl. Environ. Microbiol*., *54*(5), pp.1293-1296.

110. Sviridov, A.V., Shushkova, T.V., Zelenkova, N.F., Vinokurova, N.G., Morgunov, I.G., Ermakova, I.T. and Leontievsky, A.A., 2012. Distribution of glyphosate and methylphosphonate catabolism systems in soil bacteria Ochrobactrum anthropi and Achromobacter sp. *Appl. Microbiol. Biotechnol.*, *93*(2), pp.787-796.

111. Sviridov, A.V., Shushkova, T.V., Ermakova, I.T., Ivanova, E.V. and Leontievsky, A.A., 2014. Glyphosate: safety risks, biodegradation, and bioremediation. In *Current Environmental Issues and Challenges* (pp. 183-195). Springer, Dordrecht.

112. Pipke, R. and Amrhein, N., 1988. Degradation of the phosphonate herbicide glyphosate by Arthrobacter atrocyaneus ATCC 13752. *Appl. Environ. Microbiol., 54*(5), pp.1293-1296.F

113. Jacob, G.S., Garbow, J.R., Hallas, L.E., Kimack, N.M., Kishore, G.M. and Schaefer, J., 1988. Metabolism of glyphosate in Pseudomonas sp. strain LBr. *Appl. Environ. Microbiol , 54*(12), pp.2953-2958.

114. Daborn, P.J. and Le Goff, G., 2004. The genetics and genomics of insecticide resistance. *Trends Genet., 20*(3), pp.163-170.

115. Jennings, B.H., 2011. Drosophila–a versatile model in biology & medicine. *Mater. Today*, *14*(5), pp.190-195.

116. de Aguiar, L.M., Figueira, F.H., Gottschalk, M.S. and da Rosa, C.E., 2016. Glyphosate-based herbicide exposure causes antioxidant defence responses in the fruit fly

Drosophila melanogaster. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*., *185*, pp.94-101.

117. Kaya, B., Creus, A., Yanikoğlu, A., Cabré, O. and Marcos, R., 2000. Use of the Drosophila wing spot test in the genotoxicity testing of different herbicides. *Environ. Mol. Mutagen, 36*(1), pp.40-46.

118. Kale, P.G., Petty Jr, B.T., Walker, S., Ford, J.B., Dehkordi, N., Tarasia, S., Tasie, B.O., Kale, R. and Sohni, Y.R., 1995. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ. Mol. Mutagen, 25*(2), pp.148-153.

119. Datta, S., Singh, J., Singh, S. and Singh, J., 2016. Earthworms, pesticides and sustainable agriculture: a review. *Environ Sci Pollut Res Int*, *23*(9), pp.8227-8243.

120. Domínguez, A., Brown, G.G., Sautter, K.D., De Oliveira, C.M.R., De Vasconcelos, E.C., Niva, C.C., Bartz, M.L.C. and Bedano, J.C., 2016. Toxicity of AMPA to the earthworm Eisenia andrei Bouché, 1972 in tropical artificial soil. *Sci. rep, 6*, p.19731.

121. Casabé, N., Piola, L., Fuchs, J., Oneto, M.L., Pamparato, L., Basack, S., Giménez, R., Massaro, R., Papa, J.C. and Kesten, E., 2007. Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *J. Soils Sediments, 7*(4), pp.232-239.

122. Yasmin, S. and D'Souza, D., 2007. Effect of pesticides on the reproductive output of Eisenia fetida. *Bull Environ ContamToxicol*, *79*(5), pp.529-532.

123. García-Torres, T., Giuffré, L., Romaniuk, R., Ríos, R.P. and Pagano, E.A., 2014. Exposure assessment to glyphosate of two species of annelids. *Bull Environ Contam Toxicol*, *93*(2), pp.209-214.

124. Santadino, M., Coviella, C. and Momo, F., 2014. Glyphosate sublethal effects on the population dynamics of the earthworm Eisenia fetida (Savigny, 1826). *Water Air Soil Pollu*t, *225*(12), p.2207.

125. Santos, M.J.G., Ferreira, M.F.L., Cachada, A., Duarte, A.C. and Sousa, J.P., 2012. Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of Folsomia candida and Eisenia andrei. *Ecotoxicology*, *21*(8), pp.2113-2122.

