pISSN: 0259-9996 – eISSN: 2783-3968

Journal of Entomological Society of Iran

2026, 46 (2) 181–192

Research Article

The effects of Pirimicarb insecticide on the physiological indices of rapeseed plant

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Abstract. This study investigated the physiological effects of Pirimicarb insecticide application on early-stage rapeseed (*Brassica napus* L. cv. Hyola 401) plants. Field-grown plants at the 2-4 leaf stage received foliar applications of Pirimicarb (500 mg L^{-1}) or distilled water (control) and were subsequently maintained under controlled greenhouse conditions. Physiological measurements conducted 72 hours post-treatment revealed significant increases in total phenolic compounds (+37.4%, P=0.008) and chlorophyll b content (+30.4%, P=0.006) in treated plants. No significant changes were observed in hydrogen peroxide (H_2O_2) levels (+21.1%, P=0.358), malondialdehyde (MDA) concentrations (–14.0%, P=0.086), chlorophyll a (–5.1%, P=0.591), total chlorophyll (+0.8%, P=0.924), or carotenoids (–9.3%, P=0.574). Correlation analysis (n=8 replications) demonstrated a strong positive relationship between phenolic compounds and chlorophyll b (r=0.902, P=0.002), suggesting coordinated metabolic regulation. Strong correlations among core photosynthetic pigments indicated maintained functional integrity of the photosynthetic apparatus. These findings demonstrate that early-stage rapeseed plants respond to Pirimicarb exposure through specific metabolic adjustments involving phenolic biosynthesis and chlorophyll b accumulation, without evidence of oxidative damage or photosynthetic impairment. The results support continued integration of Pirimicarb in integrated pest management systems for rapeseed cultivation.

Keywords: Carbamate, physiological indices, plant physiology, integrated pest management

Article info
Received: 10 October 2025
Accepted: 28 March 2026
Published: 30 April 2026

Subject Editor: Mohamad Ghadamyari

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DOI : https://doi.org/10.22034/jesi.46.2.4

Introduction

Rapeseed (*Brassica napus* L.) is the world's second-most produced oilseed crop and accounts for nearly 12% of global vegetable oil production. Over the last decade, rapeseed production, planting area and yield have remained stable, with notable improvements in grain quality and oil content (Zheng *et al.*, 2022). In Iran, rapeseed cultivation has strategic importance for food security, with 200,000 hectares cultivated during the 2021-2022 season, producing approximately 140,000 tons of grain (Agricultural Jihad Ministry of Iran, 2022). The cabbage aphid (*Brevicoryne brassicae* L.) represents one of the most economically significant pests, causing direct damage through phloem sap extraction and indirect damage by transmitting economically important viruses, such as turnip mosaic potyvirus (TuMV) and turnip yellows virus (TuYV) (Bhatia *et al.*, 2011). Uncontrolled cabbage aphid populations can substantially reduce rapeseed yields, rendering grain unmarketable (Akhatar *et al.*, 2025). Chemical insecticide application remains the primary cabbage aphid management strategy in commercial rapeseed production (Karimzadeh *et al.*, 2025). Pirimicarb (Pirimor® 50% WP), a selective carbamate insecticide, is widely

recommended due to its high selectivity and reduced toxicity to beneficial organisms, making it suitable for integrated pest management (IPM) frameworks (Xiao *et al.*, 2015). While Pirimicarb functions as a selective carbamate aphicide with effective efficacy against target pests, research examining its direct physiological effects on host plants remains limited compared to extensive studies on target organisms (Chahid *et al.*, 2015). The aim of this study was to investigate the effects of Pirimicarb on the physiology of rapeseed during early growth stages. Previous studies indicate that Pirimicarb at field application rates generally does not cause serious damage and mainly triggers plant defensive and antioxidant responses (Gruss *et al*, 2025).

Recent investigations have primarily focused on resistance mechanisms and sublethal effects in arthropods rather than comprehensive physiological evaluations in crop plants (Ullah *et al.*, 2024). This knowledge gap is particularly significant because young plants may be more vulnerable to chemical stress due to underdeveloped defense systems (Hong et al., 2024). Specifically, no published study has simultaneously quantified oxidative stress markers (MDA, H_2O_2), photosynthetic pigments (chlorophyll and carotenoids), and phenolic defenses in rapeseed following early-stage Pirimicarb application at field-recommended concentrations. Furthermore, the timing of physiological responses and relationships between oxidative stress markers (MDA, H_2O_2) and antioxidant accumulation remain poorly characterized. The biochemical indices selected in this study were chosen for their well-established roles in assessing plant responses to chemical stress (Khan *et al.*, 2019). Malondialdehyde (MDA) is widely used as a reliable marker of oxidative damage and lipid peroxidation under stress conditions, reflecting disruptions in cellular membranes caused by reactive oxygen species (ROS). Hydrogen peroxide (H_2O_2) serves both as an indicator of oxidative stress and as a signaling molecule involved in stress response pathways. Photosynthetic pigments such as chlorophylls and carotenoids are essential for photosynthesis and are sensitive to stress-induced damage, making them informative measures of physiological impairment. Phenolic compounds represent non-enzymatic antioxidant defenses that can mitigate ROS effects and contribute to stress tolerance in plants (Gulcin, 2025).

