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Bioaccumulation Of Herbicide, Butachlor® In Bean Leaves At 6 Weeks After Application At Pre-Emergence

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Abstract: *The over-use and/or indiscriminate application of herbicides by most rural farmers in Nigeria are major factors that contribute to bioaccumulation of chemical agents and environmental contamination in the society. Butachlor® (N-butoxymethyl-2-chloro-2, 6-diethylacetamide), a systemic herbicide is widely used to control weeds in different parts of Nigeria.Bioaccumulation of the herbicide,butachlor® in beans (Phaseolus vulgaris) leaves was evaluated at 6 weeks following cultivation of the crops and application of the herbicide at pre-emergence.A normal or recommended concentration of 260 mg/m^{2}of the herbicide, anda 12% and 24% increase (290 and 320 mg/m^2 respectively) above the normal concentration were separately applied to the experimental legume crops planted in different plots. Gas chromatography and mass spectrometry (GC-MS) were used to determine the presence and also the concentration of bioaccumulatedherbicide in the cultivated bean leaves at6 weeks. The control beans without exposure to herbicide had no trace of butachlor® detected in the leaves but the test beans which were treated with 260mg/m^2 (recommended concentration) had 0.10 ppm while 290 and 320 mg/m^2 induced a correspondingly increased bioaccumulation value of 0.13 and 0.20ppm.Butachlor® was found to havebioaccumulated minimally in beans forages at 6 weeks but the herbicide residues became accumulated with increased use of concentrations above the recommended application rate.*

Keywords: *Bioaccumulation, Herbicide Butachlor, Chromatography, Contaminant*

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I. Introduction

Modern agricultural crop production targeting bumper output reliesheavily on considerableapplication of herbicides to control weeds. In Europe alone, 140,000 tons of pesticides are sprayed on farm lands each year with fruits and vegetables being the most contaminated [1, 2].Over a span of three years (2009-2100), an average of 4,277 gallons of Gallery 75®, a selective pre-emergent broadleaf herbicide with isoxaben 75% as the active ingredient was applied per year to crops in Northern Arizona University campus [3]. In Nigeria however, the annual herbicide application rate is not closely monitored but there is evidence of a staggering increase in the use of herbicides even among rural communities. The ever-growing involvement of rural farmers with inadequate expertise encourages incessant and repeated application of herbicides thereby enhancing bioaccumulation of chemical agents in the environment. Herbicides do not only contaminate water, soil and air but may also concentrate in edible crops above the maximum residue limits.

Butachlor®containing N-butoxymethyl-2-chloro-2,6-diethylacetamidedeveloped byMonsanto Company, USA is a popular selective systemic herbicide in most rural settings and widely acknowledged for its effectiveness in Nigeria. It is used as a pre-emergent herbicide for the control of broadleaf weeds and annual grasses in rice fields, wheat, cotton, vegetable and leguminous crops. Ground water fromseveral tube wells adjacent to rice fields in Philippines were once contaminated up to a level of 0.163 ppb with Butachlor® [4].

The present research sought to assess bioaccumulation of butachlorin bean leaves at 6 weeks following application at pre-emergence.

II. Materials and Methods

2.1. Reagents and chemicals

Herbicide butachlor (98.4%purity) purchased from Monsanto, USA,analytical grade acetone and n-hexane obtained from Sigma Aldrich,USA and anhydrous sodium sulphate (BDH,England) were used.

2.2. Plant Material

Bean seeds (*Phaseolus vulgaris)* were sourcedlocally within Nsukka municipality, Enugu State, Nigeria.

2.3. Field Location and soil analysis

The Field area for the experiment was conducted at the Research farm of the Department of Crop science, University of Nigeria, Nsukka (UNN) for a period of eight (8)weeks. The geographical coordinates of the Research farm were appropriately determined using a compass. Soil analysis and characterization was carried out in the Department of Soil Science, UNNwithBouyoucous Hydrometer method [5]. One hundred grammes (100g) of soil were collected from a depth of 0-15cm within the planting site for the investigation.The physicochemical properties of the soil were determined in line with standard procedures.

