



Allelopathic activity of rapeseed leaf residues exposed to ultraviolet radiation on two soybean varieties

Nassar Ghani Sabri, Alaa Hussein Ali

Department of Biology, College of Science, University of Mosul, Iraq

Abstract

The study was conducted in a greenhouse at the University of Mosul to investigate the effects of irradiated and non-irradiated plant residues on rapeseed leaves, using ultraviolet rays (200-280 nm) at varying quantities (0, 10, 20 g/kg of soil). The experiment utilized a completely randomized design (CRD) with three replicates to ensure reliable results. The addition of irradiated and non-irradiated rapeseed leaf residues significantly reduced growth indicators, including plant height, leaf area, total chlorophyll, and relative water content, with decreases of 16.23%, 18.58%, 17.98%, and 19.56%, respectively, at the 20 g/kg dosage. Conversely, these residues enhanced proline and superoxide dismutase levels, increasing by 16.62% and 103.3%, respectively, when 20 g/kg of irradiated residues were applied. The study also noted significant differences between the Al-Shaima and Indian soybean varieties; the Indian variety exhibited lower values in most traits except for proline and superoxide dismutase, where it showed higher levels compared to Al-Shaima. The interaction effects revealed that the Al-Shaima variety had the highest values for most traits under control conditions, while the Indian variety excelled in proline and superoxide dismutase when treated with 20 g/kg of irradiated residues.

Keywords: Plant residues, radiation, allelopathic, SOD

Introduction

Soybean *Glycine max* is a member of the Leguminosae family and is among the world's most significant crops. It ranks first in terms of cultivated area and global production of oilseed crops (Mishra *et al*, 2024) [12]

It is considered a crop of economic, industrial and nutritional importance. Its seeds contain a high percentage of oil ranging between (14-24)% and the protein percentage reaches between (30-50)% (Bello *et al*, 2023) [4] It is considered an important crop because its seeds contain all the essential amino acids in addition to some minerals, vitamins and unsaturated fatty acids (Rotundo *et al*, 2024) [15] It is also used as a concentrated feed for animals and to improve soil fertility by using the root nodule bacteria *Rhizobium saponicum* to fix atmospheric nitrogen in the soil, in addition to providing plants with the necessary growth needs (Umoh *et al*, 2023). [19]

Allelopathy was defined by (Lee *et al*, 2003) [10] as a common biological phenomenon that results from the release of chemical compounds from a certain organism, such as a plant, and from its various parts into the environment They affect the growth and development of other organisms, whether positively or negatively, beneficially or harmfully, depending on the nature of the chemical compounds and their concentrations. Plants, fungi, algae, and viruses all create secondary metabolites known as allelopathic chemicals, and originate from pathways such as shikimic acid or acetate acid (Al-Deliemy and Abdul-Ameer, 2023) [20] The majority of the chemicals supplied to the soil come from the breakdown of plant leftovers. When a plant dies, its remains decompose and allelopathic compounds are released into the environment This process is influenced by a variety of elements, such as the type of plant waste, the quality of the soil, and the conditions of decomposition. These factors can limit the effectiveness of compounds released from plants on other living organisms, in terms of whether they are highly toxic, non-toxic, or stimulating to plants (Kamal, 2020). [9]

Materials and Methods

As part of the study, experiments were carried out in the greenhouse at the University of Mosul's Department of Life Sciences and College of Science to study the allelopathic properties of rapeseed crop residues (*Brassica napus* L.) irradiated and non-irradiated by ultraviolet rays with intensity (200-280) nanometers and its effect on seed germination, certain traits of vegetative growth, and physiological and biochemical characteristics of two varieties of soybean (*Glycine max* L.). A greenhouse experiment was conducted using three duplicates of each treatment in a completely randomized design (C.R.D.) to demonstrate the effect of treatment with irradiated and non-irradiated rapeseed residues on some phenotypic, physiological and biochemical characteristics of soybean plants. Irradiated and non-irradiated rapeseed residues were mixed with soil at levels of (0.10, 20) g/kg. Then they were placed in plastic pots and their nozzle were covered with perforated aluminum foil and incubated for two weeks. After that, 10 seeds were planted in each pot of soybean plant. Two weeks after planting, the number of seedlings was reduced to five seedlings and watering was done with normal water on a regular basis for all treatments. After 60 days of planting, some physiological and biochemical characteristics and vegetative growth characteristics were studied

Characteristics studied in the laboratory experiment

1. Plant height (cm): For five natural seedlings, the height of the plant was measured from above the soil's surface to its tallest point.
2. Leaf area (cm²/plant) was calculated by calculating the dry weight of discs of known area to the total dry weight of plant leaves based on the method of (Roy *et al*, 1981).

