

Research Article

Assessing the Biological Sensitivity of Certain Agricultural Plants to Some Common Herbicides by Using the GR₅₀ Test

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Article info: -

- Received: 2 November 2023

- Revised: 14 November 2023

- Accepted: 20 November 2023

- Published: 4 December 2023

Keywords:

GR₅₀, Herbicides, Atrazine, Linuron, Metribuzin, Pendimethalin

Abstract:

The bioassay experiments were conducted at the Faculty of Agriculture, Tanta University, to determine the sensitivity of various plant seedlings to the inhibitory effects on their growth caused by several herbicides, namely atrazine, metribuzin, pendimethalin, and linuron. The plant species used included oat, wheat, barley, soybean, cucumber, and mallow (molokhia). The primary aim was to identify the most sensitive plant species for estimating residue levels of these herbicides in agricultural environments, utilizing an economical and cost-effective approach. The results revealed a positive correlation between herbicide concentrations and their biological effects on the seedlings of all the studied plant species. Sensitivity to the toxic effects of these herbicides was demonstrated at levels lower than one part per million for all plant species. Oat was the most sensitive plant to the toxic effects of atrazine and metribuzin, while wheat exhibited the highest sensitivity to pendimethalin. In contrast, mallow (molokhia) and cucumber exhibited statistically similar sensitivity to linuron. In conclusion, the seedlings of these plant species can be effectively employed for monitoring residual herbicides in agricultural environments in an economical, straightforward, and highly accurate manner within the range of parts per million

1. Introduction

Herbicides are chemical substances employed in agricultural fields to control and eradicate undesired plants, particularly weeds, and have demonstrated significant positive impacts on agriculture. These effects include heightened crop yields, reduced labor expenses, and the prevention of crop losses due to weed competition. However, weeds constitute a substantial constraint on global agricultural production, leading to an average yield reduction of 10% on a global scale (Chauhan, 2020). In certain instances, uncontrolled weed growth can even result in a complete loss of yield, reaching 100% for specific crops (Fernandez-Cornejo et al., 2004). Effective weed population management facilitated by herbicides allows farmers to enhance crop quality and quantity by mitigating weed emergence time, density, and interference with cultivated crops. Additionally, herbicides offer the advantage of reducing the dependence on labor-intensive and costly practices like manual weeding and tillage (Sharma et al., 2021). The United States Department of Agriculture (USDA) conducted a study that revealed a significant surge in herbicide usage between 1952 and 1980, witnessing a nine-fold increase (USDA Economic Research 2006). Despite the obvious benefits of herbicides in control-

ling weeds and increasing crop productivity, herbicides have high environmental and health effects, which are represented in the emergence of herbicide-resistant weeds, water pollution, soil scraping, and toxicity to non-target organisms. Herbicides can also damage the crops they are applied to, most, if not all, research studies have shown that hoeing applications were better than herbicides due to the damage caused by herbicides to the host plant. They can reduce photosynthetic activity, biomass, and crop yields by disturbing the physiological and biochemical processes. Herbicides also, could cause oxidative stress, hormonal imbalance, and metabolic disturbances in crops (Sharma et al., 2018). Therefore, herbicides should be used in the recommended doses and timings to avoid injury to crops.

The GR₅₀ is a biological parameter that measures the herbicide dose required to reduce plant Dry weight by 50% compared with the untreated control (Trainer et al., 2005). The GR₅₀ was used to determine the different plants' sensitivity to herbicides and to compare the herbicides' efficacy. Several studies have used GR₅₀ to assess the weeds' and crops' response to herbicides and to identify herbicide-resistant biotypes (Trainer et al., 2005 and Sharma et al., 2018). These studies also enable us to

determine the level of selectivity of these herbicides and their ability to impact the target plant (weed) without affecting the host plant (economic crop) (Elsherbini et al., 2018). The GR₅₀ is used to establish the optimal dose and timing of herbicide application to achieve efficient weed control and minimize crop injury.