2.4. Field study and application of herbicide, butachlor®

Beds constructed each with $10m^2$ dimension and a 0.5m distance maintained between the beds had 1m furrow spacing. A plant spacing of 75cm between rows with a 50cm within rows was adopted for the bean planting. The experimental site for cultivation of the beans was delineated according to varying herbicide treatment plots as T_1, T_2, T_3 andT_4. The crops planted on Plot T_1served as the control and were not exposed to the herbicide; those on plot T_2 were treated with the recommended (normal) herbicide Butachlor® active ingredient rate of 260 mg/m^2 at pre-emergence, crops planted on T_3 were exposed to 290 mg/m^2 (12% increase above normal) of the herbicidewhile beans on PlotT_4 were treated with 320mg/m^2(24% increase above normal) respectively. The normal herbicide application rate for butachlor® in beans is 260 mg/$m^2$of the active ingredient/haatpre-emergence[6]. The cultivated beans wereallowed to growth for 6 weeks before their leaves were subjected to analysis for bioaccumulation of the herbicide.

2.5. Plant Extraction for analysis of bioaccumulation of the herbicide

Procedures to assess bioaccumulation of the herbicide were initiated at 6 weeks following cultivation of the beans. Fresh leaves weighing 20 g from each of the experimental bean crops in Plots were harvested, washed with deionized water, chopped into smaller particles and blended with 10 ml deionized water.Extraction of the herbicide from the plant leaves was carried out by solvent extraction method using acetone/n-hexane at a ratio of 1:1. A volume (200ml) of the solvent mixture (acetone and hexane) was added, shaken for 3minutes and then filtered through a 10g anhydrous sodium sulphatein a funnel. Filtrates were centrifuged and the supernatant removed before the extract was concentrated in a rotary evaporator [7].

2.6.Analysis of the extracts from the experimental bean leaves for bioaccumulation of the herbicideButachlor®

Analysis of the separate extract of the experimental bean leaves from the crops planted on Plots T_1, T_2, T_3 andT_4 at 6 weeks was carried out with gas chromatography/mass spectrometry (GC-MS). Beans from T_1 (control) had no exposure to herbicide, T_2 were treated with normal concentration of the herbicide active ingredient (260 mg/m^2); T_3 were exposed to 290 mg/m^2 and T_4 were treated 320 mg/m^2 at pre-emergence respectively. Standard stock solutions of n-hexane and working solutions of the extract at varying concentration ranges, 0-2mg/l were separately prepared.The working solution was prepared by dissolving each extract in an appropriate volume of hexane. Then a volume, 1.0 µl each of the standard and sample were injected into the Thermo-Scientific Trace GC coupled to DSQ 11 mass spectrometer equipped with an AS 3000 auto sampler and a split/split–less injector. The column used was a TR-5MS(30×0.25 mm i.d.), 0.25m d.f coated with 5% diphenyl-95% polydimethylsiloxane operated with pre-determined oven temperature and time settings. Initial temperature was 140^{o}C/min, increasing temperature: 8^{o}C/min,and final temperature: 300^{o}C held for 5 min. Injection temperature and volume was 250^{o}C and 1.0 µl respectively; injection mode: split/split 15:1; Carrier gas: helium at 30cm/s; linear velocity and inlet pressure 99.8KPa, detector temperature was 280^{o}C. Components of the standards and each sample were detected on the basis of their retention indices and confirmed by comparison of spectra values with reference compoundspectra from the library of the National Institute of Standard and Technology (NIST) database [8]. The concentration of bioaccumulated herbicide in sampled extracts from the different experimental bean leaves was determined by extrapolation from the standard curve generated by graded concentrations of the herbicide, butachlor®.