Leaf area (cm²/plant) = disc area (2cm) × dry weight of disc (g) + dry weight of plant leaf (g) / dry weight of disc (g)

3. Relative water content was determined using the following formula in accordance with the methodology of Turner (1981)^[18] and Schonfeld *et al.* (1988):^[17]
Relative water content % = (Fresh weight - Dry weight)/ (Swelling weight - Dry weight) × 100
4. Calculating the total amount of chlorophyll: Total chlorophyll was estimated by weighing 100 mg of fresh leaves from each sample of soybean plants, crushed them in a ceramic mortar with 10 ml of 80% acetone, and then centrifuged at 3000 rpm for 15 minutes. At wavelengths of 645 and 663 nanometers, the absorbance of the filtrate was measured using a Spectrophotometer (Arnon, 1949).^[2] The following equation was used:
Total chlorophyll content = 20.2(A645) +8.02(A663)
5. Proline determination in leaves: The concentration of the amino acid proline in soybean leaves was measured using a spectrophotometer at a wavelength of 520 nm in accordance with the procedure of Bates *et al.* (1973)^[3]
6. SOD activity estimation (µg/ml/min): The method described in (Misra and Fridovich, 1972)^[13] was used to detect absorbance and superoxide dismutase activity (unit/1-gram fresh weight) in plant leaves using a spectrophotometer at a wavelength of 480 nm (Bosco *et al.*, 2007).^[5]

Results

Plant height (cm)

The results of Table (1) showed that adding plant residues to rapeseed leaves had a significant effect on the plant height trait of soybeans and led to a significant decrease, as the lowest value (13.8) cm was recorded in the Indian variety compared to the Al-Shimaa variety. Regarding the comparison of plant residue averages, the results showed a significant decrease in all treatments compared to the control treatment, where the lowest plant height (12.9) cm was recorded when adding 20 gm of irradiated material, with a decrease rate of (16.23) % compared to the control. In terms of interaction between varieties and plant residues, the results showed significant differences, as the lowest value (12.7) cm was recorded when adding 20 gm of irradiated material in the Indian variety.

Table 1: Effect of plant residues of rapeseed leaves on plant height (cm) for two soybean varieties

residues average	Indian v.	Al-Shimaa v.	Varieties plant residues
15.4 a	15.0 ab	15.7 a	control
14.3 b	13.9 cd	14.6 bc	10 gm irradiated plant residues
12.9 c	12.7 e	13.0 de	20 gm irradiated plant residues
14.9 ab	14.5 bc	15.2 ab	10 gm non-irradiated plant residues
13.2 c	13.1 de	13.3 de	20 gm non-irradiated plant residues
	13.8b	14.4a	variety average

Leaf area (cm²)

The results of Table (2) showed the effect of adding plant residues to rapeseed leaves on the leaf area trait of two soybean varieties. From comparing the averages of the varieties It is evident to us that the two types' averages differ

significantly, as the highest leaf area (14.3) cm² was recorded in the Al-Shimaa variety.

In terms of comparing the averages of plant residues, it was noted that there were clear significant differences, since all treatments showed a notable decline in comparison to the control treatment, with the lowest value (12.7) cm² was recorded when adding 20 gm of irradiated material, with a decrease rate of (18.58) % compared to the control.

As for the interaction between the varieties and plant residues, the results showed significant differences, as the lowest value (12.5) cm² was recorded when adding 20 gm of irradiated material in the Indian variety.