Also, GR₅₀ is used to estimate the herbicide residues in the environment, water, and soil, and to track the effects and movement of those herbicides in different soil layers. Herbicide residues are the quantity of herbicides that stay in the soil for an extended time and can harm subsequent crop development and production or pollute water supplies. GR₅₀ can help to determine the half-life, bioavailability, leaching potential, and herbicide degradation rate in different soil types and under various climatic conditions (El-Sherbeni and Ashry, 1985; and El-Sherbeni 1989 and Paula et al., 2023). GR₅₀ can also help to identify the most sensitive bio-indicator species for each herbicide and to evaluate the risk of crop damage or environmental pollution (Hassan et al., 1989). GR₅₀ can be calculated by conducting dose-response experiments with different herbicides and plants in controlled or field conditions. GR₅₀ can also be estimated by using mathematical models or analytical methods (Trainer et al., 2005 and Sharma et al., 2018).

The primary objective of this scientific investigation is to establish the plant species exhibiting heightened sensitivity to several widely in-use herbicides by quantifying their GR₅₀ values. By doing so,

Table 1: List of The Herbicides used

Herbicide (Common name)	Herbicide (Trade name)	Structural formula
Atrazine	Gesaprim (80% WP)	
Linuron	Afalon (50% WP)	
Metribuzine	Sencor(70% WG)	
Pendimethalin	Stomp(45.5% CS)	

we intend to employ that biological assessment as a means to estimate the presence of herbicide residues in different environmental compartments, for instance, water and soil. The study seeks to provide valuable insights into the potential impacts of these herbicides on various plant species, thus contributing to an inclusive understanding of their ecological implications and supplementary in the development of effective environmental management strategies.

2. Materials and methods

In pursuit of scientific research objectives, a series of laboratory experiments were undertaken at the Faculty of Agriculture, Tanta University, to identify the most sensitive bio indicator species concerning certain conventional herbicides.

2.1. Herbicides and chemicals used

A commercial formulation of atrazine, in the form of Gesaprim 80% W.P. by Syngenta (Basel, Switzerland); linuron, as Afalon 50% W.P. by American Dupont Company; metribuzin, in the form of Sencor 70% W.G. by Taiwan Bellarchem Corporation; and pendimethalin, as Stomp 45.5% C.S. by BASF Germany were used. These herbicides were applied under the label-recommended rates Table 1. It is important to note that all chemicals utilized in this study were of analytical grade, as presented in Table 2.

Table 2: List of the chemicals used

Compound	Purity	Formula	Source	Country
Ammonium sulfate	99.9%	(NH ₄) ₂ SO ₄	ADWIC	Egypt
Calcium nitrate	99.0%	Ca(NO ₃) ₂ .4H ₂ O	Aldrich	USA
EDTA	99.4%	EDTA	Aldrich	USA
Ferrous sulfate	99+%	FeSO ₄ .7H ₂ O	Chem-Lab	Belgium
Potassium sulfate	99%	K ₂ SO ₄	Panreac	Spain
Potassium dihydrogen phosphate	99.99%	KH ₂ PO ₄	Aldrich	USA
Potassium nitrate	99%	KNO ₃	Aldrich	USA
Potassium hydroxide	99.9%	KOH	ADWIC	Egypt
MgSO ₄	99.9%	MgSO ₄	ADWIC	Egypt
Sodium chloride	99.9%	NaCl	ADWIC	Egypt

2.2. Soil Sampling

Soil samples were obtained from the Experimental Farm at the Faculty of Agriculture, and collected at a depth of 0 to 20 cm. These samples were cautiously homogenized, air-dried, and then passed through a 5-mm sieve to ensure uniformity and eliminate any coarse debris. The physical and

chemical properties of the soil samples were assessed using Klute's (1986) and Page's (1982) methods. The results indicated that all soil samples exhibited a clay texture, displaying a relatively uniform consistency without notable variations in texture. Additionally, it was observed that the soils were neither saline nor sodic, as depicted in Table 3.