2.7. Statistical Analysis

All data obtained were subjected to One-way analysis of variance (ANOVA) andDuncan new multiple range post hoc test. Differences at $p < 0.05$ were considered significant; values were expressed as mean ± SE.

III. Results

3.1. Field Location and soil analysis

The Research farm inNsukka Campus of the University is located within longitude 07° 29N latitude 06 51E and 400 miles above sea level. The soil sample textural class from the field area was found to be sandy clay loam. It had a total sand particle size of 74%, clay 19.3% and silt 7%. The organic matter content, organic carbon and cation exchange capacity were 1.86%, 1.08% and 10.80 mol/100 kg respectively while the soil pH was 5.4 (Table 1).

Table 1: The physicochemical properties of soil sample from the field area.

Properties	**Description and values**
Soil textural class	Sandy clay loam
Soil particle size (%)	Total sand (74%), clay (19.3%), silt (7%)
Organic matter	1.86%
Organic carbon	1.08%
Soil pH	5.4
Cation exchange capacity	10.80 mol/100 kg

3.2. Analysis of extracts from beans leaves for bioaccumulation of the herbicide,Butachlor®

The findings showed thatbeans leaves forage from Plot T_1 (control) had 0 or no bioaccumulated herbicide; forages from Plot T_2 , T_3 and T_4 exposed to 260, 290 and 320 mg/m^2 of the herbicide,butachlor®had 0.10, 0.13 and 0.20 ppm amounts of bioaccummulated herbicide respectively.

3.2.1. Butachlor® standard

Aretention time of 14.96 min.for butachlor® elution with the peak at 15.04 min, relative abundance and molecular structure were observed (Fig. 1a-c).

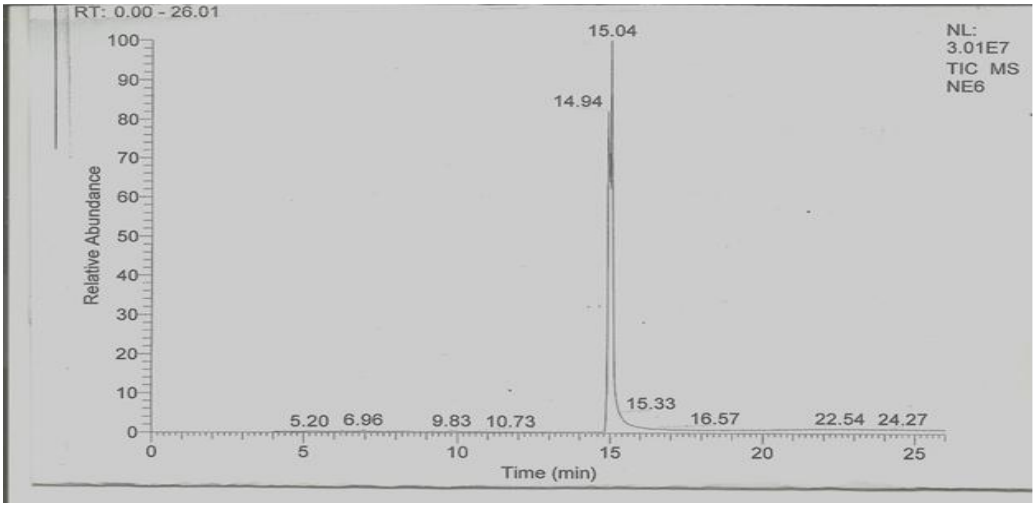

Figure 1a: Chromatogram of Butachlor® standard showing the retention time for elution of the herbicide at 14.94 min with the peak observed at 15.04 min.

