Pol. J. Environ. Stud. Vol. 32, No. 3 (2023), 2623-2636
DOI: *10.15244/pjoes/161871* ONLINE PUBLICATION DATE: 2023-04-06

Original Research

Hazardous Impacts of Clopyralid, Benazolin, and Clethodim on Seed Germination and Seedling of Oil Rapeseed (*Brassica napus*)

Zhang Hong[1*#], Yahya Faqir[1#], Ghulam Mujtaba Kalhoro[1], Muhammad Talib Kalhoro[1], Abdul Rasheed Kaleri[1], Ali Murad[1], Kang Xiaohui[1], Jahansher Qambrani[2], Wang Fukai[1], Gao Zhixian[1], Mohammad Adeel[3]

[1]Southwest University of Science and Technology, Mianyang (621010) Sichuan, China
[2]Mir Chakar Khan Rind University, Sibi (82001), Balochistan, Pakistan
[3]Lasbela University of Agriculture, Water, and Marine Sciences Dera Murad Jamali Camps, Balochistan, Pakistan

Received: 27 July 2022
Accepted: 4 March 2023

Abstract

In order to illustrate their tolerance mechanism, four leading cultivars of *Brassica napus* (Mianyou 15, Guohaoyou 8, Yiyou 21, and Mianyou 16) were investigated to study the effects of Clopyralid, Benazolin, and Clethodium on physio-chemical and ultrastructural attributes of *Brassica napus* at during seed germination stage, by various concentrations (0.0, 0.5, 0.7, 1.0, 1.3 μg/10 mL) of Clopyralid, Benazolin, and Clethodium to rape seedlings in terms of various physiological and biochemical measurements and microscopic analysis. The main results were as follows: low concentration (0.5 μg/10 ml) of Clopyralid, Benazolin, and Clethodium had no significant effect on *Brassica napus* CVS. It was found that with the increasing of treated concentrations, the growth of *Brassica napus* was inhibited more when the concentration of Clopyralid, Benazolin, and Clethodium was higher than 1.3 $\mu g \cdot L^{-1}$. It inhibited the net photosynthetic rate, chlorophyll content, chlorophyll fluorescence value, and anti-enzyme activities of *Brassica napus*. Moreover, it increased lipid peroxidation, accumulated reactive oxygen species, and damaged the ultrastructure of chloroplasts and mitochondria in mesophyll cells. In addition, the ability of resistance to the stress of Clopyralid, Benazolin, and Clethodium in all cultivars shows higher sensitivity which is more effective. Therefore, this study defined the effect of selected herbicides on *Brassica napus*, and the findings have great significance in extending the calculation by field application of those herbicides.

Keywords: oil rapeseed, herbicide, *Brassica napus*, seed germination, root responses

#Contributed equally to this work and are co-first authors.
*e-mail: zhanghong316@nwafu.edu.cn

Introduction

Winter oilseed rape (*Brassica napus L.*) is an essential crop that can overcome the increasing demand for edible oil and contend economically with cereal crops [1, 2]. After Canada and India, China is the largest producing country for *B. napus*. Globally 31 million hectares of rapeseed are cultivated, and 60 million tons of rapeseed are produced annually [3]. The rapeseed production area in China increased to 6.57 million ha, 20% of the world's total rapeseed planting area. Per hectare yield in the area is approximately 2000 kg/h, which counts for 65.4% of in USA and 58.1% of the European regions. While cultivation in intensive conditions for experiments in this area, the maximum yield was reported to be over 3000 kg/h, which is significantly higher than extensive cultivation on farms [4]. The use of herbicides, weed management, and proper planting density plays a significant role in boosting rapeseed yield and per-acre production. With the rapid upsurge in the human population, there is a need to augment vegetable oil rapidly. Conventional agriculture relies on using pesticides to control weeds, insects, and diseases to save the quality and quantity of crop production; especially herbicides are an indispensable tool for achieving high yields. Notwithstanding, studies have confirmed that only 45% of sprayed compounds achieve the target crop, although 30% become dispersed, 10% are wasted because of transportation activities (leaching, volatilization, and overflow), and 15% reach the soil. Furthermore, approximately 1% of the substance applied achieves the objective. Herbicides have been used for over since few decades in all major crops and on most weed species [5].

For pesticide toxicity in crops and fruits, concentrations of 0.05 ppm or less in vegetables and other foods can be tolerated. However, for prevalence in soil, even low quantities are undesirable; for example, the determination of herbicide residues is mostly to measure the decomposition rate and if a phytotoxic amount can sustain from one growing season to the next. Herbicide residues may cause crop damage if continued use in mono-cropping. Generally, an average herbicide application rate is 1 liter per acre, assuming the herbicide is present in the upper 5 cm of soil layer; this counts for 2-ppm residues [6]. Therefore, the herbicide persistence level is 0.I-ppm representing only such primary treatment subjects. Except for Clopyralid, Benazolin, and Clethodim herbicides, deposits of this extent typically do not lead to succeeding Crop damage. Two considerations with respect to rotation are herbicide carryover and disease cycles.

One of the characteristics of modern agriculture is the simplification of crop rotation. Whereas, various experimental methodologies are utilized to assess herbicide activity in the soil. Agricultural diversity plays an important role in protecting biodiversity, the agricultural environment, and crops from pests, diseases, and weeds. Most herbicides from the sulfonylurea and imidazolinone families have rotation restrictions of 2 to 3 years concerning canola. However, only a few studies indicate an increase in the adsorption of anionic herbicides when applied to amended soils. The targeted aim of this study was to determine the physio-chemical effect of the three different herbicides, including Clopyralid, Clethodim, and Benazolin. The results of the study will help to understand better the binding and reactivation process of chemicals to crops. Therefore, it is worth looking for plants that can diversify the species composition of contemporary agricultural plants. The most important advantages of canola/rice, maize soybean, and rotation include the control of grass weeds during the canola season and disease reduction [7]. The primary objective of this investigation is to summarize the most generally applied methodologies for analyzing and evaluating seed germination and seedling development. Oilseed crop yields consistently decline drastically because of weeds, global warming, and pests [8-10]. That has widened a big gap between demand and edible oil supply across China and the world [11]. Noxious weeds are unwanted plants that grow out of place, and herbicides are frequently used to prevent weed growth and development. Recently, the use of herbicides for weed management has been increasing, and several herbicides are recommended for use in oilseed crops in China and applied as pre and post-emergence of weeds. Meanwhile, injudicious application of herbicides may bear specific hazards such as the most desired, and adverse herbicide use may add more to the long-term loss of their effectiveness, particularly environmental degradation. Indiscriminate use of herbicides brings several threats and harms human health and the environment, as well as loss of efficacy over long-term use, and weeds become resistant against these herbicides [12]. The chemical herbicides contaminate the soil and severely damage the non-target plants, even the main crop [13]. Furthermore, extreme misuse of such agrochemicals may bring stress to plants, which causes harm to crops and the agroecosystem.

Meanwhile, these chemicals have poisonous effects on non-targeted species and human and animal-associated health threats [14, 15]. Numerous herbicides are recognized to contain certain solvents that make them inaccessible to the target, damage soil fertility, and contaminate soil, water, and plants. Clopyralid, Benazolin, and Clethodim have commonly used herbicides against weeds to enhance oilseed crop production [16-19]. Their persistent application into the environment, combined with their innate biological mechanism, has paved the way to research the toxic consequences of herbicides in natural and farming environments [10]. However, relatively little research has been concluded on broadly applied herbicides, as they affect seed germinations, limit seedlings' development and root expansion, and result in water pollution at even low concentrations. Keeping in view the current analyses in the present study, Clethodim had hazardous effects even at lower levels on seeds germination and seedlings of *B. napus* varieties, with concentrations of such chemicals observed varying from 0.7 ng/10 mL, which can be observed in the results.