Table 3: Physical and Chemical Characteristics of the Studied Soils

EC dS/m	PH	Cations Meq/l				Anions Meq/l				Particle size distribution%		
		Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Co ₃ ⁻	HCO ₃ ⁻	Cl ⁻	S o ₄ ⁻	Clay	Silt	Sand
3.66	7.91	4.47	0.25	13.98	12.24	1.88	12.75	38.79	17	49.5	33.5

2.3. Nutrition solution

A nutrient solution was employed to accomplish the plants' nutritional requirements. This solution comprises two separate components, each stored separately and later combined at the time of application.

The first component of the nutrient solution was prepared by creating specific molar concentrations. Subsequently, a measured quantity of each solution was combined to produce a 280 ml volume,

which was then made up to a total of 1000 ml using distilled water (refer to Table 4 for details).

To prepare the second component, namely the Fe solution, 8.22 g of EDTA was dissolved in 26 ml of 1.0N KOH. Subsequently, 6.2 g of FeSO₄.7H₂O was dissolved in a small volume of distilled water. The two solutions were then mixed, and the total volume was adjusted to 250 ml using distilled water. The Fe solution was stored in a dark place without a stopper.

Table 4: Composition of the first nitrification solution (molar concentrations and volumes used).

No.	Salts chemical formula	Dissolved weight(g) / l H ₂ O	Molar Conc.	Solution volume taken
1	NaCl	58.45	1.0	20
2	KNO ₃	101.10	1.0	40
3	MgSO ₄	120.376	0.5	20
4	(NH ₄) ₂ SO ₄	132.14	0.5	20
5	KH ₂ PO ₄	136.20	0.5	120
6	Ca(NO ₃) ₂	164.01	1.0	40
7	K ₂ SO ₄	174.27	0.5	20
8	H ₂ O	distilled water		720
Total volume				1000 ml (1l)

2.4. Sensitivity of plant species to herbicides (Bioassay experiments)

To assess the sensitivity of some plant species to widely utilized herbicides for weed control in maize crops (adhering to the guidelines provided by

the Ministry of Agriculture, Egypt). Wheat, barley, soybean, and oat seeds were obtained from El-Gemmayzeh Agricultural Research Center, Gharbia Governorate, Egypt. On the Other hand, Cucumber and mallow seeds were purchased from The Egyptian Company for Seeds, Agricultural and Industrial Chemicals (El-Neanaey), Tanta, Egypt. These plant species were selected for evaluation to determine their responsiveness to the specified herbicides (Table 5).

The bioassay procedure involved utilizing plastic pots (with a volume of 237 ml) containing 200 g of dry, fine soil each. To achieve the specified concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, 1,1.6, 2, and 3.2 ppm, 5 mL of the nutrient solution, 1 mL of the Fe solution, and the necessary volumes of a 1000 ppm solution to achieve the aforementioned concentrations were combined. The final solution volume was adjusted to 20 mL using distilled water. Vigorous mixing was carried out before adding the solution to each plastic pot. The application of herbicides onto the soil surface was conducted using a hand sprayer equipped with a nozzle tip. Subsequently, the topsoil was thoroughly mixed and left to dry.

Table 5: Plant species used in the study, varieties, and sources of acquisition

No.	Plant species	Variety	Obtuded from
1.	Wheat	Giza 95	El-Gemmayzeh Agricultural Research Center, Gharbia Governorate, Egypt
2.	Barley	Giza 124	
3.	Soybean	Giza 111	
4.	Oat	-----	
5.	Cucumber	Prince	The Egyptian Company for Seeds, Agricultural and Industrial Chemicals (El-Neanaey), Tanta, Egypt.
6.	Mallow (molokhia)	-----	

The reduction percentage was determined using the following equation:

$$\text{Reduction \%} = \left(\frac{\text{Control Dry Weight} - \text{Treatment Dry Weight}}{\text{Control Dry Weight}} \right) \times 100$$

Consequently, the obtained reduction percentages were plotted on logarithmic probit graph paper against the corresponding herbicide concentrations. The statistical analysis of the results was conducted following the method of (Behrens 1970; Reisler 1972; Akobundu et al., 1975).