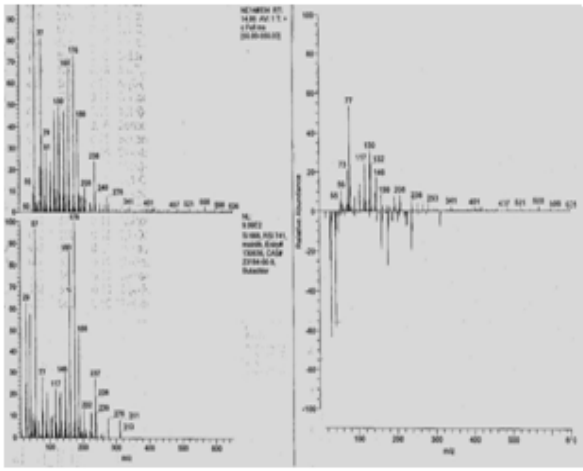

Figure 1b: Mass Spectrometry of the standard.

Fig 1c: Molecular structure of the standard

3.2.2. Extract from control bean leaves (Plot T_1)

Bioaccumulation of butachlor® was not detected; the retention time range of the standard (14.94-15.04 min) was not observed in the chromatogram (Fig. 2).

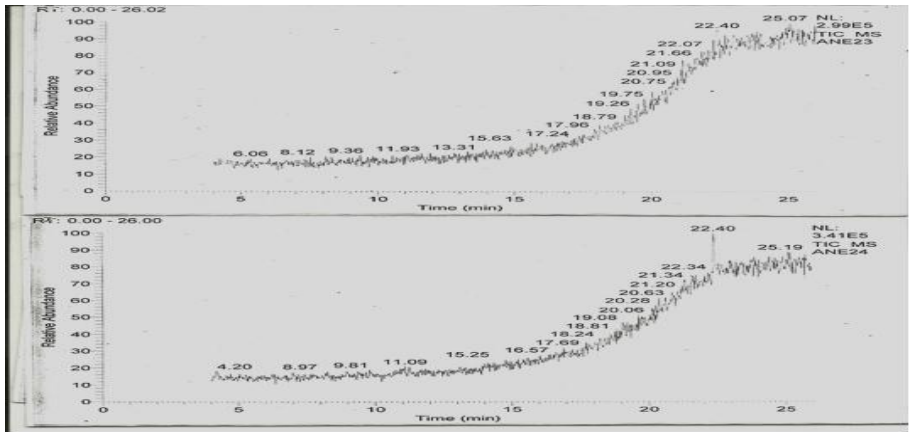

Figure 2: Extract from leaves of control beans without exposure to herbicide in Plot T_1; retention time range of the standards (14.94 -15.04 min.) was not observed, hencebutachlor® was not detected.

3.2.2. Extract from bean leaves treated with recommended herbicide concentration (260 mg/m^2) in Plot T_2

The chromatogram showed a retention time of 14.53 min and a spectrum with minimal concentration of 0.10 ppm bioaccumulated herbicide,butachlor® (Fig. 3a and 3b).