Historically herbicide application has become common to control annual or perennial weed populations [20]. However, herbicide-based weed control is deemed suitable, though it is considered an ideal, simplistic, and cost-effective technique for managing weeds in different crops [21, 22]. The herbicides usually applied to work invasive weed plants regarded as weed killers [15] can improve crop growth [23, 24]. On the contrary, the high herbicides applications may suppress the shoot growth, root, germination, and development of main crops [25].

To the best of our knowledge is concerned. Still, no reports consider the effects of Clopyralid, Benazolin, and Clethodim on seed germination nor on the shoot and root development of *B. napus*. Following seed germination, the proper growth of roots is vitally essential for plant establishment and sustainability, and any disruption to this procedure could affect plant productivity. As a result, studies of seed germination and root development in soil toxins are relevant. Further particularly, our study attempts to identify the consequences of Clopyralid, Benazolin, and Clethodim affecting seed germination and (ii) evaluate whether pre-emergent herbicides applications on *B. napus* reduce seedling growth, which potentially decreases crop yields.

Material and Method

The present research was performed at the Laboratory of Life Science and Engineering of Southwest University of Science & Technology, China, in September 2019. Four varieties of *Brassica napus* Mianyou 15, Guohao you 8, Yiyou 21, and Mianyou 16 used in this research were acquired from the local seed market. The viability of seeds was confirmed by soaking in deionized water overnight. After imbibition, the seed coat was cut with a scalpel to expose the embryos and then put in 1% tetrazolium chloride (TTC) solution and observed for staining under the light microscope. Embryos showing red color were considered viable, and seed viability was above 90% for all varieties.

Seed Germination Assay

Before germination assessment, the seeds were sanitized with 75% ethanol for 5 minutes and then rinsed with sterile distilled water to avoid any superficial contamination before the germination trial. The experiment was conducted using 90 mm Petri dishes layered with (Whatman NO. 1) double filter paper moistened with sterile distilled water or herbicide solutions at different concentrations. Three kinds of herbicides, i.e., Clopyralid (Chemical formula: $C_6H_3Cl_2NO_2$), Benazolin ($C_9H_6ClNO_3S$), and Clethodim ($C_{17}H_{26}ClNO_3S$), applied as (control 0.0, 0.5, 0.7, 1.0, 1.3 µg/10 mL) concentrations were prepared with deionized water. Germination assay was carried out by placing 15 seeds of uniform size in each Petri dish as a treatment with three replicates, and the trial was repeated. All Petri plates were arranged in a complete randomized block design and incubated at 20ºC for 7 days in the dark, while 2 mL of water was added every day to the plates to retain moisture. A seed was considered germinated if it sprouted about 1mm from the radical. For data collection, four seedling roots from each replicate were selected randomly. The seeds that failed to germinate were checked for viability per the TTC test described above and considered dead if they did not show any staining.

The root length and diameter were measured after collecting seed germination parameters and data. Three seedlings from each replication were randomly selected; root elongation and diameter were measured with a digital Vernier calliper.

Oxidative Stress

Oxidative stress was evaluated by calculating Hydrogen peroxide (H_2O_2) levels per protocol adapted from [26] with minor modifications. Embryonic material of seed 0.2 gram, homogenized in 1 mL of 0.2% TCA and centrifuged at (RCF) 12,000-x g for 10 minutes. 150 µl of solubilized supernatant supplemented with 0.2 mL of 20 mM Gomori buffers (pH 7.0) and 0.5 mL of 1 M KI. The final product's absorbency was examined at 320 nm, and hydrogen peroxide values were performed and examined on a standard calibration curve.

Antioxidant Enzymes

Antioxidant enzyme activity evaluated by macerated 0.1-gram embryonic material immersed in 2 mL buffer composed of (100 mmol 1^{-1} Gomori buffers, [pH 7.8]), 100 mmol $^{-1}$ Ethylenediaminetetraacetic acid (EDTA), 1 mmol $^{-1}$ L–ascorbic acid), and 3% PVP-(M/V) by following [27].

Catalase

Following the method proposed by [26] Catalase test was done as a combination of 30 mM Gomori buffers (pH 7.0), 150 mM-hydrogen peroxide, and purified water at 20ºC. The catalase activity was determined by applying the Hydrogen peroxide solubilization for 3 minutes at 30-second hiatuses, observing variations in Complex III activity at 140 nm, with a molar-extinction coefficient observed by [28] of 0.0394-mM^{-1} cm^{-1}. Whereas the ascorbate-peroxidase content was assessed at 28ºC in a reaction medium comprising 30 mM Gomori buffers (pH 7.0) and 5 mM

L-ascorbic acid ($C_6H_8O_6$), 0.5 mM of hydrogen peroxide (H_2O_2), and purified water (H_2O_2).

The phenotypic symptoms of herbicide toxicity on seedlings of all the varieties of *B. napus* were observed under a light microscope. Three seedlings selected from each treatment with three replicates were used to evaluate herbicide toxicity.

Statistical Analyses

The results were described and subjected to each treatment value characterized by the mean±standard deviation (SD) of three biological replications. In addition, SPSS statistics 23, origin 2021, and MS Excel 2016 were used to ensure the statistical analysis of data. A comprehensive, accurate homogeneity of variance for the significance of the related ratios and mean values were statistically analyzed for variance analysis (ANOVA) to show a correlation in all four varieties of B. napus and herbicides using a student t-test at $P\text{-}value<0.05^{*}$.

Results

Response of Different Herbicides Application to Seed Germination of *B. napus*

Seeds germination % was assessed for four varieties of *B. napus*, exposed to different herbicides, with five treatments and three replications. The results revealed that increasing the concentration of Clopyralid, Benazolin, and Clethodim had a particular effect on the emergence of *B. napus* and significantly inhibited the germination of all tested varieties. The control treatment (0.0 ng Herbicide) resulted in the maximum seed germination for all cultivars (86% in Mianyou15, 90% Guohaoyou8, 87% Yiyou21, 95% Mianyou16) and showed a significant difference compared to herbicides treatments. On the other hand, Clopyralid negatively affected seed germination in all cultivars (16.66% in Mianyou15, 23.33% Guohaoyou8, 16% Yiyou21, in the case of Benazolin (10% in Mianyou15, 32.54% Guohaoyou8, 36.88% Yiyou21, 53.33% Mianyou16) and Clethodim (12% in Mianyou15, 17.54% Guohaoyou8, 11% Yiyou21, 45.67% Mianyou16), increased concentrations (1.3ng/10mL) resulted from a considerable diminution in seed germination. Meanwhile, the Mianyou16 variety showed more resistance against these herbicides as compared to other varieties (Table 1-Table 3).

Response of Herbicides on the Root Elongation of *B. napus*

The root length of sprouts exposed to the five consistent treatments of Clopyralid, Benazolin, and Clethodim was recorded after seed germination at the seedling stage. Whereas the maximum root elongation found in the control treatment of Mianyou15 showed 45.81±1.12 mm, Guohaoyou8 showed 57.21±1.25 mm, Yiyou21 showed 40.27±1.24 mm, and Mianyou16 was 20.44±1.32 mm. However, the minimum roots observed in treatment with 1.3 μ g/10 mL Clethodim to the Mianyou15 showed no growth, Guohaoyou8 was 14.99±1.32 mm, Yiyou21 12.44±1.22 mm, and Mianyou16 was 3.1±0.45 mm $P<0.001^{***}$, the compared to control treatments. The root length of the *B. napus* under five treatments of the Benazolin herbicides was observed after seed germination during the seedling stage. The smallest root length was found at 6.24±1.14 mm, Guohaoyou8 was 8.99±1.22, Yiyou21 was 16.44±1.29 mm, Mianyou16 was 20.45±1.14 mm $P<0.001^{***}$ in the treatment 1.3 μg/10 mL, compared to control treatments. The root length of the *B. napus* under five treatments of the Clethodim herbicides was observed after seed germination at the root development stage. The miniature root length was noticed in the treatment of 0.7 μg/10 mL, where Mianyou15 was 10.12±0.56 mm, Guohaoyou8 was 6.87±0.65 mm, Yiyou21 was 5.44±0.32 mm, Mianyou16 was 19.87±0.23 mm as compared to control treatments, respectively, with a significant value *P<0.001****** (Table 1-Table 3).

