INTERNATIONAL JOURNAL OF RESEARCH CULTURE SOCIETY
Monthly Peer-Reviewed, Refereed, Indexed Journal
Volume - 7, Issue - 12, December - 2023
ISSN(O): 2456-6683
[ Impact Factor: 6.834 ]
Publication Date: 31/12/2023
IJRCS

DOIs:10.2017/IJRCS/202312019 --:-- Research Paper / Article / Review

# Evaluating the safety of herbicide by bioassay techniques: A review

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***Abstract:*** *An incredible amount of herbicide is used in agroecosystems to manage weeds, causing great public concern about the negative impacts on the environment. Herbicides can threaten agricultural safety and influence water and soil resources, human and animal health, non-target plants, and ecosystems. Herbicide residue means any specified substances in soil, agricultural commodities, food, etc. resulting from the use of an herbicide. The term includes any derivatives of a herbicide, such as by-products, metabolites, reaction products, and impurities considered to be of toxicological significance. Herbicide residues in soils are undesirable mainly because they may injure sensitive crops in a cropping system, so restrict the choice of crops in a particular cropping system. Herbicides that are persistent include triazines, phenylureas, sulfonylureas, dinitroanilines, and imidazolinones. The gauge by which we can predict herbicide persistence is the half-life of the herbicide. Bioassays are experiments that use living things to test the toxicity of chemicals. Bioassays are used to determine if herbicide residues are present in soil at a high enough concentration to adversely affect plant growth. Cucumber, sorghum, mustard, soybean, oat, and minor millets are some commonly used indicator plants in herbicide bioassays. Herbicide residue analysis can be carried out using field bioassay, pot bioassay, and analytical methods (i.e., Gas chromatography and High performance liquid chromatography). Bioassays offer several advantages, such as being cost-effective, sensitive, and ecologically relevant, making them valuable tools for monitoring herbicide contamination and assessing the environmental impact of herbicides. When bioassay techniques are used in conjunction with other analytical methods, bioassays provide a comprehensive approach to assess herbicide contamination and support informed decision-making for environmental management and agricultural practices. Continued research and development in bioassay techniques are essential to enhance their sensitivity, specificity, and reliability. By addressing these challenges, scientists can further improve the accuracy of herbicide residue analysis and contribute to better understanding the potential risks associated with herbicide contamination.*

***Key Words:*** *Herbicide, bioassay techniques, pot, field, soil residual life, persistence, agricultural safety*

## 1. INTRODUCTION:

Today, an incredible number of agrochemicals (herbicides, insecticides, fungicides, nematicides, *etc*.) are used in agroecosystems to manage weeds and other pests. This extensive application of pesticides causes great public concern about the negative impacts on the environment and human health. In the current scenario, approximately 4 million tons of pesticides are used annually worldwide in agroecosystems, of which 40% are herbicides, 30% are insecticides, and 20% are fungicides [1]. The chemicals used for killing or inhibiting the growth of plants are known as herbicide. Herbicides use harmful chemicals that can be detrimental to the health of the crop and soil and are toxic to humans. More than 90% of herbicides are misused, and as a result, they are lost in the environment, do not reach the target site, and do not effectively reduce weeds in crops [2]. Now a days, several incidents have been reported about the negative impacts of herbicides and their residues on different crops like cereals, oilseeds, vegetables, *etc*. Herbicide when applied

to soil to control weeds should not remain in soil for long period. The length of time an herbicide remains active in soil is called 'soil persistence' or 'soil residual life'. Herbicides, as the most commonly used pesticides, can threat agricultural safety and influence water and soil resources, human and animal health, food safety, non-target plants, and ecosystem function and structure [3]. The negative impact of herbicide residues could be categorized as inhibition of seed germination, reduction of dry weight, destruction of plants, *etc*., but residual herbicides provide an extended of time for weed control. Bioassay and analytical methods are used for herbicide residue analysis.

**2. Persistence and residue of herbicides :**

A herbicide is said to be persistent if it is present in the soil in its original or closely related form but in phytotoxic forms even after its mission is accomplished [4, 5], and the quantity that exists is referred to as residue. Herbicide families that are persistent include triazines, uracils, phenylureas, sulfonylureas, dinitroanilines, isoxazolidinones, imidazolinones, and certain plant growth regulators belonging to the pyridine family [6].