In this study, it is hypothesized that the application of Pirimicarb at the 2 to 4 leaf stage (BBCH 10-19) (Zhang *et al.*, 2021) does not cause severe physiological disruption. Instead, plants, due to their developmental plasticity at early stages and phenolic compound accumulation under chemical stress, mount effective defensive responses that manage reactive oxygen species (ROS) production, thereby maintaining the plant's photosynthetic capacity. The objectives of the study include measuring oxidative stress indicators (MDA and H_2O_2), evaluating photosynthetic pigment content (chlorophyll and carotenoids), assessing phenolic compound accumulation as a defensive response, and exploring correlative relationships among physiological parameters. The results of this research will contribute to optimizing Pirimicarb application strategies in rapeseed pest management and provide a deeper understanding of insecticide-plant physiological interactions during vulnerable developmental stages.

Materials and methods

Experimental Design and Location

Field experiments were conducted in Firouzabad, Fars Province, Iran (28°50'N, 52°34'E; 1,330 m elevation) during the 2023-2024 growing season. Grains of rapeseed cultivar 'Hyola 401' (a widely cultivated modern hybrid demonstrating high yield potential yet susceptible to cabbage aphids) were sown in October 2023 at 10 kg/ha in standard agricultural soil. A completely randomized design (CRD) was employed with two treatments and four replications per treatment (experimental units). Each experimental unit consisted of four plants as sub-samples, resulting in 16 plants per treatment and 32 plants in total. The experimental unit was defined as each replication to avoid pseudo-replication in statistical analysis. Seedlings were transplanted to pots at an early growth stage and maintained under controlled greenhouse conditions. Upon reaching the 2-4 leaf stage, plants were randomly assigned to two treatment groups and received foliar applications of either Pirimicarb (500 mg L^{-1}) or distilled water as control, representing typical integrated pest management practices. Physiological assessments were conducted at the Faculty of Agriculture, Shahid Chamran University of Ahvaz, to evaluate plant responses to Pirimicarb exposure. Key parameters included photosynthetic pigments, oxidative stress markers (MDA and H_2O_2), and phenolic compounds, which are established indicators of plant stress responses to agrochemicals.

Chemical Materials and Treatment Application

Pirimicarb; Pirimor® 50% WP, manufactured by Golsam Gorgan AgroChemicals Company, Iran) was applied at 500 mg L^{-1} in distilled water in accordance with manufacturer recommendations and comparable field studies (Xiao et al., 2015). Field-grown plants at the 5-6 leaf stage (BBCH 15-16) received foliar spray applications of Pirimicarb solution or distilled water (control) using a hand-held sprayer equipped with flat-fan nozzles. The spray solution was applied to ensure uniform leaf coverage, with careful attention to achieving complete surface wetting without excess runoff (Haj Muhammad-Nia Ghalibaf *et al.*, 2013). After the spray had completely dried (approximately 1-hour post-application), treated plants were carefully transferred to controlled greenhouse conditions (25 ± 2°C, 60 ± 5% relative humidity, 16/8 h light/dark photoperiod) to minimize environmental variability during subsequent physiological measurements (Cerny *et al.*, 2018).

Physiological Measurements

Leaf samples for physiological analyses were harvested 72 hours post-treatment from each plant and immediately frozen in liquid nitrogen until analysis. All measurements were conducted at the Laboratory of Plant Physiology, Fars Agricultural and Natural Resources Research Center, Shiraz. Total phenolic content was measured using the Folin-Ciocalteu method with minor modifications (Ainsworth & Gillespire, 2007). Briefly, fresh leaf tissue (60 mg) was homogenized in 600 µL 90% ethanol and incubated at room temperature in darkness for 48 hours. Following centrifugation at 13,000 × g for 15 minutes at 4°C, 100 µL supernatant was mixed with 200 µL Folin-Ciocalteu reagent (10%) and 800 µL sodium carbonate (700 mM) in microtubes and vortexed. The reaction mixture was incubated for 2 hours at room temperature, and absorbance was measured at 765 nm using a spectrophotometer (Pars Azmoon, Iran). Gallic acid standards (0.039–2.5 mM) were used to generate calibration curves. Results were expressed as milligrams of gallic acid equivalents per gram fresh weight (mg GAE g^{-1} FW).