Effect of Herbicides on the Root Diameter of *B. napus*

The root diameter of the *B. napus* exposed to five treatments of three herbicides was recorded after seeds sprouted at the seedling stage. However, in this experiment, a significant interaction between herbicide applications was observed at the high dose of herbicides (1.3 μg/10 mL). The roots were swollen and deformed and observed an increase in root diameter. The results by the Clopyralid showed in Mianyou15 2.23±0.031, Guohaoyou8 showed 2.19±0.091 mm, Yiyou21 showed 2.51±0.041 mm, Mianyou16 showed 2.61±0.081 mm, and the lowest root diameter was observed in control treatments where Mianyou15 at 1.4±0.045 mm, Guohaoyou8 showed 1.98±0.074mm, Yiyou21 as 1.78±0.074 mm, and Mianyou16 at 1.88±0.045 mm, respectively. Similarly, the Benazolin herbicide was also found effective for the root diameter of all varieties. The results showed that the maximum root diameter of 1.3 μg/10 mL of Mianyou15 revealed 2.31±0.023 mm, Guohaoyou8 showed 2.43±0.072 mm, Yiyou21 was 2.78±0.013 mm, Mianyou16 measured 1.79±0.05 mm, respectively, with a significant value $P<0.001^{***}$. The *B. napus* seeds were treated with five different Clethodim herbicide concentrations. The root diameter was confirmed subsequent root development when seedlings after seed germination. The results showed that a high root diameter was assessed at 1.3 n g/10 mL 2.98±0.056 mm, Guohaoyou8 at 2.32±0.061 mm, Yiyou21 shown at 2.12±0.051 mm, and Mianyou16 revealed 2.37±0.055 mm as compared with control treatments with a significant value $P<0.001^{***}$ (Table 1-Table 3).

Table 1. Effect of Clopyralid on the Seed germination, Root development of *B. napus*.

Clopyralid				
Varieties	Treatments (ng/10mL)	Growth parameters		
		Seed germination %	Root Length (mm)	Root Diameter (mm)
Mianyou 15	0.0	86±2.1	45.41±0.97	1.4±0.045
	0.5	81.33±1.98	34.50±0.82	1.2±0.041
	0.7	33.33±1.7***	18.12±1.20 c	0.99±0.071
	1.0	30.53±1.3***	8.45±1.45 A	0.97±0.022
	1.3	16.66±0.99 ***	0.00±0.00 A	0.86±0.031 *
Guohaoyou 8	0.0	90±2.1	57.91±1.32	1.98±0.015
	0.5	73±1.98 **	48.17±1.21 *	1.44±0.074
	0.7	60±1.7 ***	37.14±1.17 ***	1.24±0.022
	1.0	36.66±1.3 ***	26.15±0.93 ***	1.00±0.035
	1.3	23.33±0.99 ***	14.99±0.47 ***	0.71±0.041 **
Yiyou 21	0.0	87±1.87	39.77±0.97	1.78±0.074
	0.5	56.66±1.55 ***	36.15±0.82	1.56±0.015
	0.7	40±2.1 ***	28.48±1.20 *	1.11±0.022
	1.0	33.33±1.1 ***	20.15±1.45 ***	0.56±0.035
	1.3	16±1.3 ***	12.44±1.22 ***	0.35±0.041 **
Mianyou 16	0.0	95±1.87	20.44±1.32	1.88±0.045
	0.5	70±1.55 ***	14.11±1.21	1.74±0.041
	0.7	50±2.1 ***	8.12±1.17	1.42±0.071
	1.0	26.66±1.1 ***	3.10±1.23	0.99±0.022
	1.3	20±1.3 ***	2.44±0.45 ***	0.89±0.031*

Significant at p-value (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$) as computed by the LSD Test. Note: V1 =Mianyou 15, V2 = Guohaoyou 8, V3 = Yiyou 21, V4 = Mianyou 16.

Table 2. Effect of Benazolin on the Seed germination, Root development of *B. napus*

Benazolin				
Varieties	Treatments (ng/10mL)	Growth parameters		
		Seed germination %	Root length (mm)	Root diameter (mm)
Mianyou 15	0.0	80±1.41	42.71±1.25	1.98±0.045
	0.5	73.2±1.25	30.50±1.42	1.71±0.074
	0.7	53.33±1.11*	18.12±1.61	1.56±0.018
	1.0	40±0.88**	10.45±2.1 ***	1.23±0.093
	1.3	10±1.23 ***	6.24±1.14 ***	0.00±0.00 ***
Guohaoyou 8	0.0	96.0±2.14	53.91±1.41	2.1±0.039
	0.5	81.12±2.1	41.17±1.20	1.85±0.078
	0.7	60±1.22 *	25.14±1.54	1.24±0.022
	1.0	60.0±1.22 *	14.15±1.24 ***	1.00±0.019
	1.3	36.66±1.23 ***	8.99±1.22 ***	0.93±0.012

Table 2. Continued.

Yiyou 21	0.0	90±2.14	39.77±1.41	1.90±0.047
	0.5	83.33±1.22	31.15±1.22	1.89±0.021
	0.7	78.33±2.1*	29.48±1.29	1.40±0.025
	1.0	56.66±1.22 ***	22.15±1.12 ***	1.21±0.036
	1.3	40±1.23 ***	16.44±1.22 ***	0.78±0.013 *
Mianyou 16	0.0	98±1.41	60.11±1.25	1.79±0.05
	0.5	91±1.25	48.44±1.42	1.74±0.025
	0.7	80±1.11	35.47±1.61	1.62±0.049
	1.0	80±0.88	29.14±2.10 ***	1.44±0.084
	1.3	43.33±1.23 ***	20.45±1.14 ***	1.21±0.019

Significant at p-value (*$p\leq 0.05$, **$p\leq 0.01$, ***$p\leq 0.001$) as computed by the LSD Test. Note: V1 =Mianyou 15, V2 = Guohaoyou 8, V3 = Yiyou 21, V4 = Mianyou 16.

Table 3. Effect of Clethodim on the Seed germination, Root development, of *B. napus*

Clethodim				
Varieties	Treatments (ng/10 mL)	Growth parameters		
		Seed germination %	Root length (mm)	Root diameter (mm)
Mianyou 15	0.0	93±2.1	45.81±1.12	1.98±0.074
	0.5	29±1.54***	10.55±1.32 ***	1.71±0.056
	0.7	8±1.2 ***	4.31±0.41 ***	0.98±0.00 ***
	1.0	2±0.74 ***	0.00±0.00***	0.00±0.00 ***
	1.3	0.0±0.0 ***		
Guohaoyou 8	0.0	96.0±2.14	57.21±1.25	2.10±0.081
	0.5	2.2±2.2 ***	26.11±1.24 ***	1.85±0.042
	0.7	2.81±0.81 ***	5.41±0.84 ***	1.24±0.061
	1.0	0.00±0.00 ***		
	1.3	0.00±0.00 ***		
Yiyou 21	0.0	89±1.54	40.27±1.24	1.90±0.047
	0.5	33.33±1.22 ***	31.00±1.22	1.89±0.041
	0.7	6.66±2.32 ***	7.12±1.41 ***	1.40±0.051
	1.0	3.33±0.47 ***	0.00±0.00 ***	0.00±0.00 ***
	1.3	0.0±0.0 ***		
Mianyou 16	0.0	94.00±2.10	64.11±1.45	1.79±0.014
	0.5	86.66±1.54	37.12±1.27 ***	1.74±0.081
	0.7	13.33±1.20 ***	26.34±1.31 ***	1.62±0.055
	1.0	5.00±0.74 ***	0.00±0.00 ***	0.00±0.00 ***
	1.3	0.00±0.00 ***		

Significant at p-value (*$p\leq 0.05$, **$p\leq 0.01$, ***$p\leq 0.001$) as computed by the LSD Test. Note: V1 =Mianyou 15, V2 = Guohaoyou 8, V3 = Yiyou 21, V4 = Mianyou 16.