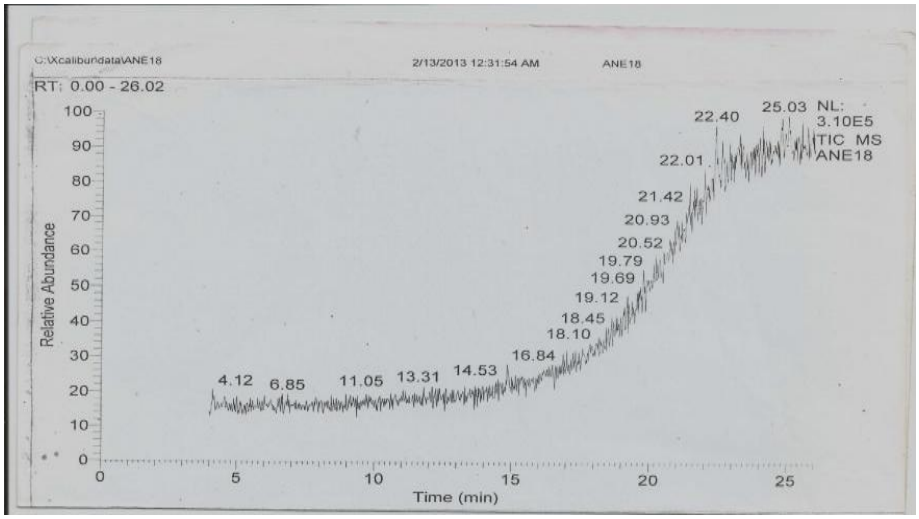

Figure 3a: Chromatogram of leaf extracts from beans treated with 260 mg/m^{2}in Plot T_2; Retention time of detected butachlor® was 14.53min.

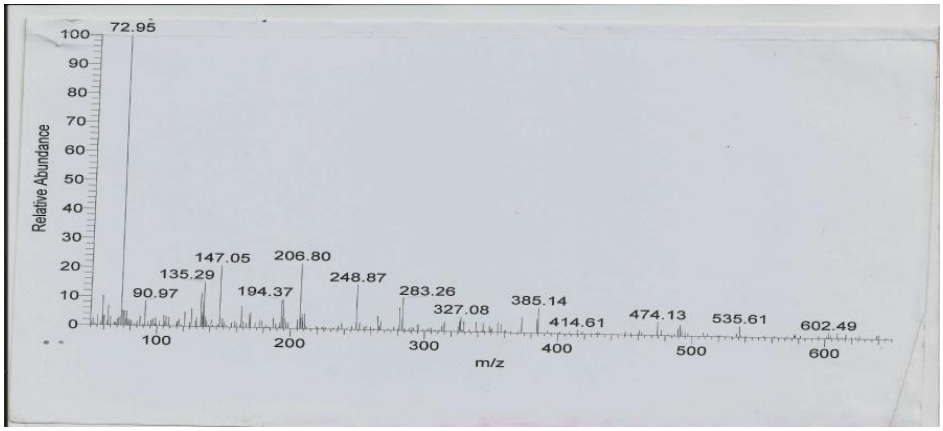

Figure 3b: Mass spectrum of beans leaf extract from Plot T_2 treated with herbicide,butachlor® at 260 mg/m^2

3.2.2. Extract of leaves from bean crops treated with 12% increase above herbicide recommended concentration (290 mg/m^2) on Plot T_3

The chromatogram showed a retention time of 14.86 min and a spectrum with relative abundance of 0.13 ppm of bioaccumulated herbicide,butachlor® (Fig. 4a and 4b).