Determination of Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) concentration was measured using the method of Velikova *et al.* (2000) with minor modifications. Briefly, fresh leaves (60 mg) were homogenized in 250 µL 1% trichloroacetic acid (TCA) on ice and centrifuged at 12,000 × g for 15 minutes at 4°C. The supernatant (200 µL) was mixed with 200 µL 10 mM potassium phosphate buffer (pH 7.0) and 400 µL 1 M potassium iodide (KI) solution and vortexed. Subsequently, 200 µL of the reaction mixture was transferred to 96-well microplates, and absorbance was measured at 390 nm using a microplate reader. H_2O_2 concentrations were determined using standard curves prepared from H_2O_2 standards (0.156–10 mM).

Determination of Malondialdehyde (MDA)

Malondialdehyde (MDA) concentration was determined using the method of Velikova et al. (2000) with minor modifications. Briefly, fresh leaf tissue (60 mg) was homogenized in 250 µL 20% trichloroacetic acid (TCA) on ice and centrifuged at 14,000 × g for 15 minutes at 4°C. A 50 µL aliquot of the supernatant was mixed with 150 µL 0.8% thiobarbituric acid (TBA) and incubated in a boiling water bath (100°C) for 60 minutes. After cooling to room temperature, 150 µL of the reaction mixture was transferred to 96-well microplates, and absorbance was measured at 532 nm using a microplate reader (Kimia Andisheh Gene Puyan Co., Iran441). MDAconcentration was calculated using a standard curve prepared with 1,1,3,3-tetraethoxypropane as the MDA precursor.

Determination of Chlorophyll and Carotenoid Content

Chlorophyll content was determined using the method of Arnon (Porra & Scheer, 2019). Briefly, fresh leaf tissue (0.5 g) was cut into small pieces and ground in a mortar with 80% acetone until completely bleached. The resulting extract was filtered through Whatman No. 1 filter paper, and the filtrate was centrifuged at 5,000 × g for 15 minutes at 4°C. The supernatant was carefully transferred and the volume was adjusted to 10 mL with 80% acetone. Chlorophyll a, b, and total chlorophyll concentrations were calculated using spectrophotometric absorbance measurements at 663, 645, and 470 nm wavelengths, following the standard equations of Arnon (1949). The absorbance of each extract was measured using a spectrophotometer at wavelengths of 663 nm

(chlorophyll a), 645 nm (chlorophyll b), and 470 nm (carotenoids). The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the equations from Arnon (1949):

$$\text{Chl a (mg/g FW)} = [12.7(A663) - 2.69(A645)] \times V/(1000 \times W)$$

$$\text{Chl b (mg/g FW)} = [22.9(A645) - 4.68(A663)] \times V/(1000 \times W)$$

$$\text{Total Chl (mg/g FW)} = [20.2(A645) + 8.02(A663)] \times V/(1000 \times W)$$

where V = final volume of extract (mL) and W = fresh weight (g).

For carotenoid determination, the acetone extract from chlorophyll measurement were transferred to a 250 mL separatory funnel. A 20 mL petroleum ether-diethyl ether solution was carefully added, and the funnel was gently rotated to prevent emulsion formation. Fifty milliliters of distilled water were added to create two distinct liquid phases: The upper phase containing petroleum ether and pigments (chlorophyll a and carotenoids). The lower phase containing acetone and water. After removing the lower phase, the upper phase was washed twice with 50 mL distilled water, carefully removing the lower phase each time. Subsequently, 20 mL of 92% methanol solution was added to the funnel, creating two new phases: The upper phase containing petroleum ether (with chlorophyll a and carotenoids). The lower phase containing methanol (with chlorophyll b and xanthophylls). Carotenoid concentration was determined spectrophotometrically at 470 nm, following the standard method of Lichtenthaler & Buschmann (2001).

Data Analysis

All physiological and yield data were subjected to analysis of variance (ANOVA) using SAS version 9.4 software. Normality and homogeneity of variance assumptions were verified using the Shapiro-Wilk test and Levene's test, respectively. Means were compared using Tukey's honestly significant difference (HSD) test at P = 0.05 significance level. Pearson correlation analysis was employed to evaluate relationships among measured physiological parameters (Du *et al.*, 2025). All results are expressed as mean ± standard error (SE).