126. Zhou, C.F., Wang, Y.J., Yu, Y.C., Sun, R.J., Zhu, X.D., Zhang, H.L. and Zhou, D.M., 2012. Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm Eisenia fetida?. *Ecotoxicology*, *21*(8), pp.2297-2305.

127. Verrell, P. and Van Buskirk, E., 2004. As the worm turns: Eisenia fetida avoids soil contaminated by a glyphosate-based herbicide. *Bull Environ Contam Toxicol*, *72*(2), pp.219-224.

128. Zaller, J.G., Heigl, F., Ruess, L. and Grabmaier, A., 2014. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Sci rep*, *4*, p.5634.

129. Bariuan, J.V., Reddy, K.N. and Wills, G.D., 1999. Glyphosate injury, rainfastness, absorption, and translocation in purple nutsedge (Cyperus rotundus). *Weed Technol*, pp.112-119

130. Claus, J.S. and Behrens, R., 1976. Glyphosate translocation and quackgrass rhizome bud kill. *Weed Sci*, *24*(2), pp.149-152.

131. Blum, R.R., Isgrigg, J. and Yelverton, F.H., 2000. Purple (Cyperus rotundus) and yellow nutsedge (C. esculentus) control in bermudagrass (Cynodon dactylon) turf. *Weed Technol*, *14*(2), pp.357-365.

132. Devlin, R.M., Karczmarczyk, S.J., Zbiec, I.I. and Koszanski, Z.K., 1986. Initial and residual activity of glyphosate and SC-0224 in a sandy soil. *Crop Prot*, *5*(4), pp.293-296.

133. Balah, M.A., Zidan, Z.H., Dahroug, A.S. and Abdel-Rahman, A.G., 2006. Response of Cyperus rotundus weeds to glyphosate herbicide with some adjuvants under greenhouse conditions. *Journal of Agricultural Science of Mansoura University*, *31*(3), pp.1653-1667.

134. Zandstra, B.H. and Nishimoto, R.K., 1977. Movement and activity of glyphosate in purple nutsedge. *Weed Sci*, *25*(3), pp.268-274.

135. Khan, H., Marwat, K.B., Hassan, G. and Khan, M.A., 2012. Chemical control of Parthenium hysterophorus L. at different growth stages in non-cropped area. *Pak J Bot* , *44*(5), pp.1721-1726.

136. Norsworthy, J.K., Burgos, N.R. and Oliver, L.R., 2001. Differences in weed tolerance to glyphosate involve different mechanisms. *Weed Technol*, *15*(4), pp.725-731.

137. Abu-Irmaileh, B.E. and Jordan, L.S., 1978. Some aspects of glyphosate action in purple nutsedge (Cyperus rotundus). *Weed Sci*, *26*(6), pp.700-703.

138. Wang, C.Y., 2001. Effect of glyphosate on aromatic amino acid metabolism in purple nutsedge (Cyperus rotundus). *Weed Technol*, *15*(4), pp.628-635.

139. Gill, J.P.K., Sethi, N., Mohan, A. and Sharma,A., 2018. A novel method for the extraction of glyphosate from its commercially available formulation. *AASCIT Journal of Environment*, *3(1)*, pp.18-23.

140. Hosangadi, B.D. and Dave, R.H., 1996. An efficient general method for esterification of aromatic carboxylic acids. *Tetrahedron lett.*, *37*(35), pp.6375-6378.

141. Vaughan Jr, J.R., 1951. Acylalkylcarbonates as acylating agents for the synthesis of peptides. *J. Am. Chem. Soc. 73*(7), pp.3547-3547.

142. Chen, F.M. and Benoiton, N.L., 1987. The preparation and reactions of mixed anhydrides of N-alkoxycarbonylamino acids. *Can. J. Chem.*, *65*(3), pp.619-625.

143. Tarbell, D.S., Yamamoto, Y. and Pope, B.M., 1972. New method to prepare Nt-butoxycarbonyl derivatives and the corresponding sulfur analogs from di-t-butyl dicarbonate or di-t-butyl dithiol dicarbonates and amino acids. *Proc. Natl. Acad. Sci.*, *69*(3), pp.730-732.

