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Treatment With Glyphosate and Its Effect on The Concentration of Three Types of Amino Acids and The Concentration of The Pesticide in The Vegetative Parts of Pea and Oat Plants

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A B S T R A C T

The current study was conducted in the greenhouses and laboratories of the Department of Biology/College of Education for Pure Science/University of Mosul during the academic year 2023-2024 to demonstrate the effect of the pesticide glyphosate (a herbicide) at four concentrations on the concentration of three types of amino acids and the concentration of the pesticide in the vegetative parts of pea and oat plants, in addition to the control treatment.

The results showed that treatment with the pesticide glyphosate, at the fourth concentration (2.10) ml/5 liters, led to a decrease in the concentration of the three amino acids: aspartic acid, glutamic acid, and serine, as they reached (17.39), (219.61), and (245.83) ppm , respectively, compared to the control treatment for each amino acid. As for the concentration of the pesticide in the vegetative parts of the two plants, it was found that there was an increase in the concentration of the pesticide in the two plants whenever the concentration of the pesticide used in the research increased compared to the control treatment, as the concentration of the pesticide glyphosate in the vegetative parts of the pea reached (64.71) ppm, while the concentration of the pesticide in the oat reached (59.77) ppm when treating the plant with the pesticide at a concentration of (2.10) ml/5 liters compared to the control treatment, which amounted to (5.91) and (1.91) ppm in the vegetative parts of pea and oat plants, respectively.

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Introduction

The term “pesticides” includes all compounds used to get rid of or control pests, including insecticides, herbicides (harmful weed), rodenticides, molluscicides, nematicides, and fungicides [1]. Pesticides have a positive side, as they improve crop yields all over the world and contribute greatly to meeting nutritional needs [2]. Weeds or wild herbs are natural herbs that grow in the wild without human cultivation, weeds have negative effects on soil and crops including competing with crops for space, nutrients, light, and moisture in the soil, which reduces crop productivity. Herbicides are usually used to eliminate harmful weeds, but chemicals carry a potential threat to human well-being if used incorrectly [3].

Modern agriculture requires the use of pesticides to increase productivity to a level that meets the consumption [4], as the increase in the world population from (1.5) million to (6.9) billion people between 1900 and 2000 led to an increase in the demand for food, which put pressure on the agricultural sectors, and without the use of pesticides, there will be a significant loss in the production of fruits, vegetables, and even grain production, which will have a negative impact on agricultural production and make it unable to meet consumption needs[5]. Detailed information about knowledge and awareness of the environmental risks associated with the use of pesticides among farmers is essential in making policies to reduce environmental risks, as the danger lies in the extensive use of pesticides or their mishandling and incorrect disposal. Therefore, safety measures are the best way to prevent the harmful effects of pesticide use, as the appropriate dose distinguishes poison from treatment, also, the use of pesticides affects human and animal health and produces new generations of resistant bushes, and the use of slow-degrading pesticides for a long period leads to their accumulation in the nutrients and soil, as they can be assimilated by plants and accumulate in edible parts[6].

The use of pesticides in the wrong concentrations has created enormous environmental concerns [7], as this has resulted in environmental pollution, including soil pollution, water pollution through surface runoff or filtration, and air pollution through air currents, which in turn are transmitted to plants and animals and then into human food [8]. In this study, two types of plants were used, the first is peas (*Pisum sativum* L.), which is grown in late summer/early fall (August - October). It is grown in some parts of the world because its seeds have a high nutritional value, consisting of high-quality proteins and loaded with dietary fiber, starch, carbohydrates, and micronutrients, including minerals and vitamins [9].