3. Results and discussion

Certain plant species were subjected to a series of laboratory experiments to assess their sensitivity to commonly used herbicides in maize cultivation. The objective was to identify the most sensitive plant species to these herbicides, which would be subsequently utilized in investigating the environmental residues of these herbicides in soil and water. Six

For each plant species under investigation, ten seeds/grains were consistently dispersed and placed at a depth of one cm from the soil surface. Afterward, the plastic pots were arranged on a large metal plate, and sub-irrigation was utilized as the watering method to provide a consistent and controlled water supply to the plants throughout the study. This systematic approach ensured uniformity in the seed placement and irrigation process. Untreated soil was designated as the control group, while each treatment was replicated four times to ensure statistical rigor. After ten days, the seedlings were thinned to four per pot, carefully maintaining this consistent plant density across all experimental units. Then, a 5 ml of nutrient solution was added to each pot.

The seedlings of each plant species were carefully cut at the soil surface precisely 30 days after planting. Subsequently, the fresh weights of the seedling stems were meticulously measured. To obtain the dry weights, the seedling stems were dried in an oven for 24 hours at a temperature of 60°C, then one hour at 130 °C.

plant species were selected for the study based on previous research that had demonstrated their sensitivity to the studied herbicides. These plant species include wheat, barley, oats, soybean, cucumber, and mallow (molokhia).

A series of preliminary experiments were conducted using the six selected plant species to assess their sensitivity to the studied herbicides (atrazine, linuron, pendimethalin, and metribuzin). These experiments aimed to identify the two most sensitive plant species for each herbicide among the studied plants. Subsequently, a bioassay was performed on the identified sensitive plants for each herbicide to determine the plant with the highest sensitivity to each herbicide, as previously mentioned. Below are the results obtained.

3.1. Atrazine

The inhibitory effect of atrazine on oat (*Avena sativa*) and soybean (*Glycine max*) seedling (the most sensitive plant toward the atrazine herbicide) plant growth has been studied. The data presented in Table 6 and Fig 1 illustrate the impact of atrazine herbicide on the growth of oat and soybean seedlings, as indicated by a reduction in seedling dry weight percentage (GR₅₀).

Based on the obtained results, it can be concluded that oats exhibited the highest sensitivity to the growth-inhibiting effect of the atrazine herbicide on seedlings, with a GR₅₀ value of 0.298. In contrast, soybean demonstrated lower sensitivity to atrazine herbicide, recording a GR₅₀ value of 0.399. Furthermore, it is noteworthy that there exists a positive correlation between herbicide concentrations and their inhibitory effects on seedling growth.

When considering the value slope, it is observed that the toxic mode of action of atrazine herbicide on both plants is quite similar, with toxicity curves being nearly parallel to each other.

3.2. Metribuzin

The growth-inhibitory effect of the herbicide metribuzin on seedlings of cucumber (*Cucumis sativus*) and oat (*Avena sativa*), which are the most sensitive plants to its growth-inhibitory effects, has been investigated. The results obtained have been summarized in Table 7 and illustrated in Fig 2. The results indicated variations in the response of the two plants to the growth-inhibitory effect of the herbicide metribuzin. Oat was found to be the most sensitive plant to the inhibitory effect of this herbicide, with a GR₅₀ value of 0.306, while cucumber exhibited a less sensitive response with a GR₅₀ value of 0.656. Moreover, the mode of toxic action of the herbicide differed noticeably between the two plants due to the variation in their steepness of response. It is also noteworthy that the relationship between herbicide concentration and the biological effect on both plants is concentration-dependent.

3.3. Linuron

The growth-inhibitory effect of the herbicide linuron on seedlings of mallow (molokhia) (*Corchorus olitorius*) and cucumber (*Cucumis sativus*), both of which exhibited the highest sensitivity to its growth-inhibitory effects, has been investigated. The results obtained have been recorded in Table 8 and visualized in Fig 3. From the obtained results, a positive correlation between herbicide concentrations and their inhibitory effects on the growth of both plant species is evident. However, the inhibitory effect and the mode of toxic action of the herbicide differed between the two plants due to significant variations in

both the value slope and the GR₅₀ values. The results revealed that mallow (molokhia) is the most sensitive plant to the growth-inhibitory effect of the herbicide linuron, with a GR₅₀ value = 0.398 ppm, while cucumber exhibited a slightly lower sensitivity with a GR₅₀ value = 0.521 ppm.

