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Molecular diagnosis of ALS and ACCase genes and detection of potential mutations and responsible for herbicide resistance in *Raphanus raphanistrum* L. weed from five Iraqi governorates

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Abstract

A field experiment was conducted during the winter season of 2022-2023 in one of the agricultural experimental fields at Ibn Al-Bitar Vocational School, located in Al-Hussainiya district of Kerbala governorate. The aim was to evaluate the efficacy of certain chemical herbicides in controlling wild radish weeds and to identify potential mutations in the ALS and ACCase genes responsible for resistance to some chemical herbicides. The experiment was designed according to a randomized complete block design (RCBD) with a split-plot arrangement and three replications. The main plots included five groups of wild radish seeds from five Iraqi governorates (Najaf, Karbala, Babylon, Wasit and Diwaniyah), while the subplots included four chemical herbicides (Navigator, Tatsteler, Mark Zone and Decimate), in addition to the untreated control. Laboratory results showed the presence of missense mutations in the ALS (Synthase acetolactate) gene in the seeds of Karbala, specifically a missense mutation at codon 349. For the seeds from Najaf, a frame shift mutation was identified, affecting codons 165, 166, 167, 168, 169 and 170. Additionally, a missense mutation was detected in the ACCase (Acetyl-CoA carboxylase) gene in the seeds of Babylon in codon 229. Regarding the field results, the seeds from Wasit weeds excelled by exhibiting the lowest average weed height and chlorophyll content after 60 and 90 days of spraying (60.82 cm and 33.12 SPAD, 80.25 cm and 37.57 SPAD for the two periods, respectively). In contrast, the seeds from Diwaniyah wild radish (*Raphanus raphanistrum* L.) weeds excelled in terms of control and inhibition percentages after 60 and 90 days of spraying (41.63% and 31.52%, 39.71% and 30.32% for the two periods, respectively). Additionally, Navigator herbicide was notably effective in reducing the height of wild radish weeds, decreasing chlorophyll content, and achieving the highest control and inhibition percentages.

Keywords: *Raphanus raphanistrum* L., Weeds resistance, ALS gene, ACCase gene, Chemical herbicides

Introduction

Wild radish (*Raphanus raphanistrum* L.) is a herbaceous plant belonging to the Brassicaceae family. This species is known for its rapid growth and widespread distribution in temperate regions worldwide [1]. Wild radish typically grows in the spring and early summer, reaching a height of approximately 30-60 cm. It is characterized by its lobed leaves and branched stems, and it bears flowers in a range of colors from white and pale yellow to pink and purple. The seeds are small and can remain dormant in the soil for several years, enhancing the plant's ability to spread and regrow under various environmental conditions [2]. Wild radish possesses unique biological traits that make it an important subject of study in agriculture and ecology. It can adapt to a wide range of environmental conditions, including poor and dry soils, and it exhibits a strong competitive ability against other plants. These attributes make it a weed that is often difficult to control [3]. Wild radish can pose a significant challenge to farmers, as it can interfere with the growth of agricultural crops and reduce their productivity. Therefore, understanding the dynamics of its spread and effective control methods is crucial for the efficient management of agricultural lands [4,5].

Chemical herbicides are one of the most effective and widely used tools in agricultural systems to eliminate or limit the spread of weeds. Herbicides play a fundamental role in modern agricultural practices by reducing competition between weeds and crops for vital resources such as water, light, and nutrients, thereby enhancing crop productivity and quality [6].

Herbicide resistance in weeds poses a critical challenge in agricultural crop management. This issue predominantly stems from the intensive and repeated application of a single herbicide type, which facilitates the emergence of genetic mutations in certain weed species, enabling their survival and reproduction across successive generations [7]. The development of resistance is driven by repeated herbicide exposure, resulting in the natural selection of the most resistant individuals within the weed population. These resistant individuals harbor genetic mutations that confer an ability to withstand herbicide treatments, leading to their increased prevalence in subsequent generations [8,9]. This phenomenon is particularly problematic in several major crops globally, contributing to significant economic losses. Addressing this challenge necessitates the implementation of novel, multifaceted management strategies, including the use of herbicides with diverse modes of action, crop rotation, and integrated weed management approaches. Additionally, there is a growing emphasis on the discovery of new herbicides and the optimization of existing ones to mitigate the risk of resistance development [10]. A comprehensive understanding of the biochemical and genetic mechanisms underlying herbicide resistance is essential for devising effective solutions. Consequently, this study seeks to assess the efficacy of specific herbicides in controlling wild radish weeds and to identify potential mutations in the ALS and ACCase genes that confer resistance to these chemical herbicides.