Table 2: Effect of plant residues of rapeseed leaves on leaf area (cm²) of two soybean varieties

residues average	Indian v.	Al-Shimaa v.	Varieties plant residues
15.6 a	15.2 ab	16.0 a	control
14.2 c	14.1 cd	14.3 bcd	10 gm irradiated plant residues
12.7 e	12.5 f	12.8 ef	20 gm irradiated plant residues
14.8 b	14.6 bc	15.0 bc	10 gm non-irradiated plant residues
13.4 d	13.2 ef	13.5 de	20 gm non-irradiated plant residues
	13.9 b	14.3 a	variety average

Relative water content (%)

The results of Table (3) indicated that adding plant residues to rapeseed leaves had a significant effect on the relative water content of soybeans and led to a significant decrease, as the lowest value for the studied trait (61.8) was recorded in the Indian variety. Regarding the averages of plant residues, the results showed that all treatments significantly decreased when compared to the control treatment, with the addition of 20 grams of irradiation material recording the lowest value (57.0), and the percentage of decrease reached (17.98) compared to the control. Regarding the interaction between the varieties and plant residues, the results indicated the presence of significant differences, as the lowest value (54.6) was recorded when adding 20 gm of irradiated material in the Indian variety.

Table 3: Effect of rapeseed leaf residues on the relative water content (RWC) of two soybean varieties

residues average	Indian v.	Al-Shimaa v.	Varieties plant residues
69.5 a	68.5 ab	70.4 a	control
63.5 c	62.8 de	64.2 cd	10 gm irradiated plant residues
57.0 e	54.6h	59.3 fg	20 gm irradiated plant residues
66.7 b	66.3 bc	67.0 bc	10 gm non-irradiated plant residues
59.0 d	56.7 gh	61.2ef	20 gm non-irradiated plant residues
	61.8 b	64.4 a	variety average

Total chlorophyll (mg/g fresh weight)

Through the results of Table (4), we can see the effect of adding plant residues to rapeseed leaves on the total chlorophyll content of the soybean plant. From comparing the averages of the varieties, it becomes clear to us that there are significant differences between the averages of the two varieties, as the highest value for the studied trait (1.72)

was recorded in the Al-Shimaa variety. Regarding the averages of plant residues, the results showed a significant decrease in all treatments compared to the control treatment, as the lowest value (1.48) was recorded when adding 20 gm of irradiated material, with a decrease rate of (19.56) % compared to the control. In terms of interaction between varieties and plant residues, significant differences were found, as the lowest value (1.40) was recorded when adding 20 gm of irradiated material in the Indian variety.

Table 4: Effect of plant residues of rapeseed leaves on the estimation of total chlorophyll (mg/g fresh weight) for two varieties of soybeans

residues average	Indian v.	Al-Shimaa v.	Varieties plant residues
1.84 a	1.78 b	1.90 a	control
1.65 c	1.56 e	1.73 bc	10 gm irradiated plant residues
1.48 d	1.40 g	1.55 ef	20 gm irradiated plant residues
1.75 b	1.67 cd	1.82 ab	10 gm non-irradiated plant residues
1.53 d	1.45 fg	1.60 de	20 gm non-irradiated plant residues
	1.57 b	1.72 a	variety average

Proline (micromol/g fresh weight)

The results of Table (5) showed the effect of adding plant residues to rapeseed leaves on the proline content of two soybean varieties. By comparing the averages of the varieties, it became evident to us that the two types' averages differed significantly, as the lowest value (3.76) was recorded in the Al-Shimaa variety. Regarding the comparison of plant residue averages, all therapies significantly outperformed the control treatment, according to the data, as the highest two values (4.21) and (4.13) were recorded when adding 20 gm of irradiated and non-irradiated, respectively, where the percentage of increase reached (16.62) % and (14.40) %, respectively, compared to the control treatment, noting that there are no significant differences between these two treatments. Regarding the interaction between varieties and plant residues, significant differences were observed, as the highest value (4.32) was recorded when adding 20 gm of irradiated material to the Indian variety, and the lowest value (3.42) was recorded in the control treatment in the Al-Shimaa variety.

Table 5: Effect of plant residues of rapeseed leaves on the estimation of proline (micromol/gram fresh weight) for two varieties of soybeans

residues average	Indian v.	Al-Shimaa v.	Varieties plant residues
3.61 c	3.80 def	3.42 g	control
3.87 b	4.05 a-d	3.68 efg	10 gm irradiated plant residues
4.21 a	4.32 a	4.10 abc	20 gm irradiated plant residues
3.78 bc	3.95 cde	3.60 fg	10 gm non-irradiated plant residues
4.13 a	4.25 ab	4.00 bcd	20 gm non-irradiated plant residues
	4.07 a	3.76 b	variety average

Superoxide dismutase (microgram/ml/minute)

The results of Table (6) indicated that adding plant residues to rapeseed leaves had a significant effect on the content of

superoxide dismutase in soybeans and led to a significant increase, as the highest value (0.058) was recorded in the Indian variety compared to the Al-Shaima variety.