3.4. Pendimethalin

From the preliminary experiment results, it was concluded that seedlings of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) plants exhibited the highest sensitivity to the inhibitory effects of the herbicide Pendimethalin. Further analysis yielded the tabulated results in Table 9, which are also illustrated in Fig 4. The results demonstrated a positive relationship between the concentrations of the herbicide used and their inhibitory effects on the seedling growth of these plants. Additionally, the herbicide's potency in inhibiting the growth of seedlings of both plant species was found to be quite similar, as indicated by the proximity of the GR₅₀ values. The GR₅₀ value for wheat and barley was 0.327 and 0.392 ppm respectively. However, statistically and within confidence intervals, there was no significant difference in the sensitivity of the two plants to this herbicide, although wheat exhibited slightly higher sensitivity. On the other hand, the mode of toxic action of the herbicide differed between the two plants, as evidenced by variations in the probit slope values, despite both plants belonging to the same botanical family.

The results can be summarized as follows:

- There is a positive relationship between herbicide concentrations and their biological effects on the seedlings of the studied plants.
- In some cases, the mode of toxic action of a single herbicide was consistent for both of the studied plant species, as observed in the case of the impact of atrazine on both oats and soybeans.
- However, in the remaining cases, the mode of toxic action of the herbicide differed among the different plant species.
- Oat was the most sensitive plant to the toxic effect of both atrazine and metribuzin, while wheat exhibited higher sensitivity to the growth-inhibitory effect of the herbicide pendimethalin. On the other hand, mallow (molokhia) and cucumber had similar sensitivity to the biological effect of the herbicide linuron.

These results align with findings from prior studies. Brinkman et al. (1980) and Fuscaldo et al. (1999) demonstrated the potential utility of oats in estimating residues of atrazine and metribuzin in soil due to its high sensitivity to the inhibitory effects of

these herbicides on seedling growth. Conversely, Simard et al. (2017) and Haq et al. (2022) highlighted the sensitivity of cucumber and mallow (molokhia) to the herbicide linuron. Moreover, several researchers (Chopra et al., 2015; Chen et al. 2019; Jiang et al., 2022) have also suggested the use of wheat and barley in estimating pendimethalin residues.

thalin residues. For the herbicide linuron, either cucumber or mallow (molokhia) seedlings can be used for residue estimation. Due to the positive correlation between the herbicide concentrations and their biological effects on these plants. This determination method is considered an economical and straightforward approach, capable of detecting these herbicides at levels lower than one part per million.

In conclusion, we can employ oat seedlings for determining the residues of both atrazine and metribuzin, as well as wheat seedlings for pendime-

Table 6: Atrazine sensitivity assessment: GR₅₀ values comparison in soybean and barley seedlings

Parameters	Oat (<i>Avena sativa</i>)							GR ₅₀	Confidence limits		Value Slope
	Concentrations(ppm)								Lower	Upper	
	Control	0.1	0.2	0.4	0.6	0.8	2				
Dry weight	0.41 ± 0	0.32 ± 0.01	0.25 ± 0.007	0.19 ± 0.003	0.14 ± 0.007	0.06 ± 0.003	0.05 ± 0.012	0.298	0.187	0.474	1.251
R%		21.95	39.02	53.66	65.85	85.37	87.8				
Soybean (<i>Glycine max</i>)											
Dry weight	1.24 ± 0.019	1.01 ± 0.012	0.9 ± 0.013	0.73 ± 0.004	0.48 ± 0.006	0.23 ± 0.007	0.2 ± 0.007	0.399	0.284	0.561	1.401
R%		18.55	27.42	41.13	61.29	81.45	83.87				