Materials and Methods

A field experiment was conducted on November 15, during the winter season of 2022-2023, at one of the agricultural experimental fields of Ibn Al-Bitar Vocational School, located in Al-Husayniyah district, Karbala governorate. The field is situated at a longitude of 44°E and a latitude of 32°N. The purpose of the experiment was to evaluate the efficacy of certain chemical herbicides in controlling wild radish weeds, as well as to identify potential mutations in the ALS and ACCase genes responsible for resistance to some chemical herbicides. The experiment was designed according to a randomized complete block design (RCBD) with a split-plot arrangement and three replications. The main plots consisted of five groups of wild radish seeds from five Iraqi governorates (Najaf, Karbala, Babylon, Wasit and Al-Diwaniyah), while the subplots included four chemical herbicides (Navigator, Tatsteler, Mark zone and Decimate), in addition to the weedy treatment.

After soil preparation, including plowing and smoothing, wild radish seeds were sown an average of 0.5 g per experimental unit. When the weeds reached the appropriate stage for control (4-6 leaf), herbicides were applied as foliar sprays according to the recommendations of the manufacturer (Table 1). Additionally, narrow-leaved weeds were removed from the experimental units that were treated with non-selective herbicides.

Table (1): Herbicides used in the study according to recommendation.

Herbicide	Recommendation
Navigator	125 L ha^{-1}
Tatsteler	400 g ha^{-1}
Decimate	0.5 L ha^{-1}
Mark zone	2.4 L ha^{-1}

RNA extraction

Following the implementation of the herbicide control procedure, 10 plants exhibiting resistance were selected. The aim of this selection was to investigate the molecular identification of the ALS gene, responsible for amino acid synthesis, and the ACCase gene, which is involved in fatty acid synthesis. This research was conducted in the laboratory of the Department of Field Crop Sciences at the College of Agriculture, University of Kerbala, Iraq.

For each plant, labeled samples were collected for RNA extraction at the onset of control results. Subsequently, the control results were monitored in wild radish weeds to differentiate between herbicide-resistant and sensitive plants. Molecular diagnostics of the genes under investigation were performed using primers specific to the mRNA sequences (Table 2). Reverse transcription polymerase chain reaction (RT-PCR) was conducted on RNA samples extracted from the leaves of all studied wild radish groups, employing the 2X AddScript RT-PCR SYBR Master kit. The final reaction volume was adjusted to 25 µl with distilled water. The reaction mixtures were prepared in sterile tubes, with one tube designated for each gene and an additional tube serving as a nucleic acid-free negative control. Components were mixed using a micropipette and centrifuged to ensure the final volume was consistent. The mixtures were then subjected to thermal polymerization using an instantaneous thermal cycler, following the specific program parameters for each gene (Table 3).

Table (2): Primers used in reverse transcription polymerase chain reaction (RT-PCR) specific for ALS and ACCase genes diagnosis.

Gene Name		Forward (5'---3')	Reverse (5'---3')
ALS	ALS1	GCTGATATCCTCGTCGAAGC	ACGCCAGCAACAGATCACTA
	ALS2	CTCGAGGCTTTTGCGAGTAG	CACCACTTGGGATCATTGG
ACCase	ACCase 1	AGAGCTTTGGGTTCTTCGTG	CTCGGGGACAGTTACCAAAC
	ACCase 2	GGTGGTGGTAAAGGCATTAGG	CCCCACCGACTACAGAGAGA

Table (3): Program of reverse transcription RT-PCR conditions for ALS and ACCase genes amplification.

Stage	Temperature (°C)	Time	Number of courses
cDNA synthesis	50	20 min	Hold
Denaturation Initial	95	10 min	Hold
Denaturation	95	30 s	40
Annealing	60	30 s	
Extension	72	2 min	
Extension	72	5 min	Hold

Reverse transcriptase polymerase chain reaction (RT-PCR)

The ALS and ACCase genes were identified using primers designed by us with NCBI tools, as detailed in table 2, the reaction mixture was prepared in a sterile tube for each genotype, including a tube without DNA as a negative control. The components of the mixture were carefully combined using a micropipette, comprising: 10 µl of Taq PCR Premix, 1 µl each of forward and reverse primers for the target gene, 5 µl of DNA, and 8 µl of distilled water. This mixture was then centrifuged to ensure a final volume of 25 µl.

The prepared mixture was transferred to a PCR device for amplification. To determine the fragment sizes of the PCR products and DNA Ladder marker, electrophoresis was conducted. First, 1 g of agarose was dissolved in 100 ml of 1X TBE buffer and heated to boiling. Once the solution cooled between 40 and 50 °C, 2 µl of red safe dye was added .Meanwhile, 3 µl of the PCR products were combined with 5 µl of loading buffer. The gel casting pot was prepared, and a comb was placed to create wells in the agarose gel. The dissolved agarose was poured into the pot and allowed to solidify at room temperature. After solidification, the comb was carefully removed to avoid damaging the wells. The tray was then positioned in the electrophoresis device, and TBE buffer was added until the Agarose layer was submerged by about 1 mm. The PCR products were loaded into the wells of the agarose gel, with a 5 µl (1 Kbp) ladder marker added to the wells on the left for size reference. Electrophoresis was conducted at 120 mA for 90 min. Afterward, the agarose gel was lifted and examined using a UV transilluminator.