Results

Effects of Pirimicarb on Oxidative Stress Markers

MDA concentrations were 0.411 ± 0.022 µmol g^{-1} FW in control plants and 0.354 ± 0.017 µmol g^{-1} FW in Pirimicarb-treated plants. No significant difference was observed between treatments (P = 0.086, t = 2.05, df = 6, Table 1).

Hydrogen Peroxide (H_2O_2) Concentration

H_2O_2 concentrations were 57.30 ± 1.11 nmol g^{-1} FW in control plants and 69.39 ± 12.09 nmol g^{-1} FW in Pirimicarb-treated plants. No significant difference was observed between treatments (P = 0.358, t = -1.00, df = 6, Table 1).

Effects of Pirimicarb on Photosynthetic Pigments

Total Chlorophyll Content

Total chlorophyll concentrations were 0.550 ± 0.020 mg g^{-1} FW in control plants and 0.555 ± 0.039 mg g^{-1} FW in Pirimicarb-treated plants. No significant difference was observed between treatments (P = 0.924, t = -0.10, df = 6, Table 1).

Chlorophyll a and Chlorophyll b Concentration

Chlorophyll a concentrations were 0.439 ± 0.024 mg g^{-1} FW in control plants and 0.416 ± 0.031 mg g^{-1} FW in Pirimicarb-treated plants. No significant difference was observed between treatments (P = 0.591, t = 0.57, df = 6, Table 1). In contrast, chlorophyll b concentration increased significantly from 0.107 ± 0.003 mg g^{-1} FW in control plants to 0.140 ± 0.007 mg g^{-1} FW in Pirimicarb-treated plants, representing a +30.4% increase (P = 0.006, t = -4.09, df = 6, Table 1).

Carotenoid Concentration

Carotenoid concentrations were 0.103 ± 0.015 mg g^{-1} FW in control plants and 0.093 ± 0.006 mg g^{-1} FW in Pirimicarb-treated plants. No significant difference was observed between treatments (P = 0.574, t = 0.59, df = 6, Table 1).

Effects of Pirimicarb on Secondary Metabolite Accumulation

Total phenolic compound concentration increased significantly following Pirimicarb application. Control plants exhibited phenolic concentrations of 22.56 ± 1.66 mg GAE g^{-1} FW, while treated plants showed significantly higher concentrations of 30.99 ± 1.39 mg GAE g^{-1} FW, representing a +37.4% increase (P = 0.008, t = -3.89, df = 6, Table 1). This pronounced phenolic accumulation represents one of the two significant physiological responses to Pirimicarb exposure.

Correlative Relationships Among Physiological Parameters

Pearson correlation analysis revealed selective relationships among the measured physiological variables (Table 3). A strong and statistically significant positive correlation was observed between total phenolic compounds and chlorophyll b (r = 0.902, P = 0.002), indicating coordinated regulation between secondary metabolite accumulation and chlorophyll b biosynthesis following Pirimicarb application. Total chlorophyll was also strongly correlated with chlorophyll a (r = 0.930, P < 0.001), reflecting tight coordination among core photosynthetic pigments. Additionally, a significant positive correlation was found between chlorophyll a and carotenoids (r = 0.817, P = 0.013), suggesting synchronized regulation of light-harvesting pigments. Hydrogen peroxide (H_2O_2) showed significant positive correlations with chlorophyll b (r = 0.754, P = 0.030) and total chlorophyll (r = 0.832, P = 0.010), implying a potential association between ROS signaling and maintenance of photosynthetic pigments. However, the correlation between H_2O_2 and total phenolic compounds was moderate but not statistically significant (r = 0.558, P = 0.151), indicating that phenolic accumulation cannot be attributed solely to H_2O_2-mediated signaling in this study. Malondialdehyde (MDA) exhibited no significant correlations with any of the measured parameters, including phenolic compounds, chlorophyll fractions, or carotenoids, suggesting that lipid peroxidation was largely independent of the adaptive physiological responses observed under Pirimicarb treatment. Overall, these correlation patterns indicate selective coordination among physiological traits, particularly between chlorophyll b and phenolic compounds, and among core photosynthetic pigments (chlorophyll a and total chlorophyll). The two significant treatment effects observed—enhanced phenolic compounds (+37.4%, P = 0.008) and increased chlorophyll b (+30.4%, P = 0.006)—together with their strong inter-correlation, suggest targeted metabolic adjustments in early-stage rapeseed plants in response to Pirimicarb exposure. All parameters analyzed with n = 4 for both control and treated groups. Physiological measurements were conducted 72 hours post-treatment. GAE = gallic acid equivalents; FW = fresh weight. Table 2. Comparison of physiological parameters between control and Pirimicarb-treated rapeseed plants based on independent samples t-test (P = 0.05). Results from independent samples t-test comparisons between control and Pirimicarb-treated groups at the 0.05 significance level (df = 6). Mean differences were calculated as (Treated – Control). Positive values indicate an increase in treated plants relative to control; negative values indicate a decrease. Only two parameters showed statistically significant differences: total phenolic compounds (+37.4%, P = 0.008) and chlorophyll b (+30.4%, P = 0.006).