Due to the multiple bioactive components in peas, peas have been utilized and transformed into various products such as pea drinks, sprouted pea products, products combined with pea flour, pea-based meat substitutes, and wrapping and packaging materials. Moreover, peas and their components can be developed into more valuable and nutritious products, peas usually have two phenotypes: round peas and wrinkled peas [10]. The second is oats (*Avena sativa*), which is an annual herbaceous plant grown in late summer and early fall from the Poaceae family. It is considered a type of grain and is used mainly as food for humans and animals. Oats are generally derived from wheat and barley grasses as a secondary crop, it is rich in protein, fiber, vitamins and amino acids. It is used as food for humans and animal feed [11]. [12] indicate that oats contain a high percentage of protein, starch, antioxidants, a high percentage of saturated fatty acids, and are gluten-free. They also help to lose weight, lower blood sugar, and reduce the risk of cardiovascular disease.

The current research aim to study the effect of the weed killer Glyphosate , wich is used by many farmers to eliminate weeds harmful to plants ,on some amino acid in the vegetative part of pea and oat plant , as well as the effect of the use of these pesticides on their accumulation in the plant part and thus their transfer through the food chain by measuring the concentration of this pesticide on both plants.

Materials and methods

The study included the use of the pesticide glyphosate (a herbicide) on two types of plants: the pea, which was planted on 11/10/2023, and the oat, which was planted on 23/11/2023, as the pesticide glyphosate was used in the following concentrations: (0.084, 1.26, 1.68, 2.100) ml/5 liters in addition to the control treatment . 5 liters were relied upon due to the need for this quantity when treating plants and to avoid repetition. Seeds of the round pea plant (*Pisum sativum* L.) of Dutch origin were obtained from local markets and the germination rate was calculated and was 97%. Oat (*Avena sativa*) seeds were also obtained from the College of Agriculture/University of Baghdad and their germination rate was 90%. The study was conducted in the wire house of the College of Education/Department of Biology, and the experiment was as follows:

The seeds were planted in a mixture soil, the percentage of sand was 76.55%, clay was 12.95%, silt was 10.5%, and the pH was 7.7. The planting was done in plastic pots with a diameter of (23) cm

and a height of (20) cm, the capacity of each pot was (5) kilograms of soil, and the distance between one pot and another was about 30 cm. The seeds were planted with 10 seeds in each pot, leaving a distance of (5) cm from the top to take into account watering. After 15 days of planting, the number of plants was reduced to (7) plants in each pot, taking into account the equality between the seeds, and the pots were irrigated with regular water at 75% field capacity.

The pea plants were sprayed with the pesticide on 27/10/2023, and the oats on 19/11/2023. The treatment was carried out by spraying with plastic sprays 40 days after the plant germinated. The pots were separated when spraying the plants by placing cork barriers and then uprooting the plants from the roots after spraying about 15 days before the plants reached the flowering stage, the plants were dried using a special oven. Then, these dried samples were ground and a known weight was taken from them to measure the amino acid content and the concentration of the pesticide glyphosate in the vegetative parts of the pea and oat plants using a German-made Sykam HPLC device, model 2012. Note that the measurement was carried out in the laboratories of the Ministry of Science and Technology/Department of Environment and Water/Pollution Treatment Center/Scientific Instrumentation Laboratory/Baghdad/Iraq, as following:

1. A weight of 0.2 grams of dry matter was taken from the plant sample (peas or oats).
2. (6 M) of HCl acid was added in a volume of 12 ml.
3. The mixture was placed in the oven at 110 ℃ for 24 hours.
4. Filtered with a paper filter.
5. Washed twice with distilled water.
6. Placed in Rotary at 50 ℃.
7. After drying, 10 ml of distilled water was added.
8. Then it was returned to the rotary until dryness.
9. (0.02 M) of HCl acid was added in a volume of 3.5 ml.
10. The acidity was neutralized by adding a base.
11. Injected into the amino acid device after adding (OPA or FMOC reagent) to the sample in order to detect the amino acids.

The measurement was done according to the following equation :

Concentration of unknown compound in the sample mg/l = Area of sample compound / Area of standard compound * 20 ppm [13] .