**Table 1: Relative persistence of some herbicides in soil**

| < 1 months | 1-3 months | 3-6 months | > 6 months |
|---|---|---|---|
| 2,4-D, Glyphosate, MCPA | Alachlor, Acetochlor, Bispyribac-sodium, Butachlor, Fluazifop-butyl, Halosulfuron, Metribuzin, Metsulfuron-methyl, Metolachlor, Oxyflurofen | Clomazone, Fluchloralin, Imazethapyr, Linuron, Oxadiazon, Pendimethalin | Atrazine, Diuron, Diquat, Picloram, Trifluralin, Simazine, Paraquat |

Source: [4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15]

**Half-life of herbicide**

The gauge by which we can predict herbicide persistence is the half-life of the herbicide. Herbicide half-life is a measure of how long it takes for 50% of a chemical to degrade. the half-life of different herbicides is furnished in Table 2.

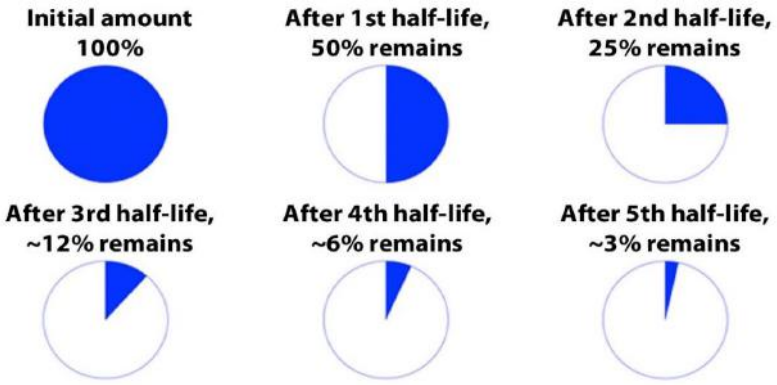


**Fig. 1: Approximate amount of herbicide remaining at the application site over time**

**Table 2: Half-life of different herbicides** [5]

| Herbicide | Half-life (Days) | Herbicide | Half-life (Days) |
|---|---|---|---|
| Atrazine | 13-58 | Metribuzin | 23-49 |
| Butachlor | 5-24 | Metolachlor | 8-27 |
| Fluazifop-p-ethyl | 8-24 | Oxyflurofen | 12-29 |
| Dithiopyr | 11-25 | Pendimethalin | 15-77 |
| Imazethapyr | 57-71 | Pretilachlor | 10-11 |
| Isoproturon | 13-21 | Sulfosulfuron | 3-27 |
| Chlorsulfuron | 31-93 | 2,4-D | 7-22 |
| Chlorimuron | 60 | Metsulfuron-Methyl | 70-147 |
| Flufenacet | 9-22 | Thiobencarb | 19-24 |
| Fluchloralin | 12-46 | Pyrazosulfuron | 16-21 |

## 3. BIOASSAY :

A bioassay or biological assay defined as an experiment for estimating the potency of a herbicide by analysis of the reaction that follows its application to living organisms [16]. Bioassays are conducted to know toxicity of known chemical solutions or unknown mixtures such as samples of soil from the environment. In spite of rapid developments in analytical methods, bioassay remains a major tool for qualitative and quantitative analysis of herbicides. In an effective bioassay, the indicator species should be sufficiently sensitive to detect even small amounts of herbicides and should express the response with increasing herbicide concentrations. A bioassay method does not measure the amount of herbicide residue present in soil, but it can indicate whether or not enough herbicide residue is present to injure a sensitive crop.

### Need of bioassay

- Bioassays is used to determine if herbicide residues are present in soil at high enough concentration to adversely affect plant growth.
- The negative impacts of herbicide residues could be categorized as the destruction of plants, decrease biomass, inhibit germination, reduce plant height and enormous crop development.
- This is a simple, economical, and direct method to determine if it is safe to seed or plant into areas previously treated with herbicides.
- Bioassays are methods that utilize living material to detect substances or determine potential toxicity of herbicides.
- It helps in the planning of succeeding crops in crop rotation.