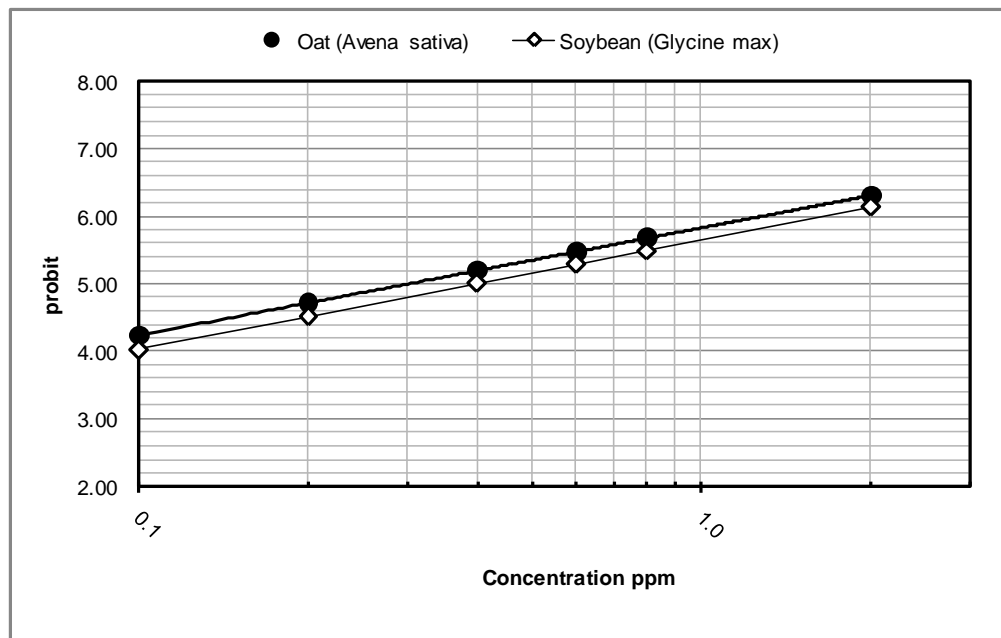


Fig. 1: Probit regression lines of atrazine herbicide inhibition effects on oat (*Avena sativa* l.) and soybean (*Glycine max*) plants.

Table 7: Metribuzin sensitivity assessment: GR₅₀ values comparison in soybean and barley seedlings

Parameters	Cucumber (<i>Cucumis sativus</i>)							GR ₅₀	Confidence limits		Value Slope
	Concentrations(ppm)								Lower	Upper	
	Control	0.1	0.2	0.4	0.6	0.8	1				
Dry weight	0.85 ± 0.023	0.75 ± 0.027	0.64 ± 0.012	0.45 ± 0.019	0.43 ± 0.007	0.34 ± 0.009	0.29 ± 0.009	0.656	0.399	1.080	1.046
R%		11.76	24.71	47.06	49.41	60	65.88				
Oat (<i>Avena sativa</i>)											
Dry weight	0.41 ± 0.015	0.31 ± 0.002	0.27 ± 0.007	0.21 ± 0.004	0.13 ± 0.003	0.06 ± 0.001	0.05 ± 0.001	0.306	0.154	0.607	0.850
R%		24.39	34.15	48.78	68.29	85.37	87.8				