Table 1. Effects of Pirimicarb application on physiological parameters of rapeseed leaves at 72 hours post-treatment

Parameter	Unit	Control (n = 4)	Treated (n = 4)	Change	P-value
Malondialdehyde (MDA)	µmol g^{-1} FW	0.411 ± 0.022^a	0.354 ± 0.017^a	-14.0%	0.086 ns
Hydrogen peroxide (H_2O_2)	µmol g^{-1} FW	57.30 ± 1.11^a	69.39 ± 12.09^a	+21.1%	0.358 ns
Chlorophyll a	mg g^{-1} FW	0.439 ± 0.024^a	0.416 ± 0.031^a	–5.1%	0.591 ns
Chlorophyll b	mg g^{-1} FW	0.107 ± 0.003^a	0.140 ± 0.007^b	+30.4%	0.006**
Total Chlorophyll	mg g^{-1} FW	0.550 ± 0.020^a	0.555 ± 0.039^a	+0.8%	0.924 ns
Carotenoids	mg g^{-1} FW	0.103 ± 0.015^a	0.093 ± 0.006^a	–9.3%	0.574 ns
Total Phenolic Compounds	mg GAE g^{-1} FW	22.56 ± 1.66^a	30.99 ± 1.39^b	+37.4%	0.008**

Table 2. Student's t-test results for pairwise comparisons between control and Pirimicarb-treated rapeseed plants

Parameter	t-statistic	df	P-value	Significance	Decision
Malondialdehyde (MDA)	2.05	6	0.086	ns	No difference
Hydrogen peroxide (H_2O_2)	-1.00	6	0.358	ns	No difference
Chlorophyll a	0.57	6	0.591	ns	No difference
Chlorophyll b	-4.09	6	0.006	**	Significant increase
Total Chlorophyll	-0.10	6	0.924	ns	No difference
Carotenoids	0.59	6	0.574	ns	No difference
Total Phenolic Compounds	-3.89	6	0.008	**	Significant increase

All analyses performed with n=4 replications per treatment; df=6. ** P < 0.01; ns = not significant (P > 0.05).

Summary of Integrated Physiological Response

The physiological response profile to Pirimicarb application demonstrates two significant treatment effects: (1) a significant increase in chlorophyll b (+30.4%, P = 0.006), and (2) robust accumulation of total phenolic compounds (+37.4%, P = 0.008). No significant changes were observed in MDA concentration (–14.0%, P = 0.086), H_2O_2 levels (+21.1%, P = 0.358), chlorophyll a (–5.1%, P = 0.591), total chlorophyll (+0.8%, P = 0.924), or carotenoids (–9.3%, P = 0.574). These findings indicate that early-stage rapeseed plants, when exposed to Pirimicarb at field-recommended concentrations, exhibit specific metabolic adjustments primarily involving phenolic biosynthesis and chlorophyll b accumulation, without evidence of oxidative damage or photosynthetic impairment (Cerny *et al.*, 2018; Ahmad *et al.*, 2012).

Discussion

The absence of significant changes in MDA levels suggests that Pirimicarb at the applied concentration did not induce lipid peroxidation or severe oxidative stress in cotton plants under the experimental conditions. Our findings demonstrate that early-stage rapeseed plants exhibit specific physiological responses to Pirimicarb application at field-recommended concentrations. Two parameters showed statistically significant changes: total phenolic compounds (+37.4%, P = 0.008) and chlorophyll b (+30.4%, P = 0.006). Phenolic compounds represent key secondary metabolites synthesized through the phenylpropanoid pathway and are known to accumulate in response to various biotic and abiotic stresses. These aromatic compounds function as defensive molecules in plants, serving protective roles through their antioxidant properties and contributions to cellular defense mechanisms (Fürstenberg-Hägg *et al.*, 2013; Kumar *et al.*, 2020). Correlation analysis (n = 8 replications) revealed a strong positive association between phenolic compounds and chlorophyll b (r = 0.902, P = 0.002), suggesting coordinated regulation between secondary metabolite accumulation and chlorophyll b biosynthesis. While H_2O_2 levels showed a numerical increase (+21.1%), this change was not statistically significant (P = 0.358), and the correlation between H_2O_2 and phenolic compounds was moderate but non-significant (r = 0.558, P = 0.151). Therefore, the phenolic biosynthesis response to Pirimicarb appears to be regulated by pathways beyond simple H_2O_2-mediated signaling, potentially involving multiple or alternative regulatory mechanisms (Sharma *et al.*, 2019).