### When should bioassay be conducted?

- When newly seeded or established plants exhibit abnormal growth/injury.
- When seeding or planting areas previously treated with herbicide known to be residual, such as imazethapyr.
- When using abandoned farmland, which may contain herbicide residues such as picloram or triazines.
- When using non-cropland, it may contain residues of chlorsulfuron and picloram.
- When purchasing livestock manure, compost, or topsoil of unknown origin.
- Additionally, if it is suspected that another product may have been contaminated with an herbicide, both the product and treated soil can be tested using a bioassay.

### Indicator species

A living organism can serve as an indicator for determining toxicity of herbicide. Microorganisms also have been used in some bioassays [17]. When using plants as indicator species for determining toxicity of herbicide is called plant bioassay. Herbicide bioassay is usually conducted with sensitive plant species referred to as indicator/test species. Sorghum, mustard, cucumber soybean, oat and minor millets are some commonly used indicators plants for herbicide bioassay.

**Table 3: Test species used for various herbicides group** [18]

| Herbicide group | Bioassay species |
|---|---|
| Aliphatic compound | Oat, millets, cucumber, barley, wheat, rice |
| Acetamides (diphenamide) | Crabgrass, oats, barley, ryegrass, pigweed |
| Acetanilide (alachlor, metolachlor) | Cucumber, ryegrass, crabgrass |
| Benzoic acid | Cucumber, oat, foxtail, pigweed |
| Dicamba | Beans, sorghum, cucumber |
| Chlorpropham | Cucumber, oat, ryegrass |
| Phenoxy derivatives (2,4-D) | Cotton, pigweed, tomato, mustard |
| Substituted ureas (diuron, isoproturon) | Cucumber, ryegrass, oats, barley, millet, sorghum |
| Thiocarbamates | Ryegrass, oat |
| Dinitroanilines (trifluralin, fluchloralin) | Oats, sorghum, rice, cucumber |
| Triazines (atrazine, simazine) | Oats, cucumber, sugar beets |

**Assessment parameter**
The response of indicator plants to herbicides can be evaluated in various ways:

**1. Germination tests**
There are some herbicides which strongly inhibit the germination of sensitive species. The carryover herbicide residue inhibits radical or shoot elongation. Typically, the root or shoot elongation is observed after a period of 24-96 hours. Cucumber, sorghum and oat are the main species used in germination tests but they are not sensitive to photosynthetic inhibitors [19].

**2. Assessment of plants**
Determination of dry weight is a common assessment used for herbicide bioassay. Plant height of indicator species also gives dependable estimation. Koren *et al*. [20] found that with thiocarbamates the height reduction was greater than weight reduction.

**3. Symptoms**
Symptoms which are typical of a certain group of herbicides or of a given compound can be used for qualitative assay and if the intensity of symptoms are dose related, it can also be used for quantitative determinations but distinguishing symptoms of element deficiency and herbicide damage is difficult. *E.g.*, Epinasty of cotton have been used to measure the effect of 2, 4-D.

**Table 4: Qualitative description of treatment effects on crop in the visuals cropping scale of 0 to 10**

| Effect | Rating | Crop description |
|---|---|---|
| None | 0 | No injury, normal |
| Slight | 1 | Slight stunting, injury, or discoloration |
| | 2 | Some stand loss, stunting or discoloration |
| | 3 | Injury more pronounced but not persistent |
| Tolerate | 4 | Moderate injury, recovery possible |
| | 5 | Injury more persistent, recovery doubtful |
| | 6 | Near severe injury, no recovery possible |
| Severe | 7 | Severe injury, stand loss |
| | 8 | Almost destroyed, a few plants surviving |
| Complete | 9 | Very few plants alive |
| | 10 | Complete destruction |

**Methods for detecting herbicide residues**
There are some analytical methods for detecting herbicide residue from various samples like soil *etc*. High performance liquid chromatography (HPLC) [21], gas chromatography (GC) [22], thin-layer chromatography (TLC) [23], high performance liquid chromatography/mass spectrometry (HPLC-MS) [24], gas chromatography/mass spectrometry (GC-MS) [25], capillary electrophoresis [26] are commonly used analytical method for evaluating herbicides residue. GC and HPLC are widely used analytical techniques for the analysis of herbicide residues in various matrices such as water, soil and crops. GC is particularly suitable for volatile and semi-volatile compounds, making it a valuable tool for the analysis of herbicides, many of which fall into these categories. GC is mostly used for glyphosate, paraquat, atrazine, simazine, 2, 4-D, *etc*. However, these methods are expensive, time consuming, complicated, required specialized equipment, extraction systems, different solvent with high purity, *etc*. Besides analytical methods, bioassay method is simple, economic, and easy method for herbicide residue analysis. The main two types of bioassay: 1) Field bioassay and 2) Pot bioassay.