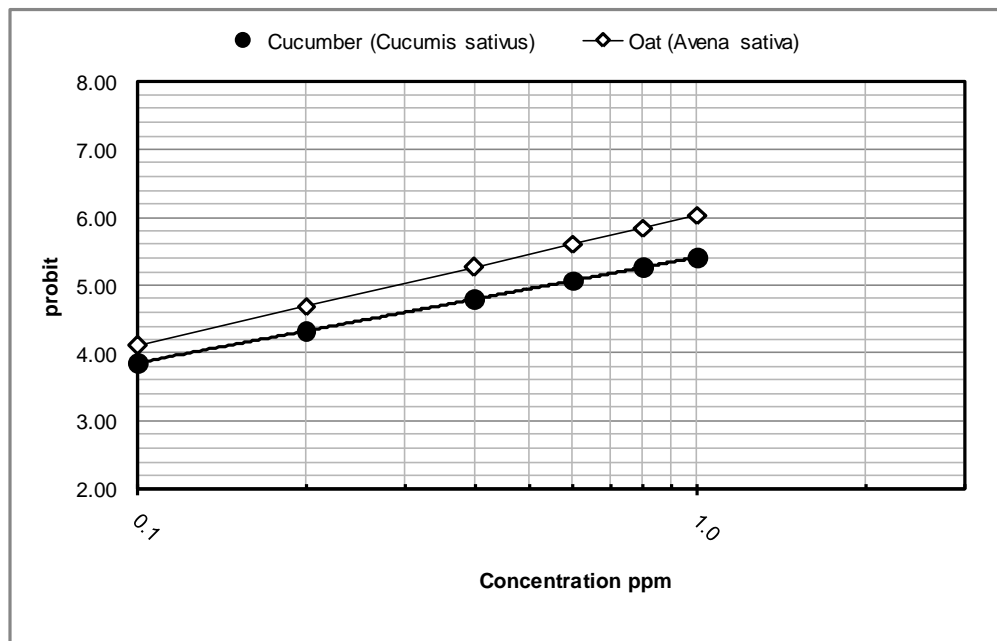


Fig. 2: Probit regression lines of metribuzin herbicide inhibition effects on Cucumber (*Cucumis sativus*) and oat (*Avena sativa*) plants.

Table 8: Linuron sensitivity assessment: GR₅₀ values comparison in mallow (molokhia) and cucumber seedlings

Parameters	Mallow (molokhia) (<i>Corchorus olitorius</i>)							GR ₅₀	Confidence limits		Value Slope
	Concentrations(ppm)								Lower	Upper	
	Control	0.1	0.2	0.4	0.8	1.6	3.2				
Dry weight	0.048 ± 0.001	0.04 ± 0.001	0.035 ± 0.001	0.027 ± 0.001	0.014 ± 0.001	0.006 ± 0	0.002 ± 0	0.398	0.262	0.603	1.399
R%		16.667	27.08	44.44	70.14	87.5	95.83				
Cucumber (<i>Cucumis sativus</i>)											
Dry	0.85 ±	0.8 ±	0.71 ±	0.6 ±	0.21 ±	0.08 ±	0.05 ±	0.521	0.347	0.782	1.655

weight	0.002	0.009	0.01	0.012	0.005	0.001	0.002				
R%		5.88	16.47	29.41	75.29	90.59	94.12				