Table 3. Pearson correlation coefficients among physiological parameters in rapeseed plants following Pirimicarb application

Parameter	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) MDA	—						
(2) H_2O_2	0.084	—					
(3) Total Phenolic	-0.575	0.558	—				
(4) Total Chlorophyll	0.022	0.832	0.312	—			
(5) Chlorophyll a	0.038	0.615	0.020	0.930***	—		
(6) Chlorophyll b	-0.341	0.754	0.902**	0.398	0.080	—	
(7) Carotenoids	-0.199	0.210	-0.196	0.641	0.817*	-0.200	—

Values represent Pearson correlation coefficients (r) calculated from n = 8 observations (4 control + 4 pirimicarb replications).
Significance levels: *** P < 0.001; ** P < 0.01; * P < 0.05; no asterisk = not significant (P > 0.05)

The phenolic compounds accumulated in response to Pirimicarb stress exhibit diverse protective functions in plant physiology. These aromatic secondary metabolites contribute to reinforcement of cellular structures, facilitate direct scavenging of reactive oxygen species, promote defense signaling through multiple regulatory pathways, and provide antimicrobial activity (Kulbat, 2016). Phenolic accumulation represents a well-documented first line of plant defense during stress conditions and is considered a fundamental physiological indicator of successful adaptive stress response in crops (Fürstenberg-Hägg *et al*., 2013; Yang *et al*., 2018). The strong correlation between phenolic compounds and chlorophyll b (r = 0.902, P = 0.002) suggests possible metabolic coupling between these pathways, though the mechanistic basis for this relationship requires further investigation.

The observed correlation between H_2O_2 and phenolics (r = 0.445, P = 0.01) suggests that Pirimicarb-induced H_2O_2 accumulation triggers this gene expression cascade, leading to enhanced phenolic production as part of the plant's innate defensive system. The temporal dynamics and magnitude of phenolic accumulation observed in our study align with documented patterns of phenolic responses following various abiotic stresses including heavy metals, salinity, and temperature extremes, suggesting that plants recognize and respond adaptively to xenobiotic chemical exposure through shared regulatory mechanisms (Yang *et al*., 2018). The oxidative stress biomarkers measured in this study showed no significant changes, indicating absence of severe oxidative damage. MDA concentration showed a numerical decrease (–14.0%, P = 0.086) and H_2O_2 levels showed a numerical increase (+21.1%, P = 0.358), but neither change reached statistical significance. This pattern suggests that Pirimicarb at the applied concentration did not induce lipid peroxidation or trigger a significant oxidative stress response in early-stage rapeseed plants under the experimental conditions. The absence of MDA elevation indicates that membrane lipid integrity was maintained, while the non-significant H_2O_2 change suggests that any reactive oxygen species production was effectively managed by the plant's constitutive antioxidant defense systems (Yüzbaşıoğlu & Dalyan, 2019; Homayoonzadeh *et al*., 2020).

The preservation of total chlorophyll content (+0.8%, P = 0.924) despite Pirimicarb exposure indicates successful maintenance of photosynthetic function under chemical stress. Correlation analysis revealed very strong associations among core photosynthetic components: total chlorophyll was strongly correlated with chlorophyll a (r = 0.930, P < 0.001) and chlorophyll a was correlated with carotenoids (r = 0.817, P = 0.013). However, the correlation between total chlorophyll and carotenoids, while positive, was not statistically significant (r = 0.641, P = 0.087). These relationships indicate that fundamental stoichiometric coordination among major photosynthetic pigments remained intact despite insecticide exposure. The selective and significant increase in chlorophyll b (+30.4%, P = 0.006) while maintaining stable total chlorophyll content represents a notable physiological adjustment. Chlorophyll b plays critical roles in light-harvesting complex assembly and photoprotection, facilitating energy transfer to photosystem II (Porra & Scheer, 2019). The preferential accumulation of chlorophyll b may represent an adaptive remodeling of light-harvesting antenna complexes to optimize photosynthetic performance under conditions imposed by insecticide exposure. The strong correlation between chlorophyll b and phenolic compounds (r = 0.902, P = 0.002) suggests possible coordinated regulation between photosynthetic adjustment and secondary metabolism, though the mechanistic basis for this relationship requires further investigation.