**Bioassay procedure**
The procedure developed by [27] is followed by many researchers with some modification. Soils treated with herbicide at different concentrations are sown with indicator/test species and after measuring the plant response by germination, plant height, dry weight, *etc*. of indicator plants standard curves are drawn. Jayakumar [28] standardized the bioassay technique for a number of herbicides, for various type of soil and brought out a quadratic model for easy assessment.

**5. FIELD BIOASSAY :**

A field bioassay used to assess the presence and potential impact of herbicide residues from the soil by using plant as test species.

Here is a general outline of how a field bioassay for herbicide residue analysis might be conducted:

- **Selecting the study area:** Choose a study site where herbicides have been applied or are suspected to be present. Ensure the site has appropriate controls (areas without herbicide application) for comparison.
- **Selecting test organisms:** Choose suitable plant species that are sensitive to the herbicide
- **Preparing test plots:** Divide the study area into test plots or treatment areas. Ensure uniform soil conditions, light exposure, and irrigation for all test plots.
- **Planting and growing plant:** Planting one or more strips of an indicator species in several locations without disturbing the soil. Allow the test plants to grow and develop symptoms of injury from any herbicide residue.
- **Control plots:** Set up control plots where no herbicide is applied. These are used as a reference for comparison.
- **Monitoring and data collection:** Regularly monitor the test organisms in both the treated and control plots. Record observations like germination percentage, plant growth and any signs of herbicide related stress or damage.
- **Statistical analysis:** Collect data over a predetermined period, which may range from weeks to months. Use statistical analysis to compare the growth and health of test organisms in treated plots with those in control plots. Analyze the data to determine if there are significant differences between the treated and control plots, indicating the presence of herbicide residues.
- **Interpretation of result:** Interpret the results of the bioassay to assess the impact of herbicide residues on the selected test organisms.
- **Reporting:** Summarize the findings in a report that includes methodology, results, and recommendations for any necessary actions, such as adjusting herbicide application rates or choosing alternative herbicides.

**POT BIOASSAY**

A pot bioassay is a commonly used method for analyzing herbicide residue in soil material. It involves growing a specific indicator plant in pots containing soil or samples collected from the field where herbicides were applied.

Here is a general overview of how to conduct a pot bioassay for herbicide residue analysis:

**Materials and equipment required for pot bioassay:**

1. **Pots:** Use clean and sterilized pots or containers.
2. **Soil sample:** Collect soil samples from the field where herbicides were applied.
3. **Test plants**: Choose a test plant species that is sensitive to herbicide you want to test for.
4. **Water:** For watering the pots.
5. **Greenhouse or controlled environment:** Depending on your resources, you may need a greenhouse or controlled environment to maintain consistent conditions.

**Procedure of pot bioassay for herbicide residue analysis:**

- **Soil collection and preparation**: Collect representative soil samples from the suspect field. Take samples from several locations in the field and combine them to make a composite sample. Take separate samples from areas where excessive residues are suspected, such as sprayer turnaround points and end rows. Do not mix these samples with the others. Sample the soil to a 6-inch depth, and divide the samples into two sections for greater accuracy. Those from 0 to 3 inches and those from 3 to 6 inches. A total of 4 kg of moist soil are needed for each bioassay and 1 kg for each laboratory analysis. Sample an area that is not suspect for use as a "check." The second option is to treat a portion of soil collected in the field with activated charcoal. If bioassay is to be performed, they should be run on the soil sample as soon as possible after they have been obtained from the field. If samples cannot be assayed immediately, store the soil in a refrigerator that is not used for food. If samples are stored in a warm environment, herbicide residue may decrease with time.
- **Prepare pot or container for bioassay**: Pots are appropriate containers in which a bioassay can be conducted. Punch holes in the bottoms of the containers to allow water drainage. Fill two or more containers (a set) with soil from each sample. Additional containers increase the accuracy of the test. In addition, fill a final set of containers with the check soil.
- **Select the indicator plant**
- **Seeding and growing bioassay species**: Sow the 10 to 15 seeds of specifically sensitive bioassay species into the submitted clean and contaminated soil samples. Make the soil wet by adding water close to field capacity. Only

one plant species is seeded per plot. Let the plant grow for at least three weeks and continue to observe them regularly. Water as required, taking extreme caution to avoid over-watering.