Oxidative Stress

Hydrogen peroxide (H_2O_2) revealed a significant enzyme activity in embryos of *B napus* varieties, while significantly greater in seeds treated with all herbicides mainly applied with Clopyralid applications where Mianyou 15 showed (0.37±0.12 μg g^{-1} FW), Guohaoyou 8 at (1.47±0.12 μg g^{-1} FW), Yiyou 21 (1.02±0.2 μg g^{-1} FW), and Mianyou 16 (1.07±0.17 μg g^{-1} FW) as compared to control treatments. However, the higher dose of all herbicides 1.3 ng/10 mL significantly increased H_2O_2 activity in the embryos of all varieties compared to control treatment $P<0.001^{***}$, respectively (Table 1).

The ascorbate peroxidase (APX) response of *B. napus* can be observed in Table 4, where minor results were found in the control treatments in all varieties. Besides that, higher results were found in the high concentration of all herbicides in all varieties and showed a significant difference $P<0.001^{***}$compared to control treatments. Meanwhile, the Mianyou16 variety was found to be more resistant against all herbicides than other varieties in this experiment.

Catalase (CAT) activity was lower in embryos as found in the control treatments, whereas Mianyou 15 was 0.37±0.12, Guohaoyou8 was 1.47±0.12, Yiyou 21 was 1.02±0.21, Mianyou16 was 0.79±0.17 mM ascorbate min^{-1}-mg^{-1}-protein respectively $P<0.001^{***}$. CAT activity in embryos was more significant in Benazolin of 1.3 μg/10mL in Guohaoyou 8 (4.92±0.14 mM ascorbate minutes^{-1} mg^{-1} protein), respectively. Therefore, a considerable interaction was found among high doses of all herbicides in all varieties for both enzymes' activities, respectively, (Table 5) as compared to control treatments (with the *P-value*$<0.001^{***}$).

Clopyralid, Benazolin, and Clethodim had an unexpected influence on roots. Furthermore, decreasing root elongation encouraged the adventitious roots in these seedlings. Therefore, it is potential because the decrease of root surface caused by interference with root elongation could be controlled, at least partially, by developing new roots. Besides that, the positive results observed following zero herbicide treatments may be due to the limitation of ROS production. On the other hand, herbicide treatments resulted in higher H_2O_2 contents in the roots (Table 6). These molecules have different reactivity levels, production sites, and the potential to cross biological membranes.

Furthermore, these roots had higher stain density than those treated with zero herbicides, implying higher superoxide levels. ROS, such as H_2O_2, have been shown to promote adventitious rooting by improving the

Table 4. Effect of Clopyralid, Benazolin, and Clethodim on Ascorbate peroxidase activity (APX) of *B. napus*.

Clopyralid Ascorbate peroxidase activity (APX) mM ascorbate min^{-1} protein				
Treatments	Mianyou 15	Guohaoyou 8	Yiyou 21	Mianyou 16
0.0	2.28±0.22	1.65±0.15	1.65±0.15	3.00±0.16
0.5	4.05±0.27	3.75±0.43 *	3.75±0.43 **	3.93±0.26
0.7	4.77±0.30 *	4.87±0.24 **	5.87±0.24 ***	5.98±0.21 **
1.0	7.39±0.34 ***	6.50±0.26 ***	7.50±0.26 ***	7.22±0.25 ***
1.3	9.99±0.51 ***	8.95±0.28 ***	9.95±0.28 ***	10.73±0.46 ***
Benazolin				
0.0	1.93±0.16	1.57±0.18	1.57±0.18	2.12±0.09
0.5	3.52±0.14 ***	2.68±0.15 *	2.68±0.15 *	3.13±0.18
0.7	4.98±0.30 ***	4.33±0.26 ***	4.32±0.26 ***	4.11±0.20 **
1.0	6.73±0.30 ***	5.79±0.24 ***	5.19±0.24 ***	6.15±0.16 ***
1.3	9.99±0.51 ***	8.95±0.28 ***	9.54±0.28 ***	10.73±0.46 ***
Clethodim				
0.0	6.18±0.24	4.87±0.18	4.87±0.18	4.39±0.30
0.5	7.68±0.33	5.34±0.25	5.14±0.25	5.75±0.22
0.7	9.99±0.51	8.95±0.28**	7.95±0.28*	10.73±0.46***
1.0				
1.3				

Significant at p-value (**p≤0.05*, ***p≤0.01*, ****p≤0.001*) as computed by the LSD Test. Note: V1 =Mianyou 15, V2 = Guohaoyou 8, V3 = Yiyou 21, V4 = Mianyou 16.

Table 5. Effect of Clopyralid, Benazolin, and Clethodim on Catalase (CAT) of *B. napus*.

Clopyralid Catalase (CAT) mM ascorbate min^{-1} mg^{-1} protein				
Treatments	Mianyou 15	Guohaoyou 8	Yiyou 21	Mianyou 16
0.0	0.73±0.03	1.06±0.09	1.50±0.17	0.84±0.04
0.5	1.31±0.03	2.62±0.10	2.05±0.10	1.16±0.04
0.7	1.42±0.09	3.92±0.09 **	2.15±0.15	1.25±0.05
1.0	2.09±0.17 ***	4.27±0.10 ***	2.65±0.15 *	2.30±0.12 *
1.3	2.95±0.14 ***	4.92±0.14 ***	3.78±0.14 **	2.52±0.14 *
Benazolin				
0.0	0.72±0.04	0.44±0.06	0.69±0.06	0.81±0.04
0.5	1.57±0.06	0.86±0.12	1.43±0.23	1.58±0.13
0.7	2.00±0.08 *	1.99±0.12	2.04±0.21	2.13±0.12 **
1.0	2.03±0.06 *	2.35±0.08 **	2.16±0.13 **	2.37±0.07 **
1.3	2.95±0.14 **	2.52±0.14 **	2.22±0.14 ***	2.52±0.14 **
Clethodim				
0.0	0.49±0.04	0.42±0.04	0.72±0.08	0.58±0.02
0.5	1.26±0.12	1.84±0.09	1.92±0.16	1.06±0.09
0.7	2.95±0.14 **	2.52±0.14 **	2.22±0.14 *	2.52±0.14 **
1.0				
1.3				

Significant at p-value (*$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$) as computed by the LSD Test. Note: V1 =Mianyou 15, V2 = Guohaoyou 8, V3 = Yiyou 21, V4 = Mianyou 16.

concentrations of solubilized carbohydrates immediately associated with the progress of adventitious roots.

Response of Herbicides on the Physical Structure, Seedling Roots, and Leaves

The herbicides (Clopyralid, Benazolin, and Clethodim) assumed toxicity on *B. napus* and were observed under a light microscope. Although well documented, control and roots at the seedling stage and herbicide-treated leaves had shown a significant disparity in their physical structure compared to control treatments seedlings, roots, and leaves. However, at higher concentrations, severe and hazardous symptoms on the seedling roots and leaves were observed. Simultaneously, the seedling leaves treated without herbicides looked healthy. This study indicates the usage of herbicides harmful to the oil rapeseeds, seedling roots, and leaves.

Correlation Analysis

In the study, we applied a heat map diagram for the Pearson correlation coefficients among various varieties, seed germination (Fig. 1), growth, and biochemical responses against the herbicides. Fig. 1, the Mianyou16 variety showed more resistance in seed germination, growth, and biochemical parameters than other varieties. Current analysis results revealed that Clethodim herbicide was more hazardous to all varieties, and Miangyou 16 variety was more resistant to all herbicides as compared to others.