Result and discussion

1- *The effect of treatment with glyphosate on the concentration of amino acids in oat***:**

It was shown from Table (1) that spraying the vegetative parts of oat plants with glyphosate pesticide at concentrations (0.084, 1.26, 1.68, 2.100) ml/5 liters led to a decrease in the content of amino acids, as treatment with the four concentrations led to a decrease in the content of the three amino acids, as the concentration of aspartic acid, glutamic acid, and serine, reached (17.39), (219.61), (245.83) ppm, respectively, compared to the control treatment, which recorded (82.22), (843.81), (692.51) ppm , respectively, for the above amino acids. The decrease in the amino acid content of the vegetative group may be the result of the interaction of the herbicide glyphosate (N-(phosphonomethyl) glycine) with the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) in the shikimate pathway in plants, which hinders the production of aromatic amino acids (AAA), which are acids that contain an aromatic ring such as phenyl(Ph), alanine(Ala), tryptophan(Trp), tyrosine and others, which contribute to the production of lignin and antimicrobial plant alexins as a means of defense in the plant against pathogens [14]. Glyphosate also reduces the plant's content of amino acids, as there is a significant decrease in the amount of serine and glutamic acid in plants treated with glyphosate [15].

2- *The effect of treatment with glyphosate on the concentration of amino acids in pea***:**

It appears from Table (2) that treating the vegetative parts of the pea with the pesticide glyphosate led to a decrease in the content of amino acids in its vegetative parts, as the plant was treated with the four concentrations (0.084, 1.26, 1.68, 2.100) ml/5 liters, and it was found that there was a decrease in the content of aspartic acid, which reached (8.62) ppm at the third concentration (1.68) ml/5 liters compared to the control treatment, which amounted to (14.8) ppm . In addition, it was found that there was a decrease in glutamic acid when it was treated with the concentration (2.100) ml/5 liters, as the glutamic acid content reached (119.9) ppm compared to the control treatment, which amounted to (370.14) ppm . There was also a decrease in the content of serine, which amounted to (187.93) ppm during the first treatment, at a concentration of (0.084) ml/5 liters compared to the control treatment, which amounted to (2144.99) ppm . Despite this, the concentration of amino acids decreased when the plant was treated with the pesticide at the four concentrations compared to control plants.

This decrease in the content of amino acids may be the result of the toxicity of pesticides, which reduces the percentage of protein and leads to the occurrence of oxidative stress, which works to decompose proteins, which consequently leads to a delay in the growth processes in the plant [16]. This is consistent with what [17] found that treatment with glyphosate led to the decrease of amino acids such as glutamic acid, aspartic acid, serine, and other acids in the plant.

3- The effect of treatment with glyphosate on the concentration of glyphosate in the vegetative parts of pea:

Table (3) shows that spraying the vegetative parts of the pea plant with the pesticide glyphosate at the four concentrations (0.084, 1.26, 1.68, 2.100) ml/5 liters led to a significant increase in the concentration of the pesticide in the vegetative parts of the pea, as it was found that the concentration of the pesticide increased during the fourth treatment (2.100) ml/5 liter, which reached (64.71) ppm compared to the control treatment, in which the pesticide concentration was (5.91) ppm , this is due to the possibility of glyphosate entering plants through four possible routes: leaves or other green tissues, stem, roots, or cut surfaces. The results indicate that unadsorbed glyphosate, which causes phytotoxicity to sensitive plants, can be continuously absorbed by the roots, metabolized, and transported to the edible vegetative parts of crops,where the pesticide can accumulate, which may cause harmful effects, especially at high concentrations [18]. In addition, the morphological characteristics of the plant, such as the structure of the leaves, the presence of a waxy, lipophilic cuticle, which is the primary barrier to glyphosate absorption, and growth habits affect the absorption and transport of glyphosate, which ultimately leads to varying levels of accumulation in different species [19], in addition, the plant's ability to absorb and transfer glyphosate from roots to shoots also affects the concentration reached in plant parts across species [20], as results indicate that the concentration of glyphosate in vegetative parts can show great variation between plant species due to differences in growth stage and the use of adjuvants to modify the absorption and transport of glyphosate into plant tissues [21]