Data recorded

Five plants were randomly taken from each experimental unit for the purpose of measuring the following traits after 60 and 90 days of control, which included plant height (cm), chlorophyll content (SPAD), dry weight of weed (g plant^{-1}) and control percentage was calculated according to the equation:

$$\text{Control\%} = \frac{\text{Number weeds in the comparison treatment} - \text{Number weeds in the control treatment}}{\text{Number weeds in the comparison treatment}} \text{x100}$$

The inhibition percentage was also calculated according to the equation:

$$\text{Inhibition\%} = \frac{\text{Dry weight of weeds in comparison treatment} - \text{Dry weight of weeds in control treatment}}{\text{Dry weight of weeds in the comparison treatment}} \text{x100}$$

Statistical analysis

The results were statistically analyzed according to the analysis of variance (ANOVA) as randomized complete block design (R.C.B.D), the least significant difference ($L.S.D_{0.05}$) test was used to compare the means [11], this is done using statistical analysis software GenStat12.

Results and discussion

Diagnosis and analysis of the ALS gene

After optimizing the RT-PCR conditions, the amplification products of the ALS gene were electrophoresed on an agarose gel. The results (Fig. 1 and 2) showed the presence of bands with molecular weights of 811 and 820 bp for the weed samples under study from all governorates (Najaf, Karbala, Babylon, Al-Diwaniyah and Wasit) to facilitate the analysis and identification of the ALS gene in wild radish.

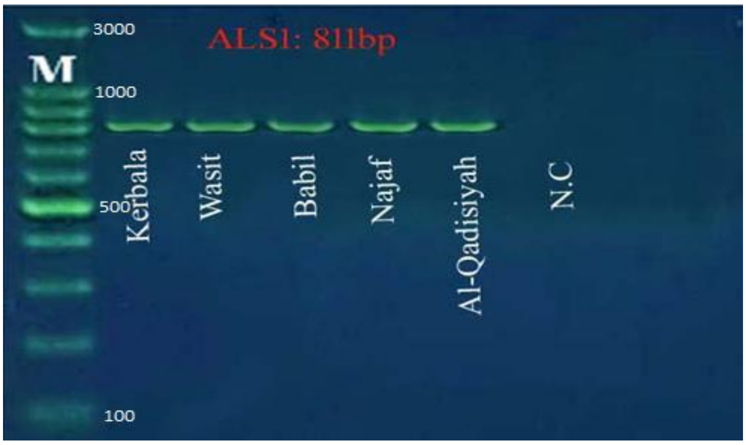

Figure (1): Transfer of PCR products for ALS gene amplification products in the seeds of the wild radish taken from five Iraqi governorates with the comparison treatment (N.C) in addition to the DNA size ladder (DNA Ladder) fixed on the left side of the figure.

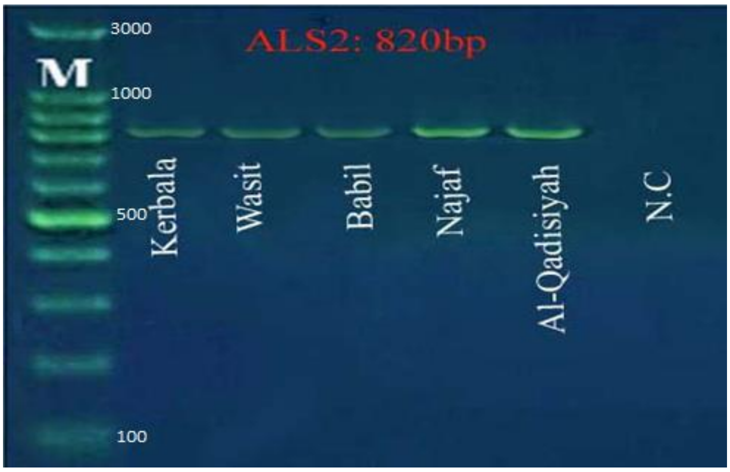

Figure (2): Transfer of PCR products for ALS gene amplification products in the seeds of the wild radish taken from five Iraqi governorates with the comparison treatment (N.C) in addition to the DNA size ladder (DNA Ladder) fixed on the left side of the figure.

