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Article

Interactive Role of Fungicides and Plant Growth Regulator (Trinexapac) on Seed Yield and Oil Quality of Winter Rapeseed

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Abstract: This study was designed to evaluate the role of growth regulator trinexapac and fungicides on growth, yield, and quality of winter rapeseed (*Brassica napus* L.). The experiment was conducted simultaneously at different locations in Germany using two cultivars of rapeseed. Five different fungicides belonging to the triazole and strobilurin groups, as well as a growth regulator trinexapac, were tested in this study. A total of seven combinations of these fungicides and growth regulator trinexapac were applied at two growth stages of rapeseed. These two stages include green floral bud stage (BBCH 53) and the course of pod development stage (BBCH 65). The results showed that plant height and leaf area index were affected significantly by the application of fungicides. Treatments exhibited induced photosynthetic ability and delayed senescence, which improved the morphological characters and yield components of rape plants at both locations. Triazole, in combination with strobilurin, led to the highest seed yield over other treatments at both experimental locations. Significant effects of fungicides on unsaturated fatty acids of rapeseed oil were observed. Fungicides did not cause any apparent variation in the values of free fatty acids and peroxide of rapeseed oil. Results of our study demonstrate that judicious use of fungicides in rapeseed may help to achieve sustainable farming to obtain higher yield and better quality of rapeseed.

Keywords: fungicides; growth regulators; leaf area index; oil quality; rapeseed; seed yield

1. Introduction

Different techniques such as osmo-priming, seed treatment, and application of chemicals used to induce growth of plants, are employed to achieve maximum seed yield [1]. Several fungicides also serve this purpose through altering cellular mechanisms of plant growth [2]. After the introduction of various modes of active fungicides, the concept of disease control gained new perspectives due to the positive physiological effects of these chemicals on plants [2]. Triazole and strobilurin treatments, along with plant growth regulators, are associated with various morphological and physiological changes in various plants; including inhibition of plant growth, decrease in inter-nodal elongation, increased chlorophyll content, enlarged chloroplast, thicker leaf tissue, increased root to shoot ratio, delayed senescence, increased antioxidant potentials, and enhancement in alkaloid production [3,4]. For example, triazole fungicides affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis [5,6]. Triazole inhibits mono-oxygenases that results in oxidation of ent-kaurene to ent-kaurenoic acid in three steps, which is an early reaction in GA biosynthesis [7]. In winter rapeseed, triazole application reduced the rate of photosynthesis by decreasing the stomatal conductance [8]. Strobilurins, another fungicide group, cause reductions in ethylene concentrations leading to degradation of cytokinins and resulting in delayed senescence [9]. Application of strobilurin fungicides maintain the photosynthetic active green leaf area for a longer period to increase the quantity of assimilates available for grain filling that can result in higher yield [9]. These fungicides were also reported previously to control lodging and to improve seed yield in cereals, but little information exists for the use of these fungicides in combination with plant growth regulators in oil seed crops. The purpose of this study is to evaluate the role of these fungicides, in combination with growth regulator trinexapac, on seed yield and oil quality of winter rapeseed in field conditions.

2. Materials and Methods

The experiments were conducted at Giessen (GI) (50°47′ N and 8°61′ E, 158 m above sea level) and Rauischholzhausen (RH) (50°45′ N and 8°39′ E, 220 m above sea level) experimental stations. The soil at RH is a loess type, while that at GI is silt clay. The mean air temperature during the growing season was 8.5 °C at GI and 9.7 °C at RH, and total rainfall from August to July were recorded as 660.5 mm and 637.5 mm, respectively. The experiments were set out as randomized complete blocks, with a factorial arrangement and four replications per treatment at each site. Six fungicides belonging to triazole or strobilurin groups, along with growth regulator Moddus (trinexapac), were applied in seven combinations (Table 1) on two winter rapeseed cultivars, “Elektra” and “NK Fair”. The treatments were applied at two growth stages of rapeseed. The first application was at the green floral bud stage (BBCH 53) and the second application was at the pod development stage (BBCH 65). The fungicides and trinexapac were applied using a CO_2-charged hand boom sprayer equipped with Tee Jet nozzles that delivered 180 L·ha^{-1}.