144. Solomon, V.R., Puri, S.K., Srivastava, K. and Katti, S.B., 2005. Design and synthesis of new antimalarial agents from 4-aminoquinoline. *Bioorganic Med. Chem*, *13*(6), pp.2157-2165.

145. Lundt, B.F., Johansen, N.L., Vølund, A. and Markussen, J., 1978. Removal of t-Butyl and t-Butoxycarbonyl Protecting Groups with trifluoroacetic acid: Mechanisms, Biproduct Formation and Evaluation of Scavengers. *Int J Pept* Protein *Res.*, *12*(5), pp.258-268.

146. Polshettiwar, V. and Kaushik, M.P., 2004. A new, efficient and simple method for the thionation of ketones to thioketones using P4S10/Al2O3. *Tetrahedron lett.*, *45*(33), pp.6255-6257.

147. Polshettiwar, V. and Kaushik, M.P., 2006. Alumina encapsulated phosphorus pentasulfide (P4S10/Al2O3) mediated efficient thionation of long chain amides. *Tetrahedron lett.*, *47*(14), pp.2315-2317.

148. Subramaniam, V. and Hoggard, P.E., 1988. Metal complexes of glyphosate. *J. Agric. Food Chem.*, *36*(6), pp.1326-1329.

149. Shabbir, A., 2014. Chemical control of Parthenium hysterophorus L. . *Pak. J. Weed Sci. Res*, *20*(1).

150. Lichtenthaler, H.K., 1987. [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol*, *148*, pp. 350-382. Academic Press.

151. Manolopoulou, E., Varzakas, T. and Petsalaki, A., 2016. Chlorophyll Determination in Green Pepper Using two Different Extraction Methods. *Current Research in Nutrition and Food Science Journal*, *4*(Special Issue Carotenoids March 2016), pp.52-60.

152. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* , *45*(4_ts), pp.493-496.

153. Fan, J., Yang, G., Zhao, H., Shi, G., Geng, Y., Hou, T. and Tao, K., 2012. Isolation, identification and characterization of a glyphosate-degrading bacterium, Bacillus cereus CB4, from soil. *J Gen Appl Microbiol*, *58*(4), pp.263-271.

154. Zou, S., Meadows, S., Sharp, L., Jan, L.Y. and Jan, Y.N., 2000. Genome-wide study of aging and oxidative stress response in Drosophila melanogaster. *Proc. Natl. Acad. Sci.*, *97*(25), pp.13726-13731.

155. Oecd, 1994. *OECD Guidelines for the Testing of Chemicals*. Organization for Economic.

156. ISO,Standard number No. 11268-1. International Standard Organization, Geneva. 1993.

157. Kobyłecka, J., Ptaszyński, B. and Zwolinńska, A., 2000. Synthesis and properties of complexes of lead (II), cadmium (II), and zinc (II) with N-phosphonomethylglycine. *Monatsh Chem Chem Mon*, *131*(1), pp.1-11.

158. Hampton, C., Demoin, D. and Glaser, R.E., 2010. Vibrational spectroscopy tutorial: sulfur and phosphorus. *Organic Spectroscopy*.

159. Heacock, R.A. and Marion, L., 1956. The infrared spectra of secondary amines and their salts. *Can. J. Chem*, *34*(12), pp.1782-1795.

160. Sreenivasulu, M., Ramesh, P. and Damodharam, T., 2015. Effect of glyphosate on chlorophyll and carotenoids in weed species (Parthenium hysterophorus L. and Cyperus rotundus L.). *International Journal of Advanced Scientific and Technical Research*, *5*(4), pp.116-123.

161. Krenchinski, F.H., Albrecht, L.P., Albrecht, A.J.P., Cesco, V.J.S., Rodrigues, D.M., Portz, R.L. and Zobiole, L.H.S., 2017. Glyphosate affects chlorophyll, photosynthesis and water use of four Intacta RR2 soybean cultivars. *Acta Physiol Plant, 39*(2), pp.63.

162. Reddy, K.N., Hoagland, R.E. and Zablotowicz, R.M., 2001. Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (Glycine max) varieties. *Journal of New Seeds*, *2*(3), pp.37-52.

163. Kitchen, L.M., Witt, W.W. and Rieck, C.E., 1981. Inhibition of chlorophyll accumulation by glyphosate. *Weed Sci*, *29*(4), pp.513-516.