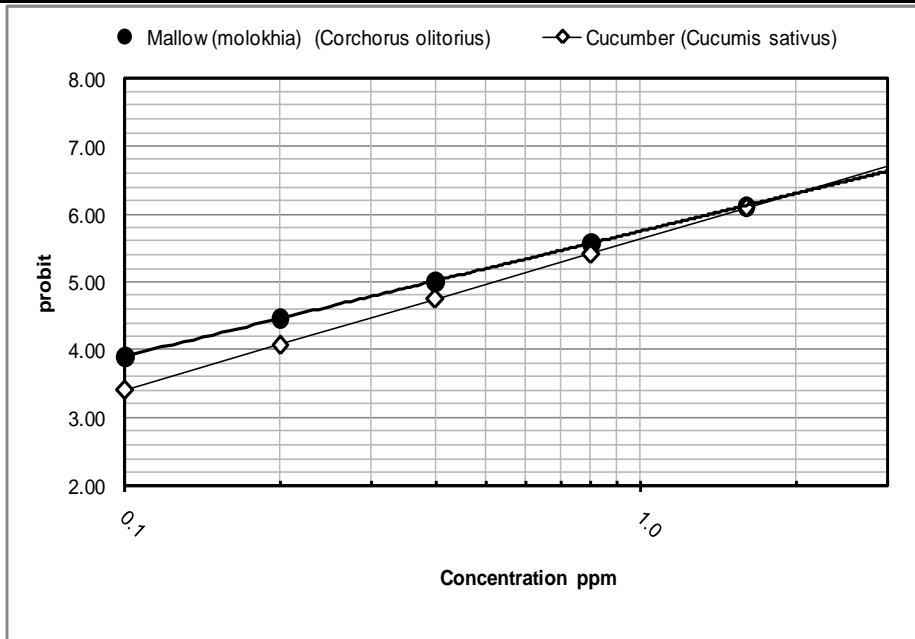


Fig. 3: Probit regression lines of linuron herbicide inhibition effects on Mallow (molokhia) (*Corchorus olitorius*) and Cucumber (*Cucumis sativus*) plants.

Table 9: Pendimethalin sensitivity assessment: GR₅₀ values comparison in wheat and barley seedlings

Parameters	Wheat (<i>Triticumae stivum</i>)							GR ₅₀	Confidence limits		Value Slope
	Concentrations(ppm)								Lower	Upper	
	Control	0.1	0.2	0.4	0.8	1.6	3.2				
Dry weight	0.28 ± 0.006	0.189 ± 0.004	0.163 ± 0.003	0.125 ± 0.002	0.116 ± 0.002	0.073 ± 0.002	0.044 ± 0.002	0.327	0.171	0.622	0.808
R%		32.62	41.9	55.48	58.57	73.93	84.17				
Barley (<i>Hordeum vulgare</i>)											
Dry weight	0.359 ± 0.006	0.304 ± 0.008	0.262 ± 0.003	0.21 ± 0.005	0.111 ± 0.001	0.038 ± 0.001	0.009 ± 0	0.392	0.194	0.793	0.954
R%		15.4	27.18	41.56	69.11	89.52	97.4				

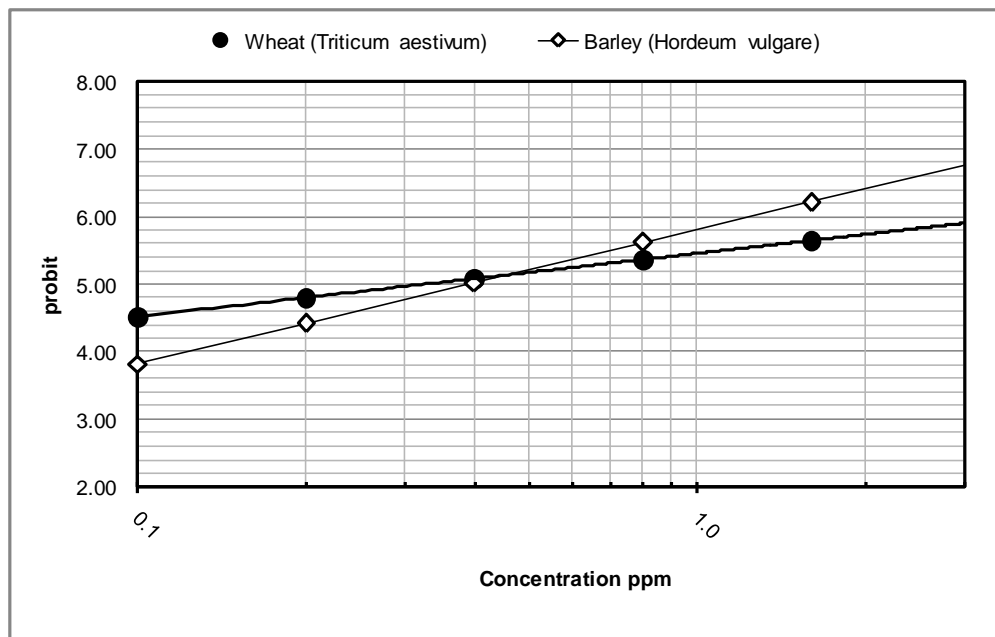


Fig. 4: Probit regression lines of pendimethalin herbicide inhibition effects on Wheat (*Triticum aestivum*) and Barley (*Hordeum vulgare*) plants.

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