- **Evaluating plant growth and injury:** To accurately diagnose herbicide injury. Depending on the type and concentration of residues, injury symptoms usually appear within 10 to 20 days after plant emergence. Examine the plants of each species for stunting of growth, yellowing or discoloration of leaves or stems, abnormal leaf and stem growth, and root swelling or stunting. The plants may be photographed two to three weeks after emergence.
- **Reporting bioassay result**: After completing a bioassay for herbicide residue diagnosis, a written report should be prepared.

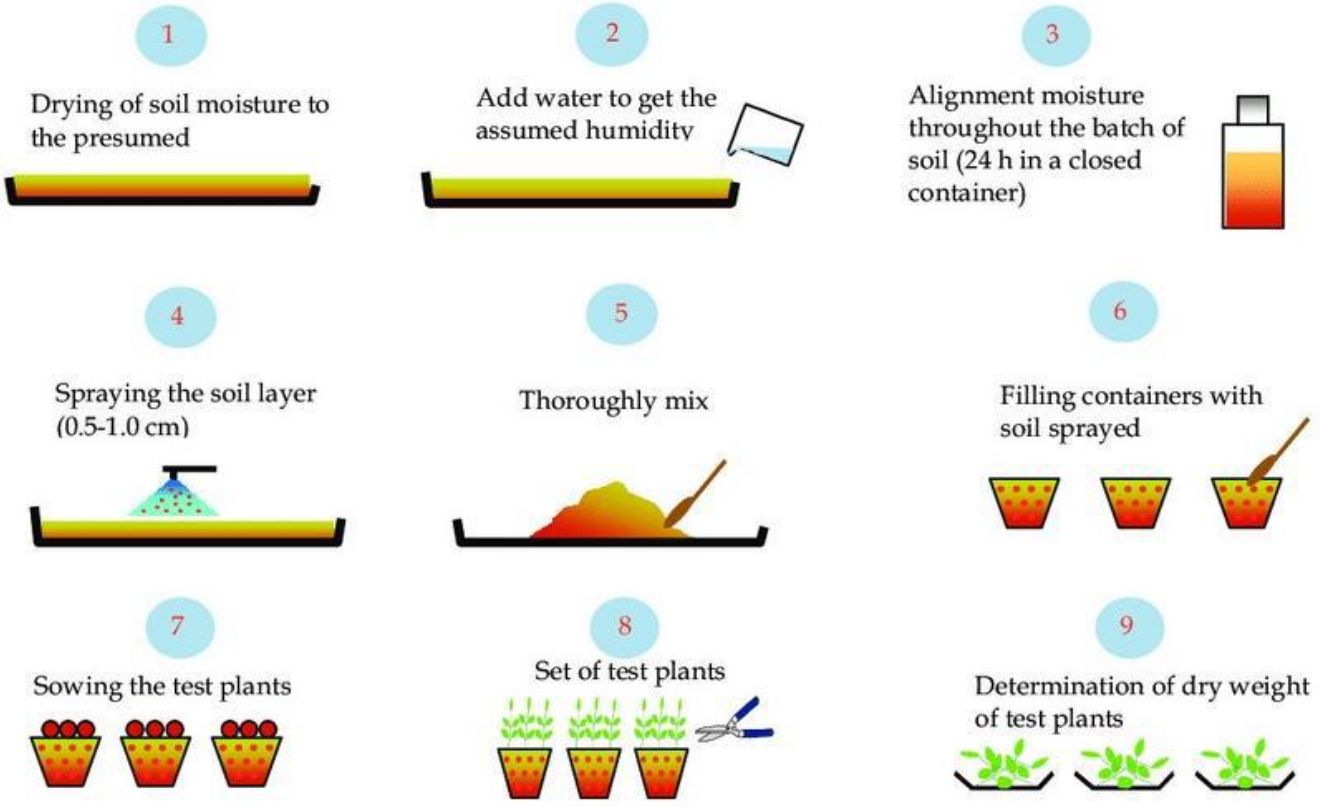


**Fig. 2: Diagram of conventional bioassay**

## 6. REVIEW OF LITERATURE :

### Review on field bioassay

The significantly highest plant stand (19.3/$m^2$) of pearlmillet under treatment oxadiargyl 150 g/ha at 20 DAS and fodder yield (9540 kg/ha) of pearlmillet under treatment oxadiargyl 60 g/ha at 20 DAS and significantly lowest plant stand (5.8/$m^2$) and fodder yield (981 kg/ha) of succeeding pearlmillet crop after harvest of cumin under treatment pendimethalin 1000 g/ha at 1 DAS which indicated pendimethalin residue were present and oxadiargyl residue were not present after harvest of cumin [29]. Bhalu [30] found that significantly highest germination (66.50%), plant height (15.26 cm) and dry matter (1.00 g/5 plants) of cotton under treatment pendimethalin 500 g/ha PE fb IC & HW at 30 DAS and significantly lowest germination (57.17 %) of cotton under treatment atrazine 500 g/ha PoE at 15 DAS fb IC & HW at 30 DAS and plant height (13.28 cm) and dry matter (0.57 g/5 plants) of cotton under treatment Atrazine 500 g/ha PE fb IC & HW at 30 DAS which indicated pendimethalin residue were not present and atrazine residue were present after harvest of pearlmillet. Punia *et* al. [31] concluded that significant highest plant height (97.4 cm) of mustard under treatment fluchloralin 0.75 kg/ha PPI and lowest plant height (39.8 cm) of mustard under treatment imazethapyr 100 g/ha PE which indicated fluchloralin had no adverse effect on mustard but imazethapyr residue were present after harvest of cluster bean.

### Review on pot bioassay

Among the herbicidal treatments germination (%) of bajra at 30 DAS significantly highest (92.53%) under treatment HW & IC at 20 DAS *fb* imazethapyr 75 g/ha PoE at 45 DAS and significantly lowest (20.00%) under treatment fluchloralin 0.9 kg/ha PE which indicated fluchloralin residue were present in groundnut field at 30 DAS and