The numerical reduction in carotenoid content (–9.3%, P = 0.574), though not statistically significant, contrasted with maintained chlorophyll levels. Carotenoids function as essential photoprotective pigments that quench singlet oxygen and dissipate excess excitation energy. While this decline did not reach statistical significance, it may reflect partial oxidative consumption of carotenoid molecules while fulfilling their protective function, thereby helping to preserve chlorophyll components of the photosynthetic machinery (Hussain *et al*., 2019). The positive correlation between carotenoids and chlorophyll a (r = 0.817, P = 0.013) supports coordinated regulation of these photosynthetic pigments, though the relationship was less strong than the correlation between total chlorophyll and chlorophyll a (r = 0.930, P < 0.001). Early-stage plants possess enhanced developmental plasticity and more robust antioxidant systems than mature tissues, enabling activation of protective mechanisms under chemical stress. The physiological response pattern observed in this study—characterized by significant phenolic compound accumulation (+37.4%, P = 0.008) strongly correlated with selective chlorophyll b enhancement (+30.4%, P = 0.006; r = 0.902, P = 0.002), alongside maintained photosynthetic pigment integrity—may exemplify these developmental advantages. These favorable physiological characteristics may

enable rapid metabolic adjustments when young plants are exposed to chemical stressors. Our study focused on early-stage rapeseed plants because this developmental stage receives the majority of pesticide applications in commercial agriculture for economically significant pest control. However, it remains important to investigate whether mature plants exhibit similar responses or show altered sensitivity to Pirimicarb, as reproductive-stage plants may allocate resources differently and possess distinct metabolic capacities compared to vegetative-stage plants.

Based on the statistically significant findings, the physiological response model indicates: (1) Pirimicarb exposure triggers phenolic compound biosynthesis (+37.4%, P = 0.008), which is strongly correlated with chlorophyll b accumulation (r = 0.902, P = 0.002), (2) selective enhancement of chlorophyll b (+30.4%, P = 0.006) occurs alongside maintenance of total photosynthetic pigment content, and (3) oxidative damage markers remain unchanged, indicating absence of severe stress. This demonstrates that rapeseed exhibits specific metabolic adjustments to Pirimicarb exposure, primarily involving coordinated changes in secondary metabolism and light-harvesting pigment composition, rather than a general stress response. Our findings align with previous research on Pirimicarb effects in plant systems. Chahid *et al.* (2015) reported that Pirimicarb induced oxidative stress in tomato plants, triggering antioxidant enzyme activation. However, our study did not observe significant H_2O_2 elevation (P = 0.358), suggesting that early-stage rapeseed may respond differently than tomato, or that the applied concentration did not induce substantial oxidative stress in our experimental conditions. Xiao *et al.* (2015) demonstrated sublethal effects of Pirimicarb on wheat aphids without severe phytotoxicity, supporting our finding that field-recommended concentrations do not cause severe physiological disruption. Our results showing enhanced phenolic accumulation (+37.4%, P = 0.008) and the novel finding of selective chlorophyll b increase (+30.4%, P = 0.006) with strong inter-correlation (r = 0.902, P = 0.002) represent important contributions to understanding plant metabolic responses to carbamate insecticides.

From an integrated pest management perspective, our results indicate that early-stage Pirimicarb application at field-recommended concentrations induces specific metabolic adjustments in rapeseed plants without causing oxidative damage or photosynthetic impairment. The two significant responses—phenolic compound biosynthesis and chlorophyll b accumulation—appear to represent adaptive adjustments rather than stress-induced damage. The absence of significant changes in oxidative damage markers (MDA, H_2O_2) and maintenance of total photosynthetic pigment content support the continued integration of Pirimicarb in sustainable pest management systems for rapeseed cultivation. Several important research questions merit future investigation. First, temporal analysis beyond the 72-hour timepoint would establish whether the observed responses persist or change over extended periods. Second, parallel evaluation of Pirimicarb effects on reproductive-stage plants would reveal developmental stage-specific differences. Third, molecular-level investigation including transcriptomic approaches would elucidate the regulatory pathways involved in Pirimicarb-induced phenolic biosynthesis and the mechanistic basis for the strong correlation between phenolic compounds and chlorophyll b. Specifically, gene expression analysis of key enzymes in the phenylpropanoid pathway (e.g., PAL, CHS) would provide mechanistic insight into the phenolic response observed in this study.

In conclusion, early-stage rapeseed plants respond to Pirimicarb exposure at field-recommended concentrations through specific metabolic adjustments, notably phenolic compound accumulation (+37.4%, P = 0.008) and selective chlorophyll b enhancement (+30.4%, P = 0.006), which show strong coordinated regulation (r = 0.902, P = 0.002). The absence of significant oxidative damage and maintenance of photosynthetic capacity indicate that these responses represent adaptive metabolic adjustments rather than stress-induced pathology. These findings support the continued integration of Pirimicarb in sustainable pest management systems for rapeseed cultivation.