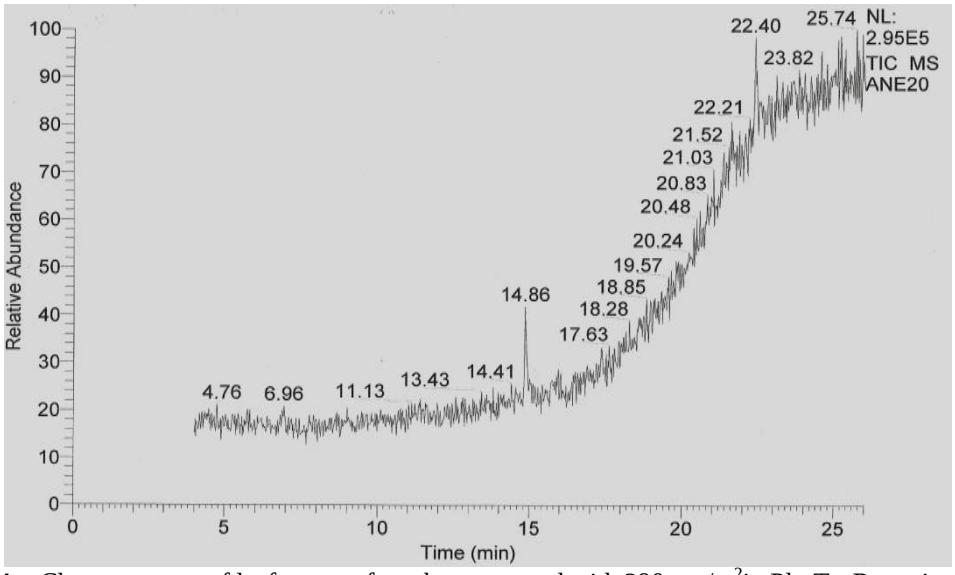

Figure 4a: Chromatogram of leaf extractsfrom beans treated with 290 mg/m^{2}in PlotT_3; Retention time of detected butachlor® was 14.86 min.

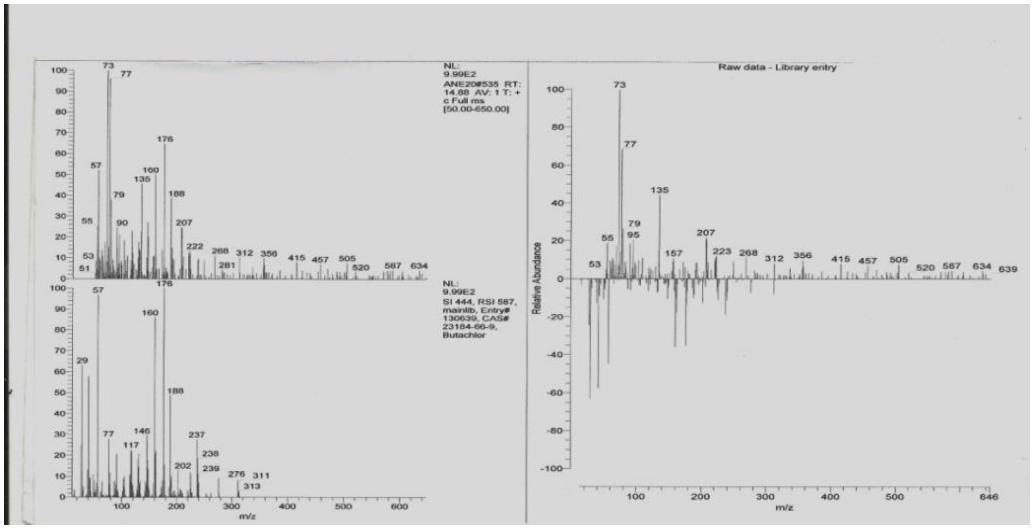

Figure 4b: Mass spectrum of beans leaf extract from Plot T_3 treated with herbicide,butachlor® at 290 mg/m^2

3.2.3. Extract of leaves from bean crops treated with 24% increase above the recommended application rate of the herbicide (320 mg/m^2) on Plot T_4

The chromatogram showed a retention time of 14.84 min and a spectrum with relative abundance of 0.20 ppm of bioaccumulated herbicide,butachlor® (Fig. 5a and 5b).