germination(%) of bajra at 60 & 90 DAS significantly highest under treatment pendimethalin 1.0 kg/ha PE and significantly lowest under treatment quizalofop ethyl 40 g/ha PoE at 45 DAS which indicated quizalofop ethyl residue were present up to 90 DAS. However, after harvest no significant impact on germination of bajra which indicated herbicides, residue was not present after harvest of groundnut so, bajra crop can safely grow after harvest of groundnut [32]. There was a significant persistence effect of oxyfluorfen on sorghum and oxadiargyl on cucumber at 30 DAS. The results also showed that post-emergence herbicides showed no effect on sorghum and cucumber when oxadiargyl, quizalofop-ethyl and fenoxaprop-p-ethyl applied at 60 DAS [33]. The germination (%), plant height as well as dry matter production of sorghum at 30 DAS significantly highest under treatment IC & HW at 15-20 DAS *fb* propaquizafop 50 g/ha PoE at 45 DAS next to weed free treatment and significantly lowest under treatment oxadiargyl 90 g/ha PE *fb* IC & HW at 30 & 60 DAS which indicated oxadiargyl residue were present in cotton field at 30 DAS [34]. Hirapara [35] noted that germination (%) of sorghum at 30 DAS significantly highest under treatment IC & HW at 20 & 45 DAS *fb* paraquat 300 g/ha as PoE at 75 DAS (directed spray) next to weed free treatment and plant height as well as dry matter production of sorghum significantly highest under treatment IC & HW at 20 DAS *fb* propaquizafop 75 g/ha PoE at 50 DAS next to weed free treatment. They also recorded that significantly lowest germination (%), plant height and dry matter production at 30 DAS under treatment oxyflurofen 180 g/ha PE *fb* IC & HW at 30 & 60 DAS which indicated oxyflurofen residue were present in pigeon pea field at 30 DAS.

## 7. CONCLUSION:

Bioassay techniques play a crucial role in herbicide residue analysis, providing valuable information about the potential effects of herbicides on living organisms and the environment. These techniques involve using living organisms, such as plants, to assess the presence and toxicity of herbicide residues in various samples, like soil. Bioassays offer several advantages, such as being cost-effective, sensitive, and ecologically relevant, making them valuable tools for monitoring herbicide contamination and assessing the environmental impact of herbicides. Herbicide bioassays are usually conducted with sensitive plant species referred to as indicator/test species. When bioassay techniques are used in conjunction with other analytical methods, bioassays provide a comprehensive approach to assess herbicide contamination and support informed decision-making for environmental management and agricultural practices.