Discussion

Crop maximization programs and developing strategies towards sustainable agriculture for environment-friendly and economic interpretation of inputs are encouraged in China. Laboratory research is possible; describe ratios and relative importance of different transport, retrofit, and control the retention process by which herbicides are dissipated in the soil. Winter rape is the most important oil plant in the world [29]. Registered herbicide applications in this regard include quinazoline, quizaloform, and chloandrpyrimidine [30]. Carmelina and winter rape belong to the same family; therefore, these herbicides may also be used for flax shepherd's purses and other small crops. However, it is important to determine the toxicity of these plants. Flax shepherd's purse herbicide. It is also important to identify the effects of herbicides

Table 4. Effect of Clopyralid, Benazolin, and Clethodim on hydrogen peroxide (H_2O_2) of *B. napus*.

Clopyralid Hydrogen peroxide µg g^{-1} FW				
Treatments	Mianyou 15	Guohaoyou 8	Yiyou 21	Mianyou 16
0.0	0.37±0.12	1.47±0.12	1.02±0.21	0.79±0.17
0.5	1.47±0.01 **	2.56±0.02 ***	1.18±0.19 *	1.35±0.04
0.7	2.20±0.15 ***	2.98±0.11 ***	2.18±0.12 ***	2.26±0.02 ***
1.0	2.07±0.21 ***	3.78±0.13 ***	2.98±0.13 ***	2.65±0.13 ***
1.3 ng/10mL	3.52±0.13 ***	3.37±0.12***	3.21±0.04***	2.79±0.20***
Benazolin				
0.0	0.29±0.04	0.39±0.09	0.13±0.08	0.23±0.08
0.5	0.36±0.09	0.40±0.11	0.31±0.07	0.31±0.07
0.7	0.40±0.11	0.92±0.10	0.92±0.10	0.62±0.10
1.0	0.90±0.11	1.39±0.05 **	1.51±0.06 **	1.32±0.06 **
1.3 ng/10mL	1.36±0.06 **	1.62±0.13 **	1.97±0.13 **	1.43±0.13**
Clethodim				
0.0	0.40±0.10	0.50±0.10	0.31±0.05	0.31±0.05
0.5	1.31±0.17	1.52±0.13 **	1.27±0.13***	0.78±0.13 *
0.7	1.73±0.19 ***	2.33±0.19 ***	1.61±0.17 ***	1.11±0.17 **
1.0	2.16±0.76 ***	2.47±0.36 ***	2.17±0.86 ***	1.34±0.44 ***
1.3 ng/10mL	3.98±0.32 ***	3.18±0.56 ***	3.28±0.86 ***	2.78±0.56 ***

Significant at p-value (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$) as computed by the LSD Test. Note: V1 =Mianyou 15, V2 = Guohaoyou 8, V3 = Yiyou 21, V4 = Mianyou 16.

on crop development [31]. As we all know, plant stress, whether biological or abiotic, will have a negative impact on the physiological state and photosynthetic activity of plants. Plant protection treatment, including weeding treatment, will cause short-term stress on crops and are toxic to harmful plants (weeds). In many cases, weeding agents directly reduce photosynthetic activity or indirectly cause damage to plants, thereby reducing photosynthetic efficiency. Herbicides that directly affect PSII usually act by inhibiting electron transport in PSII [32].

Several studies have been conducted on numerous sulfonylurea-containing herbicides for residual toxicity and persistence in crop produce and soil, especially on wheat and then in maize rotation, sunflower, rape, chickpea, and soybean. On the other hand, the effects of the separate use of pesticides resulted in variations in the peak day of physiological changes in treated plants. Further experiments showed that pesticide treatment leads to a significant decrease in polyphenol oxidase activity. Still, no significant changes in the contents of hydrogen peroxide, malondialdehyde, and electrolyte leakage index were found [33]. However, another study demonstrated that inappropriate herbicides use could reduce the oil productivity in oilseed rape [34]. Anonther study showed that, adding organic synergistic on fertile soil having low organic matter content have the potential to amend the superficial soil layer and subsurface material promotion [35]. Meanwhile, the field studies have shown that crops revealed to pre-emergent herbicide treatment may resist the preliminary circumstances of chemical stress [36]. Absorption of soil-applied herbicides can occur in root and shoot tissue in direct contact with herbicide dissolved in the soil solution or present in the vapor phase. During germination and growth, prior to soil emergence, shoot tissue is exposed to the soil solution and any herbicide dissolved in the soil solution [37]. The most significant barrier to herbicide absorption into shoot tissue is the cuticle, which covers the surface of essentially all epidermal cells of all above-ground shoot systems [38]. The cuticle is a complex and variable layer. With the exception of herbicides that may act at the plasmalemma surface, herbicides must move across the plasmalemma prior to reaching the site of action [39]. Several methods are used to determine herbicide translocation. Most commonly, translocation is determined by autoradiography of plants treated with radioactive herbicide [40].

Growers are paying much attention to both executions of cost-effective returns and profit from environment-friendly technologies' adaptation and