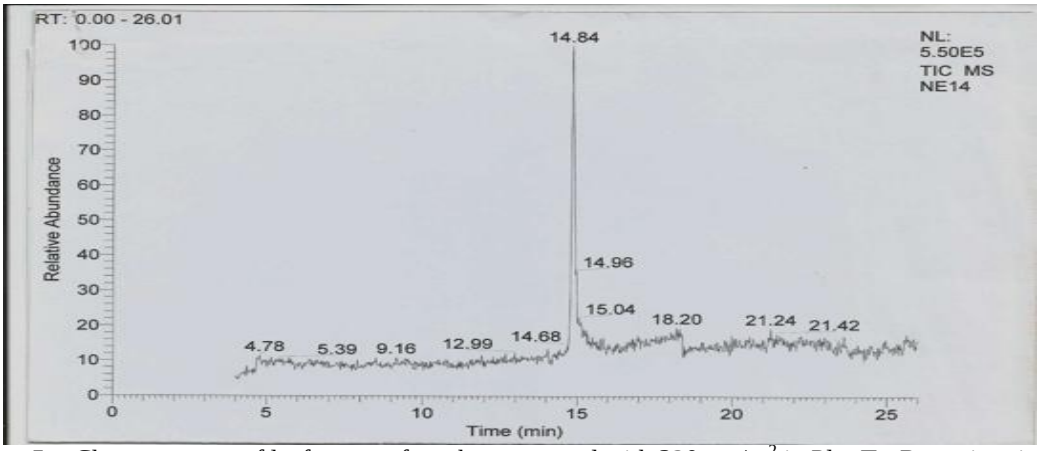

Figure 5a: Chromatogram of leaf extractsfrom beans treated with 320 mg/m^2 in Plot T_4; Retention time of detected butachlor® was 14.84 min.

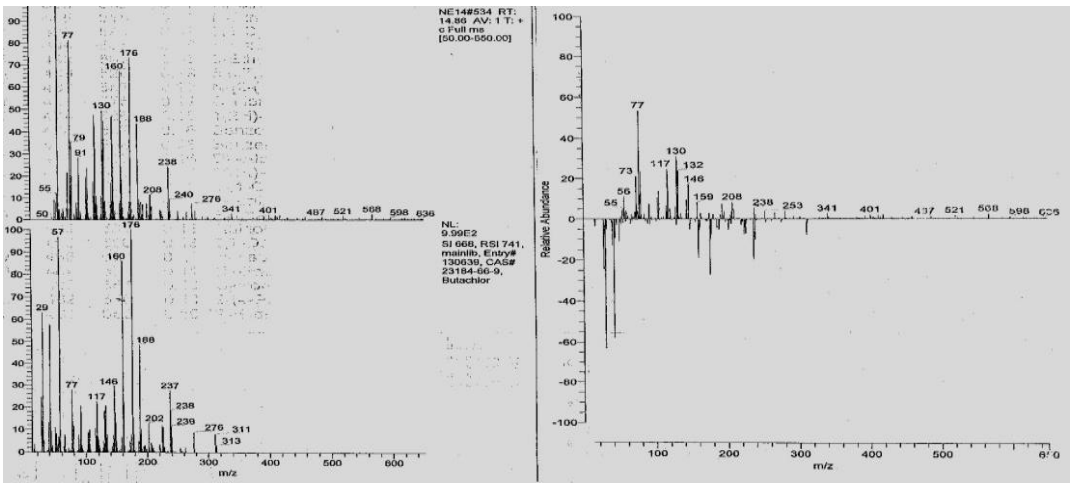

Figure 5b: Mass spectrum of beans leaf extract from Plot T_4 treated with herbicide,butachlor® at 320 mg/m^2

IV. Discussion

The study found that application of herbicide,butachlor® at recommended concentration induced a minimal level (0.10 ppm) of bioaccumulation which may be within the tolerant or acceptable residue limit in bean leaves at 6 weeks. The herbicide residues however became appreciably elevated with increase above the normal or recommended application rate. It becomes apparent that repeated and extra-label use of the herbicide,butachlor® could compound incidence of bioaccumulation of chemical agents in the environment and the potential risks associated with it. Again, the sampled soil acidic pH of 5.4 could also have affected the chemical and/or microbial degradation of the herbicide leading to enhanced persistence in the soil. Herbicide, Butachlor® degrades rapidly but under certain conditions of low temperature, and moisture with high alkalinity and absence of suitable microbial degraders, remains biologically active and persists in soils for a long time [9,10].Toxic herbicides are routinely translocated from the roots through the stem to bioaccumulate in leaves and fruits which are eaten by man and animals [11, 12].

V. Conclusion

Herbicide, butachlor® applied to cultivated beans during cultivation has potential to be absorbed and translocated through the roots to become concentrated in the legume leaves at 6 weeks. The bioaccumulation ability of the herbicide correlates with increased duration after use and the application rate above the recommended concentration.

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