## REFERENCES:

1. Rojas, S., Rodríguez-Diéguez, A. and Horcajada, P. (2022): Metal– organic frameworks in agriculture. ACS Appl. Mater. Interfaces, 14, 16983-17007.
2. Muchhadiya, R. M., Kumawat, P. D., Sakarvadia, H. L. and Muchhadiya, P. M. (2022): Weed management with the use of nano-encapsulated herbicide formulations: A review. Pharma Innovation, 11(12), 2068-2075.
3. Mehdizadeh, M. (2016): Effect of pesticide residues on agricultural food production; A case study: Sensitivity of oilseed rape to Triasulfuron herbicide soil residue. MOJ Food Processing & Technology, 2: 1-2.
4. Sankaran, S., Jayakumar, R. and Kempuchetty, N. (1993): *Herbicide residues*, pp. 211-224. Gandhi Book House, 218, Cowly Brown Road (West), Coimbatore.
5. Sondhia, S. (2014): Herbicides residues in soil, water, plants and non-targeted organisms and human health implications: an Indian perspective. Indian Journal of Weed Science, 46(1): 66–85.
6. Curran, W. S. (2001): Persistence of herbicides in soil. Agronomy Facts 36. Published by Penn State College of Agricultural Sciences research and extension programs. Available online at http://extension.psu.edu/pests/weeds/control/persistance-of-herbicides-in soil/extension_publication_file.
7. Loss, M. A. (1975): *In: Herbicides: chemistry, degradation and mode of action*. *Eds*. P. C. Kearney and D. D. Kaufman. Vol. I. Marcel Dekker Inc., New York.
8. Hager, A., Sprague, C. and McGlamery, M. (2000): *Factors affecting herbicide persistence*, pp. 323-326. In: *Illinois Agricultural Pest Management Handbook*. Available online at http:// web.aces.uiuc.edu/vista/pdf_pubs/iapm2k/chap20.pdf.
9. Sharma, N. and Angiras, N. N. 2003: Estimation of metribuzin residues in wheat fields through bioassay technique. Crop Research, 26: 219-220.
10. Sharma, N., Angiras, N. N. and Ruchi. (2006): Dissipation behaviour of butachlor in rice cropped soils of Himachal Pradesh. Crop Research 32(3): 149-152.
11. Chinnusamy, C., Prabhakaran, N. K., Janaki, P. and Govindarajan, K. (2008): Compendium on weed science research in Tami Nadu (25 years). Dept. of Agronomy, TNAU, Coimbatore. pp 220.
12. Sharma, N., Sharma, S., Kumar, S. and Joshi, R. (2013): Dissipation and harvest time residue studies of 2, 4-D in soil and wheat crop. Indian Journal of Weed Science, 45(1): 68-70.
13. Sharma, N., Kumar S., Angiras, N. N. and Sehgal, S. (2014): Evaluation of pendimethalin residues in garlic. Indian Journal of Weed Science, 46(4): 374-377.