Diagnosis of genetic mutations at the ALS gene level

The results (Table 4) revealed point mutations in the ALS gene that inhibit the action of the herbicides, Navigator and Tatsteler, due to changes in the nucleotide sequence. These changes lead to alterations in the amino acids encoded. Some wild radish seeds from certain governorates were found to match the global resistant strain registered in NCBI, while other governorates exhibited novel mutations. For example, wild radish seeds from Karbala governorate showed a missense mutation at codon 349 (CGG → GGG), which changes the amino acid from Arg → Gly. In contrast, wild radish seeds from Najaf governorate exhibited a different type of mutation known as a frame shift mutation. This was detected at codon 165 (GCT → GCG), which encodes the amino acid Ala. This mutation resulted from the deletion of one nucleotide (-T) in codon 165, causing a shift in the following nucleotide bases and leading to missense mutations in codons 166, 167, 168, 169 and 170 (ATT → TTG, GGC → GCA, AAG → AGA and AAC → ACA, respectively), which encode different amino acids (Ile → Leu, Gly → Ala, Lys → Arg and Asn → Thr, respectively). Additionally, codon 170 experienced an insertion mutation due to the addition of one nucleotide (+A), which restored the sequence to its normal path by compensating for the deletion in codon 165. These findings align with previous studies [12,13], that reported point mutations in the ALS gene leading to resistance to chemical herbicides used in controlling wild radish.

Table (4): Diagnosis of genetic mutations in ALS gene of the resistant and sensitive wild radish weeds to the action of chemical herbicides.

Position / Seed groups	165		166		167		168	
	Codon	**Amino Acid**	**Codon**	**Amino Acid**	**Codon**	**Amino Acid**	**Codon**	**Amino Acid**
Global plants resistant	GCT	Ala	GAG	Glu	ATT	Ile	GGC	Gly
Babylon resistant plants	GCT	Ala	GAG	Glu	ATT	Ile	GGC	Gly
Karbala resistant plants	GCT	Ala	GAG	Glu	ATT	Ile	GGC	Gly
Najaf resistant plants	GCG Transversion	Ala T-Frame shift mutation	AGA Deletion mutation	Arg Mis-sense muta-tion	TTG Deletion mutation	Leu Missense mutation	GCA Deletion mutation	Ala Missense mutation
Wasit resistant plants	GCT	Ala	GAG	Glu	ATT	Ile	GGC	Gly
Al- Diwani-yah resistant plants	GCT	Ala	GAG	Glu	ATT	Ile	GGC	Gly

Position / Seed groups	169		170		349	
	Codon	**Amino Acid**	**Codon**	**Amino Acid**	**Codon**	**Amino Acid**
Global plants resistant	AAG	Lys	AAC	Asn	CGG	Arg
Babylon resistant plants	AAG	Lys	AAC	Asn	CGG	Arg
Karbala resistant plants	AAG	Lys	AAC	Asn	GGG Transversion	Gly **Missense mutation**
Najaf resistant plants	AGA Deletion mutation	Arg **Missense mutation**	ACA +A **Insertion mutation**	Thr **Insertion mutation**	CGG	Arg
Wasit resistant plants	AAG	Lys	AAC	Asn	CGG	Arg
Al- Diwani-yah resistant plants	AAG	Lys	AAC	Asn	CGG	Arg

Diagnosis and analysis of the ACCase gene

After optimizing the RT-PCR conditions, the amplification products were subjected to agarose gel electrophoresis. The results (Figs. 3 and 4) revealed the presence of bands with molecular weights of 888 and 994 bp in the weed samples collected from all the studied governorates (Najaf, Kerbala, Babylon, Al-Diwaniyah, and Wasit). This process was carried out to support the analysis and identification of the ACCase gene in wild radish.

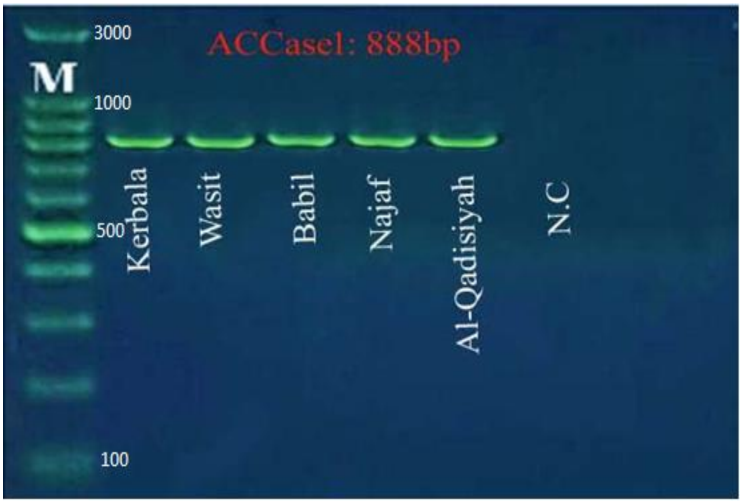

Figure (3): Transfer of PCR products for ACCase gene amplification products in the seeds of the wild radish taken from five Iraqi governorates with the comparison treatment (N.C) in addition to the DNA size ladder fixed on the left side of the figure.