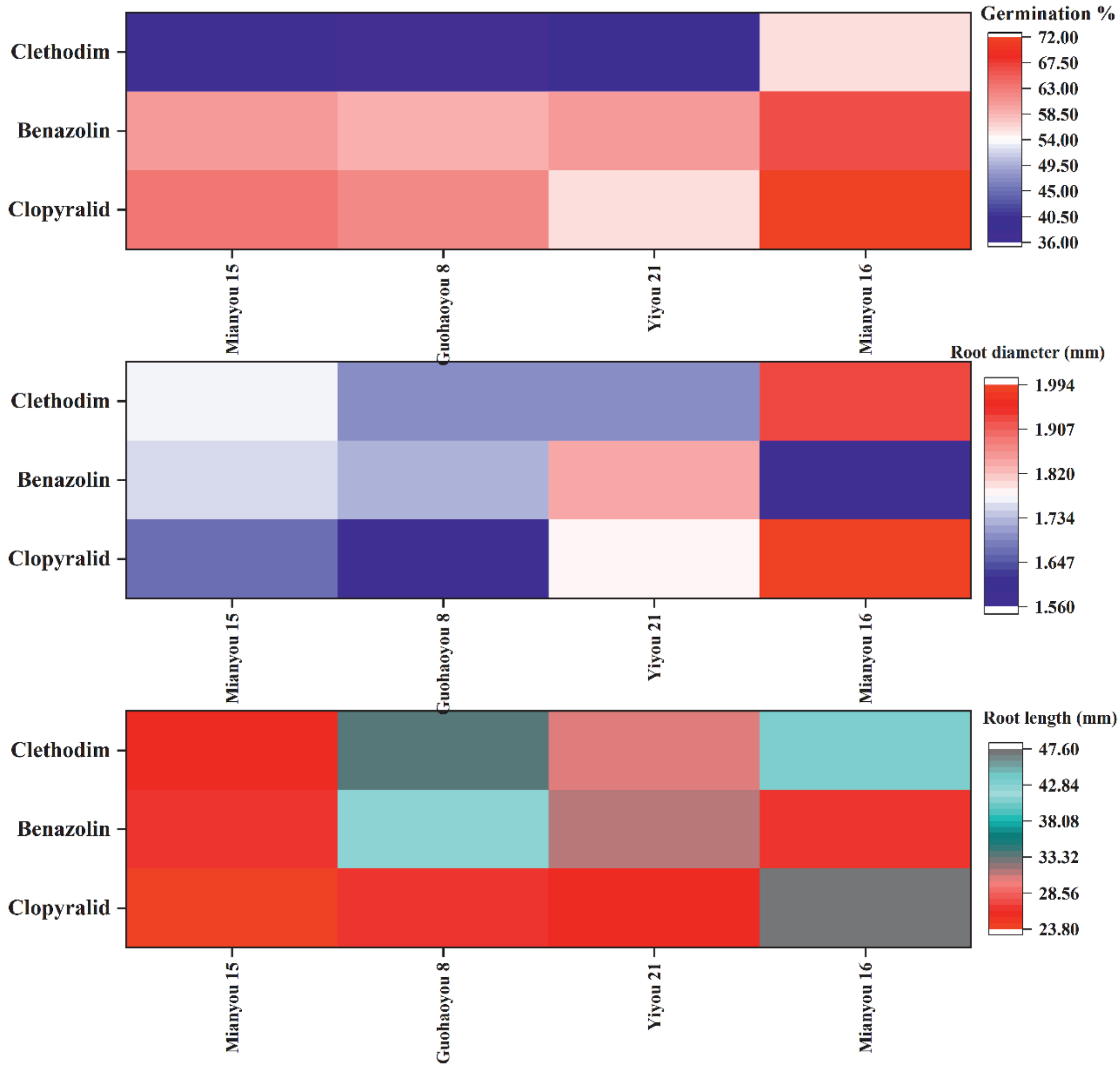

Fig. 1. The Pearson correlation coefficient analysis heat map presents the correlation between herbicides and varieties. The entire correlation between all parameters refereeing all data of all treatments (n = 12) and the correlation of seed germination, root length, and diameter, affect the various herbicides and the total parameters under distinctive treatment groups (*n = 3*). The grayscale demonstrated the highest significantly positive correlation, and the red scale indicated a highly negative correlation.

cropping patterns that guarantee high yields. We found that herbicides (Clopyralid, Benazolin, and Clethodim) toxicity adversely affected rape seedling growth. In contrast, root and shoot lengths were significantly inhibited under all three herbicide treatments. The present study's findings highlight the applications of low-herbicide concentrations that may assure a better yield and economically acceptable crop return. The simplicity and effectivity of weed control are most important, followed by reduced herbicide costs, while neither higher yields nor higher returns are critical considerations. Comparable interpretations were described for other crops treated with other kinds of herbicides [41].

Our result outcomes highlighted that seedlings of all *Brassica napus* varieties showed considerable damage after applying higher doses of all three herbicides. Indeed, the highest Clethodim dose led by stunt seedlings, plumule injuries, and root deformities suggests that the optimum dose of herbicides caused severe effects on *B. napus* seedling growth in the initial phenological stage. The reduction in photosynthesis cause chlorosis in leaves, signifying that the final productivity of the photosynthetic course is considered to safeguard the photosynthetic system to maintain plant growth [42]. Clopyralid, Benazolin, and Clethodim drastically affected all four oil rapeseed varieties (*B. napus*). The germination parameters had been undesirably affected by these chemicals. Evaluation of herbicides carried out in the present analysis, of all these three different types of chemicals excepting Clethodim, the rest of the two chemicals (Clopyralid and Benazolin) did not affect when applied on the low-level concentrations, but rather when oil rape seeds were exposed were to their higher levels of concentrations effected the rate of seed germination of

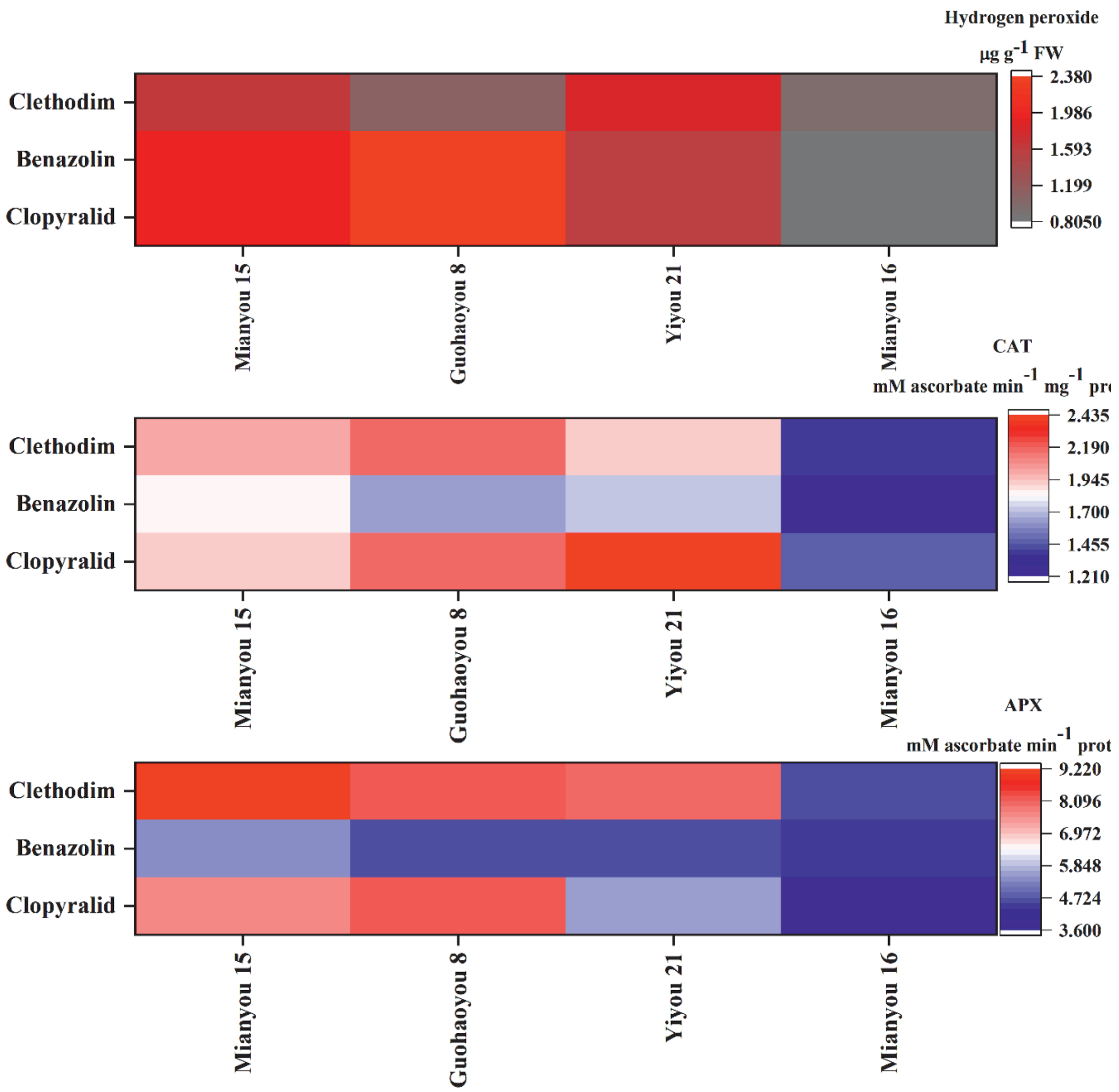

Fig. 2. The Pearson correlation coefficient analysis heat map presents the correlation between herbicides and varieties. The entire correlation between all parameters refereeing all data of all treatments (n = 12) and H_2O_2 (D), CAT (E), and APX (F) and affect the various herbicides and the total parameters under distinctive treatment groups (*n = 3*).

B. napus. Furthermore, the herbicide Clethodim was affected at low concentrations. All herbicides found affected the germination quality of seeds exposed to specific concentrations.

Previous investigation findings indicated that Clethodim is harmful to seed germination and prevents seedling emergence [43]. Recent study results mentioned that herbicides had affected rice growth and yielded a low dose of herbicide [44]. Herbicides are variously stimulated at different exposure durations and absorptions to the antioxidant enzymes and directed to suppress the oxidative stress when removing ROS formed throughout stress stages [45]. Under a specific physiological concentration range, ROS, particularly H_2O_2, behaves in the activation necessary for seed germination [46, 47], and the progressive influence of H_2O_2 on germinating seed is recorded chiefly [46]. ROS at a basal cellular level in plants is important for signalling molecules regulating diverse metabolic pathways [48]. Previous research found that the over-saturating use of herbicides in agriculture has significantly impregnated the soil, water, and environment with toxic substances [49, 50].