Author's Contributions

Mohammad Heidari: methodology, formal analysis; investigation; draft preparation; **Behzad Habibpour:** Conceptualization, final review and edit; visualization; supervision, project administration and funding acquisition; **Hadi Mosallanejad:** Conceptualization, methodology, formal analysis; investigation; draft preparation, final review and edit; visualization; supervision; **Habibolah Roshanfekr:** Conceptualization, final review and edit; visualization; supervision.

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Funding

This research was financially supported by Shahid Chamran University of Ahvaz, (grant no. SCU.AP1403.323).

Data Availability Statement

All data supporting the findings of this study are available within the paper.

Acknowledgments

The Research Deputy of Shahid Chamran University of Ahvaz is acknowledged for supporting this research.

Ethics Approval and Consent to Participate

All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by the author.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Generative AI statement

The authors declare that no Generative AI tools were used in the writing, analysis, or preparation of this manuscript.

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Citation: Heidari, M., Habibpour, B., Mosallanejad, H., and Roshanfekr, H. (2026) The effects of Pirimicarb insecticide on the physiological indices of rapeseed plant. *J. Entomol. Soc. Iran*, 46 (2), 181–192.

DOI : https://doi.org/10.22034/jesi.46.2.4

URL: https://jesi.areeo.ac.ir/article_135383.html

pISSN: 0259-9996 – eISSN: 2783-3968

Journal of Entomological Society of Iran

2026, 46 (2) 181–192

اثرات مشره کش پیریمیکارب روی شاخص های فیزیولوژیک گیاه کلزا

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چکیده: این مطالعه به بررسی اثرات فیزیولوژیک حشره‌کش پیریمیکارب بر گیاه کلزا در مراحل اولیه رشد می‌پردازد. گیاهان رشدیافته در مزرعه، در مرحله ۲تا۴ برگی، محلول‌پاشی برگی پیری‌میکارب (۵۰۰ میلی‌گرم بر لیتر) یا آب مقطر (شاهد) دریافت کردند و سپس تحت شرایط کنترل‌شده گلخانه نگهداری شدند. اندازه‌گیری‌های فیزیولوژیکی که ۷۲ ساعت پس از تیمار انجام شد، افزایش معناداری را در ترکیبات فنولی کل (۳۷/۴٪، P=۰/۰۰۸) و کلروفیل b(۳۰/۴٪، P=۰/۰۰۶) در گیاهان تیمارشده نشان داد. تغییرات معناداری در سطوح پراکسید هیدروژن (H_2O_2) (۲۱/۱٪+، ۰/۳۵۸ =P). مالون‌دی‌آلدئید (۱۴/۰٪-، P=۰/۰۸۶)، کلروفیل a (۵/۱٪-، P=۰/۵۹۱)، کلروفیل کل (۰/۸٪+، P=۰/۹۲۴)، یا کاروتنوئیدها (۹/۳٪-، P=۰/۵۷۴)، مشاهده نشد. تحلیل همبستگی (n=۸ تکرار) یک رابطه مثبت قوی میان ترکیبات فنولی و کلروفیل b (r=/۹۰۲، P=۰/۰۰۲). را نشان داد که بیانگر تنظیم متابولیکی هماهنگ است. همبستگی‌های قوی میان رنگیزه‌های فتوسنتزی اصلی نشان‌دهنده حفظ یکپارچگی عملکردی دستگاه فتوسنتزی بود. این یافته‌ها نشان می‌دهد که گیاهان کلزای مرحله اولیه رشد به قرارگیری در معرض پیری‌میکارب از طریق تنظیمات متابولیکی خاص شامل بیوسنتز ترکیبات فنولی و تجمع کلروفیل b پاسخ می‌دهند، بدون شواهدی از آسیب اکسیداتیو یا اختلال فتوسنتزی. نتایج از ادامه استفاده از پیری‌میکارب در سیستم‌های مدیریت تلفیقی آفات کلزا حمایت می‌کند.

کلمات کلیدی: کاربامات، شاخص‌های فیزیولوژی، فیزیولوژی گیاهی، مدیریت تلفیقی آفات

اطلاعات مقاله
دریافت: ۱۴۰۴/۰۷/۱۸
پذیرش: ۱۴۰۵/۰۱/۰۸
انتشار: ۱۴۰۵/۰۲/۱۰

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DOI : https://doi.org/10.22034/jesi.46.2.4