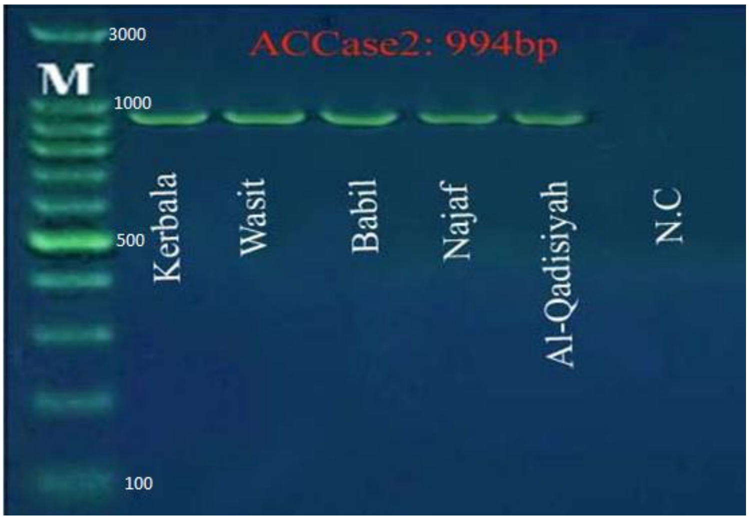

Figure (4): Transfer of PCR products for ACCase gene amplification products in the seeds of the wild radish taken from five Iraqi governorates with the comparison treatment (N.C) in addition to the DNA size ladder fixed on the left side of the figure.

Diagnosis of genetic mutations at the ACCase gene level

The results (Table 2) indicated the presence of point mutations in the ACCase gene, which is responsible for fatty acid synthesis in plants. These genetic mutations consequently affect the mechanism of action of certain chemical herbicides. One of these mutations was identified in the weeds of Babylon governorate at codon 45 (ATA → ATT), which encodes the amino acid Ile, while another missense mutation was found at codon 229 (ACA → TCA), encoding the amino acid Thr → Ser, additionally, a silent mutation was detected in the weeds of Karbala governorate at codon 156 (GTT → GTC), which encodes the amino acid Val. Another silent mutation was also identified in the weed seeds of Najaf governorate at codon 141 (CAT → CAC), which encodes the amino acid His. These findings are consistent with those of [14], who reported the presence of point mutations in the ACCase gene that contributed to the development of herbicide-resistant individuals. In contrast, the weed seeds from the Diwaniyah and Wasit governorates were found to be identical to the globally recognized resistant strain.

Table (5): Diagnosis of genetic mutations in ACCase gene of the resistant and sensitive wild radish weeds to the action of chemical herbicides.

Position / Seed groups	45		141		156		229	
	Codon	**Amino Acid**	**Codon**	**Amino Acid**	**Codon**	**Amino Acid**	**Codon**	**Amino Acid**
Global plants resistant	ATA	Ile	CAT	His	GTT	Val	ACA	Thr
Babylon resistant plants	ATT Transversion	Ile Silent mutation	CAT	His	GTT	Val	TCA Transversion	Ser **Missense mutation**
Karbala resistant plants	ATA	Ile	CAT	His	GTC Transversion	Val Silent mutation	ACA	Thr
Najaf resistant plants	ATA	Ile	CAC Transition	His Silent mutation	GTT	Val	ACA	Thr
Wasit resistant plants	ATA	Ile	CAT	His	GTT	Val	ACA	Thr
Al- Diwaniyah resistant plants	ATA	Ile	CAT	His	GTT	Val	ACA	Thr

Plant height (cm)

The results (Table 6) showed a significant difference in plant height among the governorates from which the seeds were collected. The weed seeds from Wasit governorate exhibited the lowest mean plant heights, measuring 60.82 cm and 80.25 cm for the two periods, respectively, which did not differ significantly from the weed seeds from Diwaniyah governorate, which recorded mean heights of 66.67 cm and 86.09 cm for the same periods. The reduced height of wild radish weeds in Wasit governorate might be attributed to differences in the genetic compositions among the seeds from various governorates. The variation in wild radish weed height among the seeds from the studied governorates could be due to genetic mutations in the ALS and ACCase genes, as observed in the weeds from Babylon governorate at codon 93, and in the weeds from Karbala governorate at codons 96 and 349, and in the weeds from Najaf governorate at codons 165, 166, 167, 168, 169 and 170 (Table 4 and 5). These mutations might have led to increased gene expression of these genes responsible for the synthesis of amino acids and fatty acids, which are crucial for the formation of enzymes, proteins, and cellular membranes. This additional capability could have allowed the weeds to overcome the inhibitory effects of chemical herbicides[15,16], thereby explaining their superiority in plant height compared to the weed seeds from Wasit and Diwaniyah governorates.