The scientific methodology is an experimental technique used to investigate finding and answer issues. The approaches discussed here are classified based on the characteristic of the process they typify: herbicide transportation (I), transformation (II), and retention (III). There are an unimaginable number of microbial communities in the soil, and biodegradation is one of the primary components of minimizing pesticide intensity in soil. From a thorough agricultural perspective, disturbances to germinating seeds can directly affect plant yields and any suspensions or reductions in plant development, influencing final productivity [51]. Certain dynamic models (MM5, PEARL, and PEM models) are dedicated to forecasting pesticide volatilization, although they often struggle to simulate high emission rates.

Furthermore, in evaluating herbicide volatility concerns, the existing model does not account for the process in soil, atmospheric, and agro-based influence activities [52]. Therefore, whenever the portion of the herbicides unavailable for weed management exceeds the obtainable portion, the herbicide's efficiency and durability in soil are drastically diminished, affecting seed germination and growth. The latest investigation showed that herbicides are an environmental risk [53]. Because herbicides are among the most extensively used chemicals, they are poisonous even at low-end points. They can play the role of appropriate early warning technologies to signify their involvement [54].

Conclusions

Using effective herbicides to control weeds in the fields is one of the major objectives of agronomists. This investigation analyzes and evaluates the consequences of herbicide dosage reductions on oilseed rape development and seed viability in order to minimize pesticide dependency. To improve weed control efficacy and minimize the application costs, the present study explores that herbicides application had some adverse effects on the roots and seedling leaves of *B. napus*. It was an important observation because any reduction in seed germination rate has helped us depict the reduced crop output, financial losses that inevitably incur to farmers and enhanced oil rapeseed susceptibility, and possible future financial losses to the farmers. These results demonstrated that herbicides had deleterious effects on *Brassica napus* seeds germination and the physiology of root parts and produced excessive oxidative stress on the seedlings. Therefore, much care is needed to use herbicides during oil rapeseed cultivation due to their harmful effects on the seed and root system.

We concentrated on the conceivable use and cost-effective application of three different herbicides as laboratory experiments, which can be considered demonstrative for the environment and adaptable for canola crops. This research suggests that reducing herbicide dose may be considered a persuasive agricultural practice accessible for growers to minimalize ecological exploitation and develop crop maximization strategies. However, if it is predictable that cutting the dose of some herbicides may encourage the development of herbicide resistance in some weeds, in that case, other cultural practices, such as crop rotation and alternative cultivation methods, could be encouraged.

Acknowledgments

The research was funded by the "Sichuan oil rape innovation team of national modern agricultural industry technology" (SCCXTD-2021-03).

Conflict of Interest

The authors declare no conflict of interest.

References

1. CHEW S.C. Cold-pressed rapeseed (*Brassica napus*) oil: Chemistry and functionality. Food Research International, **131** 108997, **2020**.
2. PULLENS J.W.M., SHARIF B., TRNKA M., BALEK J., SEMENOV M.A., OLESEN J.E. Risk factors for European winter oilseed rape production under climate change. Agricultural and Forest Meteorology, **272**, 30, **2019**.
3. YANG M., ZHENG C., ZHOU Q., HUANG F., LIU C., WANG H. Minor components and oxidative stability of cold-pressed oil from rapeseed cultivars in China. Journal of Food Composition and Analysis, **29 (**1), 1, **2013**.
4. WANG C., XU M., WANG Y., BATCHELOR W.D., ZHANG J., KUAI J., LING L. Long-Term Optimal Management of Rapeseed Cultivation Simulated with the CROPGRO-Canola Model. Agronomy, **12 (**5), 1191, **2022**.
5. DUKE S.O. Perspectives on transgenic, herbicide-resistant crops in the United States almost 20 years after introduction. Pest Management Science, **71 (**5), 652, **2015**.
6. SANAULLAH M., USMAN M., WAKEEL A., CHEEMA S.A., ASHRAF I., FAROOQ M. Terrestrial ecosystem functioning affected by agricultural management systems: A review. Soil and Tillage Research, **196**, 104464, **2020**.
7. DUKE S.O. Glyphosate: environmental fate and impact. Weed Science, **68 (**3), 201, **2020**.
8. KIRKEGAARD J.A., LILLEY J.M., BRILL R.D., WARE A.H., WALELA C.K. The critical period for yield and quality determination in canola (*Brassica napus* L.). Field Crops Research, **222**, 180, **2018**.
9. UPRETY D.C., REDDY V. Crop responses to global warming. Springer: **2016**.
10. VAN BRUGGEN A., HE M., SHIN K., MAI V., JEONG K., FINCKH M. and MORRIS JR J. Environmental and health effects of the herbicide glyphosate. Science of the Total Environment, **616**, 255, **2018**.
11. HE D., WANG E., WANG J. and LILLEY J. M. Genotype× environment× management interactions of canola across China: A simulation study. Agricultural and Forest Meteorology, **247**, 424, **2017**.
12. GREEN J.M. Current state of herbicides in herbicide-resistant crops. Pest Management Science, **70** (9), 1351, **2014**.
13. PARK J., BROWN M.T., DEPUYDT S., KIM J.K., WON D.-S., HAN T. Comparing the acute sensitivity of growth and photosynthetic endpoints in three Lemna species exposed to four herbicides. Environmental Pollution, **220**, 818, **2017**.
14. RODRIGUES E.T., ALPENDURADA M.F., RAMOS F., PARDAL M.Â. Environmental and human health risk indicators for agricultural pesticides in estuaries. Ecotoxicology and Environmental Safety, **150**, 224, **2018**.