Table 1. Various fungicide and growth regulator applications at two developmental stages.

Stage	Fungicide and Growth Regulator	Dose L·ha^{-1}
Floral bud development (BBCH 53)	Control	-
	Toprex	0.5
	Toprex	0.5
	Toprex	0.35
	Toprex + Moddus	0.35 + 0.5
	Folicur	1.0
	Caramba	1.0
Pod development (BBCH 65)	Control	-
	Ortiva	1.0
	Ortiva	1.0
	Ortiva	1.0
	Ortiva	1.0
	Proline	0.7
	Cantus	0.5

Triazole:Toprex (Difenoconazole 250 g·L^{-1} + Paclobutrazol 125 g·L^{-1}), Folicur (Tebuconazole 251.2 g·L^{-1}), roline (Prothioconazole 250 g·L^{-1}) and Caramba (Metconazole 60 g·L^{-1}); Strobilurin:Ortiva (azoxystrobin 250 g·L^{-1}), Cantus (Boscalid 500 g/L) (Cantus is not a strobilurin fungicide but has same mode of action like strobilurin); Growth regulator: Moddus (Trinexapac 222 g·L^{-1} + Ethyl ester 250 g·L^{-1}).

The seed-bed was prepared in the autumn prior to the experiment by chisel plowing, followed by two rounds of cultivation during the following spring. Each 5 m × 3 m plot consisted of 12 rows, and the seed was sown at a depth of 2–3cm depth to give a seeding density of 50·m^{-2}. Post emergence herbicide Butisan Top (metazachlor 12% + quinmerac 37.5%) was applied to eradicate weeds to avoid weed losses at the rate of 1.8 L·ha^{-1}at both experimental locations. In order to control insect damage Trafo (λ-cyhalothrin) was applied at the rate of 150 g·ha^{-1} at BBCH 57 in Rauischholzhausen, and Biscaya (thiacloprid 240 g·L^{-1}) was applied at different growth stages of the crop (BBCH 49 and BBCH 54) at the rate of 300 mL·ha^{-1} in GI. No disease infestation was observed at either experimental station. The previous crop at RH was winter wheat, and at GI was winter barley. A fertilizer dressing of 150kg N·ha^{-1} was applied, half at BBCH 18 and the other half at BBCH 30, whereas 72 kg S·ha^{-1} was applied at BBCH 18. Ammonium nitrate and ammonium sulfate were applied for N and S fertilization.

The leaf area index (LAI) and height of plant stands was measured after one fungicide application per week until maturity. The leaf area index was measured using a Sun Scan canopy analysis system (Delta T Company, CA, USA) [10]. The other recorded traits were the numbers of seeds per main stem, pods per main stem, length of main stem, and pod length. These parameters were assessed from 10 plants per plot across all four replicates at GI. Neither LAI nor any of the morphological traits were assessed in the RH trial. Thousand grain weight (TGW) was obtained by counting two lots of 500 seeds per sample. The extent of lodging was estimated by grading the crop on a 1 to 9 scale (1 for erect and 9 for flat) at BBCH 75. After harvest, the seed moisture content was adjusted to 9% before estimating seed yield.