The results (Table 6) indicated that all herbicides used in the study significantly reduced the height of wild radish weeds 60 days after spraying. The herbicides Navigator and Decimate were particularly effective, yielding the lowest average weed heights of 64.63 cm and 69.31 cm, respectively, compared to the untreated control, which had an average height of 79.54 cm. After 90 days, Navigator continued to show superior performance, with the lowest average height of 87.19 cm, compared to the control (weedy treatment), which had an average height of 96.99 cm. The reduction in the height of wild radish weeds following treatment with Navigator may be attributed to the chemical nature of its active ingredients, which inhibit the ALS enzyme, thereby preventing the synthesis of essential amino acids. This inhibition negatively affects cell growth and differentiation, ultimately reducing plant height [17]. Furthermore, the inhibition of the ACCase enzyme disrupts the biosynthesis of fatty acids required for cellular membrane construction, leading to impaired cell division and elongation, which also contributes to the reduction in plant height [18].

The results also revealed a significant interaction between the seeds from the studied governorates and the chemical herbicides 60 days after spraying. The weed seeds from Wasit governorate showed the best interaction with the herbicide Navigator, recording the lowest average height of 53.74 cm, whereas the weed seeds from Karbala governorate with weedy treatment the highest average height of 84.31 cm. After 90 days of control, it was observed that the weeds from Wasit governorate uniquely responded to Navigator, with an average height of 70.96 cm, while the weed seeds from Babylon governorate with weedy treatment the highest average height of 105.18 cm.

Table (6): Effect of control treatments after 60 and 90 days of spraying on the height of the wild radish weeds taken from five Iraqi governorates.

After 60 days of control						
Treatments	**Governorates' seeds under study**					**Means**
	Najaf	**Kerbala**	**Babylon**	**Diwaniyah**	**Wasit**	
Weedy	83.14	84.31	84.06	75.80	70.43	79.54
Navigator	66.73	71.90	67.20	63.61	53.74	64.63
Tatsteler	72.31	80.29	81.09	56.45	63.17	70.66
Decimate	70.92	77.76	75.23	66.45	56.19	69.31
Mark zone	78.34	61.55	80.72	71.04	60.58	70.44

L.S.D	14.07					5.42
Means	74.28	76.46	77.66	66.67	60.82	
L.S.D 0.05	7.10					
After 90 days of control						
Treatments	**Governorates' seeds under study**					**Means**
	Najaf	**Kerbala**	**Babylon**	**Diwaniyah**	**Wasit**	
Weedy	99.02	102.83	105.18	91.04	86.90	96.99
Navigator	90.14	94.06	96.28	84.52	70.96	87.19
Tatsteler	97.23	98.51	98.47	86.79	82.61	92.72
Decimate	94.64	93.40	100.47	84.66	80.14	90.88
Mark zone	96.83	92.34	101.87	87.49	80.66	91.65
L.S.D	9.38					3.61
Means	95.57	96.22	100.45	86.09	80.25	
L.S.D 0.05	6.74					

Chlorophyll content (SPAD)

The results (Table 7) indicated a significant difference in the chlorophyll index among the seeds from the governorates under study 60 days after spraying. The seeds from the weeds in Wasit governorate recorded the lowest average at 33.12 SPAD, which was not significantly different from the average of the seeds from the weeds in Najaf, Babylon, and Diwaniyah governorates, which recorded averages of 36.15, 37.16 and 34.44 SPAD, respectively. In contrast, the seeds from the weeds in Kerbala governorate recorded the highest average chlorophyll index at 40.40 SPAD. After 90 days of spraying, the seeds from the weeds in Wasit governorate again exhibited the lowest average at 37.57 SPAD, which was not significantly different from the average recorded by the seeds from the weeds in Diwaniyah governorate, which was 39.87 SPAD. The variation in the chlorophyll index among the seeds from the different governorates could be attributed to differences in the plants' ability to absorb water and nutrients, particularly nitrogen, which is a key component in the porphyrin ring, an essential compound in the construction of the chlorophyll molecule [19].