15. HAZRATI H., SAHARKHIZ M.J., NIAKOUSARI M., MOEIN M. Natural herbicide activity of *Satureja hortensis* L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. Ecotoxicology and Environmental Safety, **142**, 423, **2017**.
16. FENNIMORE S.A., BOYD N.S., KORRES N., BURGOS N., DUKE S. Sustainable weed control in strawberry. Weed Control: Sustainability, Hazards, and Risks in Cropping Systems Worldwide, 383, **2018**.
17. ZHAO Y., XU L. Dispersive solid-phase extraction based on mesoporous melamine-formaldehyde polymer for sensitive determination of five chlorinated herbicides residues in water by high-performance liquid chromatography. International Journal of Environmental Analytical Chemistry, **98 (**14), 1331, **2018**.
18. SANDÍN-ESPAÑA P., SEVILLA-MORAN B., LOPEZ-GOTI C., MATEO-MIRANDA M.M., ALONSO-PRADOS J. L. Rapid photodegradation of clethodim and sethoxydim herbicides in soil and plant surface model systems. Arabian Journal of Chemistry, **9 (**5), 694, **2016**.
19. GUO M.J., WANG Y.G., YUAN X.Y., DONG S Q., WEN Y.Y., SONG X.E., GUO P.Y. Responses of the antioxidant system to fluroxypyr in foxtail millet (*Setaria italica* L.) at the seedling stage. Journal of Integrative Agriculture, **17** (3), 554, **2018**.
20. ZHU J., WANG J., DITOMMASO A., ZHANG C., ZHENG G., LIANG W., ISLAM F., YANG C., CHEN X., ZHOU W. Weed research status, challenges, and opportunities in China. Crop Protection, 104449, **2018**.
21. WARYSZAK P., LENZ T.I., LEISHMAN M.R., DOWNEY P.O. Herbicide effectiveness in controlling invasive plants under elevated CO_2: sufficient evidence to rethink weeds management. Journal of Environmental Management, **226**, 400, **2018**.
22. LECLÈRE M., JEUFFROY M.H., BUTIER A., CHATAIN C., LOYCE C. Controlling weeds in camelina with innovative herbicide-free crop management routes across various environments. Industrial Crops and Products, **140**, 111605, **2019**.
23. BELZ R., CEDERGREEN N., DUKE S. Herbicide hormesis-can it be useful in crop production? Weed Research, **51** (4), 321, **2011**.
24. MENEGAT A., BAILLY G.C., APONTE R., HEINRICH G.M., SIEVERNICH B., GERHARDS R. Acetohydroxyacid synthase (AHAS) amino acid substitution Asp376Glu in Lolium perenne: effect on herbicide efficacy and plant growth. Journal of Plant Diseases and Protection, **123** (4), 145, **2016**.
25. AHMED H.M., AMIRI-ARDEKANI E., EBADI S. Phytotoxicity of Natural Molecules Derived from Cereal Crops as a Means to Increase Yield Productivity. International Journal of Agronomy, **2022**, **2022**.
26. GOMES M.P., BICALHO E.M., SMEDBOL E.L., CRUZ F.V.D.S., LUCOTTE M., GARCIA Q.S. Glyphosate can decrease germination of glyphosate-resistant soybeans. Journal of Agricultural and Food Chemistry, **65** (11), 2279, **2017**.
27. LIU T., LI T., ZHANG L., LI H., LIU S., YANG S., AN Q., PAN C., ZOU N. Exogenous salicylic acid alleviates the accumulation of pesticides and mitigates pesticide-induced oxidative stress in cucumber plants (*Cucumis sativus* L.). Ecotoxicology and Environmental Safety, **208**, 111654, **2021**.
28. HOMAYOONZADEH M., MOEINI P., TALEBI K., ROESSNER U., HOSSEININAVEH V. Antioxidant system status of cucumber plants under pesticides treatment. Acta Physiologiae Plantarum, **42** (11), 1, **2020**.
29. PULLENS J.W.M., SHARIF B., TRNKA M., BALEK J., SEMENOV M.A., OLESEN E.J. Risk factors for European winter oilseed rape production under climate change. Agricultural and Forest Meteorology, **272**, 30, **2019**.
30. SOBIECH Ł., GRZANKA M., KURASIAK-POPOWSKA D., RADZIKOWSKA D.J. A. Phytotoxic effect of herbicides on various camelina (*Camelina sativa* (L.) Crantz) genotypes and plant chlorophyll fluorescence. Agriculture, **10** (5), 185, **2020**.
31. BOUTIN C., AYA K., CARPENTER D., THOMAS P., ROWLAND O. Phytotoxicity testing for herbicide regulation: shortcomings in relation to biodiversity and ecosystem services in agrarian systems. Science of The Total Environment, **415**, 79, **2012**.
32. KAISER Y.I., MENEGAT A., GERHARDS R.J. Chlorophyll fluorescence imaging: a new method for rapid detection of herbicide resistance in A lopecurus myosuroides. Weed Research, **53** (6), 399, **2013**.
33. KAUR T., BRAR L.S. Residual effect of sulfonylurea herbicides applied to wheat on succeeding maize. Indian Journal of Weed Science, **46** (2), 129, **2014**.
34. DELIGIOS P.A., CARBONI G., FARCI R., SOLINAS S., LEDDA L. Low-input herbicide management: Effects on rapeseed production and profitability. Sustainability, **10** (7), 2258, **2018**.
35. GARCÍA-DELGADO C., MARÍN-BENITO J.M., SÁNCHEZ-MARTÍN M.J., RODRÍGUEZ-CRUZ M.S. Organic carbon nature determines the capacity of organic amendments to adsorb pesticides in soil. Journal of Hazardous Materials, **390**, 122162, **2020**.
36. LAZARUS B.E., GERMINO M.J. Plant community context controls short-versus medium-term effects of pre-emergent herbicides on target and non-target species after fire. Applied Vegetation Science, **25 (**2), e12662, **2022**.
37. GABA S., FRIED G., KAZAKOU E., CHAUVEL B., NAVAS M.-L. Agroecological weed control using a functional approach: a review of cropping systems diversity. Agronomy for Sustainable Development, **34** (1), 103, **2014**.
38. MIRZA N., MAHMOOD Q., MAROOF SHAH M., PERVEZ A., SULTAN S. Plants as useful vectors to reduce environmental toxic arsenic content. The Scientific World Journal, **2014**.
39. CHAUDHARY N., CHOUDHARY K.K., AGRAWAL S., AGRAWAL M. Pesticides Usage, Uptake and Mode of Action in Plants with Special Emphasis on Photosynthetic Characteristics. Pesticides in Crop Production: Physiological and Biochemical Action, 159, **2020**.
40. NANDULA V.K., VENCILL W.K. Herbicide absorption and translocation in plants using radioisotopes. Weed Science, **63** (SP1), 140, **2015**.
41. JONES P.A., BROSNAN J.T., KOPSELL D.A., ARMEL G.R., BREEDEN G.K. Preemergence herbicides affect hybrid bermudagrass nutrient content. Journal of Plant Nutrition, **38** (2), 177, **2015**.
42. DELIGIOS P.A., CARBONI G., FARCI R., SOLINAS S., LEDDA L.J.S. Low-input herbicide management: Effects on rapeseed production and profitability. Sustainability, **10** (7), 2258, **2018**.
43. RODRIGUEZ HERRERA D. Investigating the Prospect of Fine Fescue Turfgrass Seed Production in Minnesota. **2020**. https://hdl.handle.net/11299/213068
44. WU G.L., CUI J., TAO L., YANG H. Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen

peroxide in rice (*Oryza sativa*). Ecotoxicology, **19** (1), 124, **2010**.
45. LUKATKIN A.S., GAR'KOVA A.N., BOCHKARJOVA A.S., NUSHTAEVA O.V. , DA SILVA J.A.T. Treatment with the herbicide TOPIK induces oxidative stress in cereal leaves. Pesticide Biochemistry and Physiology, **105** (1), 44, **2013**.
46. WOJTYLA Ł., LECHOWSKA K., KUBALA S., GARNCZARSKA M. Different modes of hydrogen peroxide action during seed germination. Frontiers in Plant Science, **7**, 66, **2016**.
47. NIU L., LIAO W. Hydrogen peroxide signaling in plant development and abiotic responses: crosstalk with nitric oxide and calcium. Frontiers in Plant Science, **7**, 230, **2016**.
48. MITTLER R.J. ROS are good. Trends in Plant Science, **22** (1), 11, **2017**.
49. LI H., FENG Y., LI X., ZENG D. Analytical confirmation of various herbicides in drinking water resources in sugarcane production regions of Guangxi, China. Bulletin of Environmental Contamination and Toxicology, **100** (6), 815, **2018**.
50. AL-SAMARAI G.F., MAHDI W.M., AL-HILALI B.M. Reducing environmental pollution by chemical herbicides using natural plant derivatives-allelopathy effect. Annals of Agricultural and Environmental Medicine, **25** (3), 449, **2018**.
51. GOMES M.P., RICHARDI V.S., BICALHO E.M., DA ROCHA D.C., NAVARRO-SILVA M.A., SOFFIATTI P., GARCIA Q.S., SANT'ANNA-SANTOS B.F. Effects of Ciprofloxacin and Roundup on seed germination and root development of maize. Science of the Total Environment, **651**, 2671, **2019**.
52. SCHREIBER F., SCHERNER A., ANDRES A., CONCENÇO G., CEOLIN W.C., MARTINS M.B. Experimental methods to evaluate herbicides behavior in soil. Revista Brasileira de Herbicidas, **17** (1), 71, **2018**.
53. JIANG R., WANG M., CHEN W., LI X. Ecological risk evaluation of combined pollution of herbicide siduron and heavy metals in soils. Science of the Total Environment, **626**, 1047, **2018**.
54. HASENBEIN S., PERALTA J., LAWLER S.P., CONNON R.E. Environmentally relevant concentrations of herbicides impact non-target species at multiple sublethal endpoints. Science of the Total Environment, **607**, 733, **2017**.