14. Ramprakash, T., Madhavi, M. and Yakadri, M. (2014): Influence of bispyribac-sodium on soil properties and persistence in soil, plant and grain in direct seeded rice (wet), In: Proceedings of the Biennial Conference of Indian Society of Weed Science on Emerging Challenges in Weed Management. 15-17 February, 2014. Directorate of Weed Science Research, Jabalpur, pp. 281.
15. Tandon, (2014): Dissipation of anilofos in soil and its harvest residue analysis in rice, In: Proceedings of the Biennial Conference of Indian Society of Weed Science on Emerging Challenges in Weed Management. 15-17 February, 2014. Directorate of Weed Science Research, Jabalpur, pp. 275.
16. Strebig, J. C. (1988): Herbicide bioassay. Weed Research, 28(6): 479-484.
17. Hess, F. D. (1980): A Chlamydomonas algal bioassay for detecting growth inhibitor herbicides. Weed Science, 28(5): 515-520.
18. Rana, S. S. and Rana, M. C. (2014): *Advances in weed management*. Department of Agronomy, CSKHPKV, Palampur. DOI: 10.13140/RG.2.2.26235.72487. pp. 183.
19. Kratky, B. A. and Warren, G. F. (1971): A rapid bioassay for photosynthetic and respiratory inhibitors. Weed Science, 19(6): 658-661.
20. Koren, E., Foy, C. L. and Ashton, F. M. (1968): Phototoxicity and persistence of four thiocarbamates in five soil types. Weed Science, 16(2): 172-175.
21. Mehdizadeh, M., Alebrahim, M. T. and Roushani, M. (2017): Determination of two sulfonylurea herbicides residues in soil environment using HPLC and phytotoxicity of these herbicides by lentil bioassay. Bulletin of Environmental Contamination and Toxicology, 99: 93-99.
22. Zhang, L., Yuac, R., Yu, Y., Wang, C. and Zhang, D. (2019): Determination of four acetanilide herbicides in brown rice juice by ionic liquid/ionic liquid-homogeneous liquid-liquid micro-extraction high performance liquid chromatography. Microchemical Journal, 146: 115-120.
23. Haskis, P., Mantzos, N., Hela, D., Patakioutas, G. and Konstantinou, I. (2019): Effect of biochar on the mobility and photodegradation of metribuzin and metabolites in soil-biochar thin-layer chromatography plates. International Journal of Environmental Analytical Chemistry, 99: 310-327.
24. Dong, X., Liang, S., Shi, Z. and Sun, H. (2016): Development of multi-residue analysis of herbicides in cereal grain by ultra-performance liquid chromatography–electrospray ionization-mass spectrometry. Food Chemistry, 192: 432-440.
25. Yu, Q., Zhang, P., He, Y., Xu, Z., He, X., Hu, Y., Zhang, H. and He, L. (2019): Dissipation dynamics and residue of four herbicides in paddy fields using HPLC-MS/MS and GC-MS. International Journal of Environmental Research and Public Health, 16: 236.
26. Daniel, D. and Do-Lago, C. L. (2019): Determination of multiclass pesticides residues in corn by QuEChERS and capillary electrophoresis tandem mass spectrometry. Food Analytical Method, 12: 1684.
27. Crafts, A. S. (1935): The toxicity of sodium arsenite and sodium chlorate in four California soils. Hilgardia, 9: 462-498.
28. Jayakumar, R. (1987): Bioassay for detecting certain herbicide residues in soils. Madras Agricultural Journal, 74(Jun-Jul): 1.
29. Yadav, R. S., Sharma, S. K., Poonia, B. L. and Dahama, A. K. (2004): Selectivity and phytotoxicity of oxadiargyl on cumin and weeds and its residual effect on succeeding moth bean and pearl millet. Indian Journal of Weed Science, 36(1&2): 83- 85.
30. Bhalu, V. B. (2010): *Efficacy of herbicides in summer pearl millet (Pennisetum glaucum L.) and assessment of their persistence through bioassay technique*, Ph. D. (Agri.) Thesis (Unpublished), Junagadh Agricultural University, Junagadh (Gujarat).
31. Punia, S. S., Singh, S. and Yadav, D. (2011): Bio efficacy of imazethapyr and chlorimuron-ethyl in cluster bean and their residual effect on succeeding *rabi* crops. Indian Journal of Weed Science, 43(1&2): 48-53.
32. Chhatrala, M. R. (2006): *Efficacy of various herbicides and determination of their persistence through bioassay technique for kharif groundnut (Arachis hypogaea L.)*, M. Sc. (Agri.) Thesis (Unpublished), Junagadh Agricultural University, Junagadh (Gujarat).
33. Ramani, B. B. and Khanpara, V. D. (2010): Efficacy of various herbicides and determination of their persistence through bioassay technique for garlic. Indian Journal of Weed Science, 42(3&4): 198-202.
34. Chhodavadia, S. K. (2016): *Efficacy of various herbicides in Bt cotton (Gossypium hirsutum L.) and determination of their persistence through bioassay technique*, Ph. D. (Agri.) Thesis (Published - Lambert Academic Publication), Junagadh Agricultural University, Junagadh (Gujarat).
35. Hirapara, K. V. (2023): *Efficacy of various herbicides in pigeon pea and determination of their persistence through bioassay technique*, Ph. D. (Agri.) Thesis (Unpublished), Junagadh Agricultural University, Junagadh (Gujarat).