The results (Table 7) showed that treatment with chemical herbicides caused a significant decrease in the chlorophyll index after 60 and 90 days of control, as Navigator and Decimate were superior by giving averages of 29.98 and 30.79 SPAD, 39.85 and 38.23 SPAD for the two periods respectively, compared to the weedy treatment which gave 49.27 and 43.76 SPAD for the two periods respectively. The superiority of some herbicides in reducing the chlorophyll content may be due to the physiological nature of these herbicides, as Navigator herbicide inhibits the ALS enzyme and the ACCase enzyme responsible for the synthesis of amino and fatty acids, which causes a defect in the biosynthesis of chloroplasts and thus affects the efficiency of the photosynthesis process, which leads to the decomposition and damage of the chlorophyll pigment and then the death of the plant [20].Decimate also inhibits the work of the second photosystem in addition to its effect on the work of the enzyme Phytoene desaturase, which is responsible for the synthesis of carotenoids, which are pigments that assist in the process of photosynthesis, and thus reduces the chlorophyll content in the plant [21].

Table (7): Effect of control treatments after 60 and 90 days of spraying on the chlorophyll index of the wild radish weeds taken from five Iraqi governorates.

After 60 days of control						
Treatments	**Governorates' seeds under study**					**Means**
	Najaf	**Kerbala**	**Babylon**	**Diwaniyah**	**Wasit**	
Weedy	51.72	53.46	48.91	47.10	45.17	49.27
Navigator	30.23	35.42	30.66	27.91	25.76	29.98
Tatsteler	34.01	36.13	35.18	35.84	32.88	34.41
Decimate	30.12	35.72	33.09	28.12	26.89	30.79
Mark zone	37.70	41.29	37.94	33.21	34.92	37.01
L.S.D	N.S					2.16
Means	36.15	40.40	37.16	34.44	33.12	
L.S.D 0.05	4.04					
After 90 days of control						
Treatments	**Governorates' seeds under study**					**Means**
	Najaf	**Kerbala**	**Babylon**	**Diwaniyah**	**Wasit**	
Weedy	44.41	48.52	44.78	44.65	36.44	43.76
Navigator	40.24	42.46	43.75	36.90	35.92	39.85
Tatsteler	42.58	43.41	42.00	39.07	38.18	41.04
Decimate	37.10	42.65	38.94	35.80	36.65	38.23
Mark zone	43.12	45.54	45.50	42.94	40.67	43.55
L.S.D	N.S					1.80
Means	41.09	44.52	42.99	39.87	37.57	
L.S.D 0.05	3.36					

Control percentage (%)

The results (Table 8) showed a significant difference in the control percentage of wild radish weed seeds from the governorates included in the study 60 days after spraying. The highest control percentage was observed in the seeds from Babylon governorate, with an average of 41.63%, which was not significantly different from the rates observed in Diwaniyah and Wasit governorates, with averages of 41.21% and 41.25%, respectively. When measuring the control percentage after 90 days, the highest percentage was observed in the wild radish weed seeds from Diwaniyah governorate, with an average of 39.71%, which was not significantly different from the averages observed in the seeds from Karbala, Babylon and Wasit governorates, which recorded 38.91%, 35.64%, and 38.19%, respectively. The overall decline in the control percentage may be attributed to the presence of a form of resistance known as non-target-site resistance (NTSR). This type of resistance involves any mechanism that reduces the herbicide's effect on the targeted enzyme by decreasing the absorption and translocation of the herbicide within the plant tissues, increasing its degradation, or lowering the concentration of herbicides at their site of action to non-toxic levels [22]. NTSR operates through multiple mechanisms and may involve morphological traits, such as thicker epidermis that causes retention of herbicides, or more often, physiological changes resulting from alterations in the plant's environment [23].

The results (Table 8) indicated significant differences among the chemical herbicides in terms of the percentage of control 60 days after spraying. The highest control percentage were recorded with the herbicides Navigator and Decimate, which achieved averages of 53.62% and 49.77%, respectively. In contrast, the control percentages for the herbicides Tatsteler and Mark zone were lower, with averages of 41.91% and 43.67%, respectively. After 90 days of spraying, the treatment with Navigator herbicide showed the highest average control percentage of 51.93%. The superior performance of Navigator in terms of the percentage of control may be attributed to the fact that these seeds had not been previously treated with this herbicide, resulting in weaker resistance compared to those that exhibited higher resistance. herbicides that are used less frequently can be effective in eliminating weed plants due to the lack of evolutionary development of the weeds and the absence of resistant genetic mutations [24,25].

The results (Table 8) revealed a significant interaction between the seeds collected from the governorates under study and the chemical herbicide treatments after 60 days of control. The weeds from Babylon governorate responded with the highest average control percentage of 60.86% when treated with the herbicide Mark zone, while the seeds from the weeds in Karbala governorate showed the lowest response, with an average of 27.59% under the same treatment.

Table (8): Effect of control treatments on the percentage of control after 60 and 90 days of spraying in the wild radish weeds taken from five Iraqi governorates.