Table (4) shows that treating the vegetative parts of the oat plant with the pesticide glyphosate at the four concentrations (0.084, 1.26, 1.68, 2.100) ml/5 liters, that there was an increase in the concentration of the pesticide as it reached (59.77) ppm at the fourth treatment at the concentration (2.100) ml/5 liters compared to the control treatment, which amounted to (1.91) ppm, This is consistent with [22] found that treating plants with toxic substances causes an increase in the concentration of these substances within the plant tissues.

the reason may be due, as some studies have shown, to the fact that glyphosate remains on plant parts for at least a year after it is treated on plants. In the same context, climate can affect how long glyphosate remains in plant tissues, as high rates of use of glyphosate lead to increased accumulation and stability in plant tissues, especially in meristematic tissues (growing tips), which in turn can affect their ability to absorb, transport and store glyphosate residues. Therefore, in addition to plant-specific characteristics, such as species, growth stages, and levels of exposure to the pesticide, these pesticides also tend to accumulate in the roots and meristematic tissues to a greater extent and over long periods of time [24]

Conclusion

Spraying the vegetative parts of pea and oat plants with the pesticide glyphosate at four concentrations led to a decrease in the concentration of three types of amino acids. The effect of the concentrations of this pesticide varied with the concentration of amino acids, while it was noted that the concentration of this pesticide in the vegetative parts of the two plants increased with increasing concentration of the pesticide, meaning that the relationship was direct between the treatment concentration and the pesticide concentration in the vegetative parts of the pea and oat plants.

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Table 1. The effect of treatment with glyphosate (ml/5 liters) on the concentration of three types of amino acids (ppm) in the vegetative parts of oat.

No.	Amino acids (ppm)	Control	Glyphosate concentration (ml/5 liters)			
			0.084	1.26	1.68	2.10
1	Aspartic acid	82.22	16.35	12.46	17.89	17.39
2	glutamic acid	843.81	733.54	-----	316.78	219.61
3	Serine	692.51	390.49	399.40	515.29	245.83

Table 2. The effect of treatment with glyphosate (ml/5 liters) on the concentration of three types of amino acids (ppm) in the vegetative parts of pea.

No.	Amino acids (ppm)	Control	Glyphosate concentration (ml/5 liters)			
			0.084	1.26	1.68	2.10
1	Aspartic acid	14.08	73.50	71.84	8.62	-----
2	glutamic acid	370.14	797.64	461.93	129.84	119.9
3	Serine	2144.99	187.93	907.56	231.76	-----

Table 3. Glyphosate concentration (ppm) in vegetative parts of pea plants with different concentrations of glyphosate (ml/5 liters)

Pesticide conc. ml/5 liters	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Conc. (ppm)
Control	2.775	83.271	9.847	7.5	14	0.45	5.91
0.084	2.44	121.605	0	0	-	-	8.63
1.26	2.707	144.19	2.253	32.3	34.3	0.9	10.24
1.68	2.512	281.176	0	0	-	-	19.96
2.10	2.156	911.628	0	0	-	-	64.71

Table 4. Glyphosate concentration (ppm) in vegetative parts of oat plants treated with different concentrations of glyphosate (ml/5 liters).

Pesticide conc. ml/5liters	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Conc. (ppm)
Control	2.602	26.898	0.843	5.4	2.4	2.1	1.91
0.084	2.615	35.012	3.593	28.3	38.1	0.4	2.49
1.26	2.825	41.068	6.512	1.2	1.6	1.75	2.92
1.68	2.044	134.733	0	0	-	-	9.56
2.10	2.776	842.005	0	0	-	-	59.77