As for comparing the averages of plant residues, Since the highest two values (0.061) and (0.058) were obtained after adding 20 gm of irradiated and non-irradiated, respectively, we discovered a significant increase in all treatments when compared to the control treatment, and the percentage of increase reached (103.3) % and (93.3) %, respectively, compared to the control treatment, noting that there are no significant differences between these two treatments. Regarding the interaction between the varieties and plant residues, the results indicated the presence of significant differences, as the highest two values (0.070) and (0.067) were recorded when adding 20 gm of irradiated and non-irradiated, respectively, in the Indian variety, while the lowest value (0.020) was in the control treatment in the Al-Shimaa variety.

Table 6: Effect of rapeseed leaf residues on the determination of superoxide dismutase (SOD) (microgram/ml/minute) for two soybean varieties.

residues average	Indian v.	Al-Shimaa v.	Varieties plant residues
0.030 c	0.040 d	0.020 e	control
0.049 b	0.059 b	0.039 d	10 gm irradiated plant residues
0.061 a	0.070 a	0.052 bc	20 gm irradiated plant residues
0.045 b	0.054 bc	0.035 d	10 gm non-irradiated plant residues
0.058 a	0.067 a	0.048 c	20 gm non-irradiated plant residues
	0.058 a	0.039 b	variety average

Discussion and Conclusions

Agricultural lands are considered one of the main production elements that must be exploited well to ensure that they remain productive on a permanent basis. The agricultural rotation system and the type of plant grown in these lands have a clear impact on the physical and chemical properties of the soil (Faysal *et al.*, 2012).^[8] Allelopathy is a biological phenomenon in which plants secrete chemical compounds known as allelochemicals that affect the germination, growth, and survival of other organisms around them, whether the effect is positive or negative (Ain *et al.*, 2023).^[1] This phenomenon can be exploited from a practical point of view by using plant residues that aim to improve the correct agricultural applications. Through our current study, the effect of plant residues of rape leaves exposed to ultraviolet rays with intensity (200-280) nanometers on germination, growth, as well as a few physiological and biochemical traits of two types of soybeans were examined. In comparison to untreated plants, the results demonstrated a significant visual decrease in plant height, leaf area, relative water content, and total chlorophyll for two soybean varieties and all additions of rapeseed leaf residues (10 g irradiated, 10 g non-irradiated, 20 g irradiated, and 20 g non-irradiated). The greatest decrease in plant height was brought about by the addition of 20 grams of radiation. leaf area, relative water content and total chlorophyll compared to the control. This is due to

the rapeseed plant containing effective allelopathic compounds that can affect the studied traits. These compounds include (benzoic acid, caffeic acid, chlorogenic acid, vanillic acid, syringic acid). These compounds are stimulating or inhibiting depending on the additions (Šćepanović *et al*, 2021).^[16] In addition, most of the allelopathic compounds decompose through a series of intermediate compounds that show allelopathic effects. Therefore, the partial transformation of one of the compounds may lead to the formation of many compounds that show allelopathic effects (Rice, 1984).^[14] Regarding the proline and superoxide dismutase (SOD) content of soybean plants, there was a clear significant increase in all additions to the plant residues of rapeseed leaves compared to the control treatment, as the addition of 20 grams of irradiated plants recorded the highest value, followed by the rest of the treatments, up to the control treatment. It is believed that the reason for this is that plants are exposed to environmental stresses during their growth stages, which leads to physiological and biochemical changes in the tissues of the vegetative groups. This leads to the accumulation of a number of osmotic compounds such as proline, which can be considered a mechanism for increasing growth, in addition to its role in the osmotic regulation of ions and elements (Li and Chen, 2000).^[11]

As for the increase in the enzyme superoxide dismutase (SOD), The rationale is that this enzyme is one of the regulatory enzymes that controls the amount of hydrogen peroxide (H₂O₂), hence preventing the generation of OH radicals that arise from photorespiration and the oxidation of fatty acids in organelles during metabolic processes (Bhutta, 2011).^[6] In addition, many studies indicate that allelopathic compounds increase the activity of antioxidant enzymes, especially when exposed to oxidative stresses such as heat, salinity and light, as they lead to the formation of reactive oxygen species. Which leads to the destruction of electron transport systems, and therefore plants have defense mechanisms to remove reactive oxygen species, including antioxidant enzymes (Faysal, 2020).^[7]