Treatments	After 60 days of control Governorates' seeds under study					Means
	Najaf	Kerbala	Babylon	Diwaniyah	Wasit	
Weedy	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	52.73	52.41	54.70	53.19	55.08	53.62
Tatsteler	31.26	39.65	38.76	42.96	56.94	41.91
Decimate	45.95	44.93	54.42	51.15	52.44	49.77
Mark zone	29.91	27.59	58.17	60.86	41.83	43.67 3.97
L.S.D	10.32					
Means	31.97	32.91	41.21	41.63	41.25	
L.S.D 0.05	7.41					
	After 90 days of control					
Treatments	**Governorates' seeds under study**					**Means**
	Najaf	**Kerbala**	**Babylon**	**Diwaniyah**	**Wasit**	
Weedy	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	46.92	50.22	49.61	57.48	55.45	51.93
Tatsteler	32.38	36.40	46.48	36.77	48.58	40.12
Decimate	35.19	51.00	47.08	52.74	48.49	46.90
Mark zone	31.65	56.94	35.07	51.56	38.46	42.73
L.S.D	N.S					3.61
Means	29.22	38.91	35.64	39.71	38.19	

L.S.D 0.05	6.73	

Inhibition percentage (%)

The results (Table 9) indicated a significant difference among the governorates from which wild radish weed seeds were collected in terms of inhibition percentages after 60 and 90 days of spraying. The highest average inhibition percentage was recorded for the weeds from Diwaniyah governorate, with averages of 31.52% and 30.32% for the respective periods. This may be attributed to the superior control percentage observed in the seeds from Diwaniyah (Table 8), which could explain their higher inhibition percentage. Alternatively, it might be due to the absence of point mutations in the genes responsible for resistance to chemical herbicides, making them less resistant compared to their counterparts (Table 4 and 5).

The results (Table 9) showed a significant effect between the chemical herbicides in the inhibition percentage after 60 days of control. However, the treatment with Navigator herbicide was superior with an average of 37.86%, while Tatsteler, Decimate and Mark zone herbicides gave averages of 27.28%, 35.05% and 29.40%, respectively. After 90 days of control, it was also noted that Navigator herbicide was superior with an average of 36.44%, but it did not differ significantly from the average of Decimate herbicide, which amounted to 35.02%, while Tatsteler and Mark zone herbicides recorded averages of 25.07% and 26.23%, respectively, The increase in the inhibition percentage resulting from the use of Navigator and Decimate herbicides may be attributed to the role of these two herbicides in inhibiting the action of the ALS and ACCase enzymes responsible for the synthesis of amino and fatty acids, in addition to inhibiting the synthesis of carotenoids, which affects the plant's rate of performance for its vital activity and thus reduces the assimilation and accumulation of dry matter, which is positively reflected in the increase in the inhibition percentage [26].

The results also showed a significant interaction between the seeds of the seeds of the governorates included in the study and the chemical herbicides after 60 and 90 days of control, as the highest response was in the seeds of Diwaniyah governorate a percentage of 46.48% and 46.01% for the two periods, respectively, with the Tatsteler herbicide, while the seeds of Najaf governorate recorded the lowest response at a percentage of 17.18% and 15.46% for the two periods, respectively, with the herbicide Mark zon.

Table (9): Effect of control treatments on the percentage of inhibition after 60 and 90 days of spraying in the wild radish weeds taken from five Iraqi governorates.

After 60 days of control						
Treatments	Governorates' seeds under study					Means
	Najaf	Kerbala	Babylon	Diwaniyah	Wasit	
Weedy	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	37.19	39.06	39.80	33.81	39.47	37.86
Tatsteler	22.39	21.68	22.26	46.48	23.59	27.28
Decimate	34.73	31.21	38.97	35.21	35.15	35.05

Mark zone	17.18	29.13	29.53	42.11	29.09	29.40
L.S.D	5.81					2.24
Means	22.29	24.21	26.11	31.52	25.46	
L.S.D 0.05	4.17					
After 90 days of control						
Treatments	**Governorates' seeds under study**					**Means**
	Najaf	**Kerbala**	**Babylon**	**Diwaniyah**	**Wasit**	
Weedy	0.00	0.00	0.00	0.00	0.00	0.00
Navigator	37.04	38.84	37.12	33.59	35.61	36.44
Tatsteler	20.97	18.58	19.91	46.01	19.89	25.07
Decimate	33.70	35.76	38.17	34.97	32.53	35.02
Mark zone	15.46	26.96	28.96	37.06	22.74	26.23
L.S.D	4.04					2.16
Means	21.43	24.02	24.83	30.32	22.15	
L.S.D 0.05	5.62					

Based on the above findings, it can be concluded that the variation among the wild radish groups from the governorates under study may be attributed to differences in environmental conditions from one region to another. Additionally, the mutations identified in the ALS and ACCase genes are very likely to have played a role in enhancing the resistance of these weeds to chemical herbicides.

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