Reference

1. Ain Q, Mushtaq W, Shadab M, Siddiqui MB. Allelopathy: an alternative tool for sustainable agriculture. *Physiology and Molecular Biology of Plants*,2023;29(4):495-511.
2. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*,1949;24(1):1.
3. Bates LS, Waldren RP, Teares ID. Rapid determination of free proline for water-stress studies. *Plant and Soil*,1973;39(1):205-207.
4. Bello I, Adeniyi A, Mukaila T, Hammed A. Optimization of soybean protein extraction with ammonium hydroxide (NH₄OH) using response surface methodology. *Foods*,2023;12(7):1515.
5. Bosco G, Yang ZJ, Nandi J, Wang J, Chen C, Camporesi EM. Effects of hyperbaric oxygen on glucose, lactate, glycerol and antioxidant enzymes in the skeletal muscle of rats during ischaemia and reperfusion. *Clinical and Experimental Pharmacology and Physiology*,2007;34(1-2):70-76.
6. Bhutta WM. Antioxidant activity of enzymatic system of two different wheat (*Triticum aestivum* L.) cultivars growing under salt stress, 2011.
7. Faysal MS. Effect of allelopathic potential of corn, sunflower, field capacity and ascorbic acid in growth of two wheat cultivars. *Journal of Education and Science*,2020;29(2):260-278.
8. Faysal MS, Saleh FS, Hassan GQ. The effect of adding fenugreek and wheat residues on the growth and growth constants of the wheat plant *Trigonella foenum-graecum* L. *Journal of Basic Education College Research*, 2012, 12(2).
9. Kamal J. Allelopathy, a brief review. *J Nov Appl Sci*,2020;9(1):1-12.
10. Lee SB, Kim KH, Hahn SJ, Chung IM. Evaluation of screening methods to determine the allelopathic potential of rice varieties against *Echinochloa crus-galli* Beauv. var. *oryzicola* Ohwi, 2003.
11. Li ZY, Chen SY. Differential accumulation of the S-adenosylmethionine decarboxylase transcript in rice seedlings in response to salt and drought stresses. *Theoretical and Applied Genetics*,2000;100(5):782-788.
12. Mishra R, Tripathi MK, Sikarwar RS, Singh Y, Tripathi N. Soybean (*Glycine max* L. Merrill): A multipurpose legume shaping our world. *Plant Cell Biotechnology and Molecular Biology*,2024;25(3-4):17-37.
13. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*,1972;247(10):3170-3175.
14. Rice EL. *Allelopathy*, 2nd ed. Academic Press, 1984, 422. ISBN: 978-0-12-587058-0.
15. Rotundo JL, Marshall R, McCormick R, Truong SK, Styles D, Gerde JA, Rufino MC. European soybean to benefit people and the environment. *Scientific Reports*,2024;14(1):7612.
16. Šćepanović M, Sarić-Krsmanović M, Šoštarčić V, Brijačak E, Lakić J, Špirović Trifunović B, Radivojević L. Inhibitory effects of Brassicaceae cover crop on *Ambrosia artemisiifolia* germination and early growth. *Plants*,2021;10(4):794.
17. Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW. Water relations in winter wheat as drought resistance indicator. *Crop Science*,1988;28(3):526-531.
18. Turner NC. Techniques and experimental approaches for the measurements of plant water status. *Plant and Soil*,1981;58(1):339-366.
19. Umoh FO, Ekwere OJ, Udoh UM, Akwang EG. Effects of animal manure on the performance of soybean (*Glycine max* L. Merrill) grown on ultisols, Akwa Ibom State, Nigeria. *AKSU Journal of Agriculture and Food Sciences*,2023;7(2):34-44.
20. Al-Deliemy AOA, Abdul-Ameer MA. The allelopathic effect of root exudates of mungbean and maize plants affected by metabolic, fertilizer and mechanical stress factors on some indicators of germination of cowpea seeds. In: *IOP Conference Series: Earth and Environmental Science*,2023;1213(1):012026. IOP Publishing.