The Soxhlet method was used to determine seed oil content [11]. For the quantification of fatty acids, the oil was subjected to gas chromatography (Varian CP-3800) equipped with a flame ionization detector (GC-FID) through an OPTIMA-FFAP-Wax column (25 m × 0.32 mm i.d; film thickness 0.25 μm) [12]. The Dumas combustion method [13], utilized by a CHNS analyzer EA1110 type thermo Finnegan device, was used to measure the N content of the sample, and its protein content was obtained by multiplying this value by 6.25. A simple titration was applied to determine the free fatty acid (FFA) content. For this procedure, a 10 g aliquot of oil was dissolved in an equal volume of ethyl ethanol and 50 mL to 10 uL in the presence of phenolphthalein, and titrated against 0.01N NaOH until the solution reached a stable pink endpoint. The FFA content was calculated on either an oleic or a palmitic acid basis. Each sample was titrated in duplicate. The peroxide value (PV) of the oil was also obtained by titration, using 5 g oil dissolved in 30 mL acetic acid: iso-octane (3:2 *v*/*v*). Following the addition of 0.5 mL saturated potassium iodide (KI), the mixture was titrated with 0.01N sodium thiosulfate, using the starch/iodine reaction as the indicator.

3. Statistical Analysis

The experimental data were statistically analyzed using the software package PIAF (Planning Information Analysis Program for Field Trials). A general linear model was assumed, and multiple comparisons were performed using a *t*-test, with a chosen significance level of $p < 0.05$. Mean values were compared using a least significant difference test.

4. Results and Discussion

The results of this study indicate that application of fungicides at BBCH 53 and 65 increased leaf area index (LAI) of rapeseed by delaying senescence, in comparison with application of triazole (Toprex) or strobilurin (Ortiva) fungicides, as well as control treatment ,at the later stages of rapeseed in Giessen (Table 2). Maximum LAI was recorded by application of Toprex (paclobutrazole and difenoconazole) at the rate of 0.5 L/ha in combination with Ortiva (azoxystrobin) at all growth stages. This effect can be explained by prolonged photosynthetic duration of green tissues by Ortiva application at BBCH 65 as compared with its combined application with triazole fungicides. Similar results were recorded by Zhang *et al.* (2010) [4] in wheat, who postulated that Ortiva application delayed senescence by enhancing antioxidative potential and protecting the plants from harmful active oxygen species.

Our triazole treatment (Folicur + Proline) reduced LAI among double-applied treatments at later stages of rapeseed. Zhou and Leoul (1998) [13] reported that application of triazole increased the level of stress hormone abscisic acid (ABA), which favors senescence. Strobilurin performed best as the second application at BBCH 65 to delay senescence compared with triazole.

Triazole and trinexapac are anti-gibberellins that improve stem stability by inhibiting intercalary growth, which reduces the probability of lodging. Combinations of chemicals had higher LAI and reduced lodging more than single applied fungicides (Table 2). Leaf area index is the ratio of green plant material that covers a square meter of land and has a direct influence on crop vigor, root development, carbohydrate storage, and nutrient transport. Healthiness of rape plants was improved with increased LAI and hence lodging was reduced considerably.

Height of plant stands had an inverse relation with lodging, as observed by Armstrong and Nicol (1991) [14]. At both stations sole-applied Ortiva and control resulted in a severely lodged crop with a minimum plant height (PH) in comparison with other treatments (Table 2). Higher plant height (PH) with minimum lodging was recorded for Folicur + Proline over other treatments at both stations. Application of Ortiva in combination with Toprex produced healthy plants which resisted lodging. At Giessen it was observed that by reducing the concentration of Toprex from 0.5 to 0.35 L·ha^{-1} in combination with Ortiva increased lodging. Baylis and Wright (1990) [15] also observed similar results after applying paclobutrazole on winter rapeseed.

Table 2. Effect of fungicides and growth regulator on leaf area index (LAI) at GI, lodging (Lodg) and plant height (PH) of two cultivars of rapeseed at GI and RH.

Treatments	Giessen					Rauischholzhausen	
	Leaf Area Index			Lodg (1–9)	PH (cm)	Lodg (1–9)	PH (cm)
	BBCH 62	BBCH 75	BBCH 80	BBCH 80	BBCH 80	BBCH 80	BBCH 80
Control	7.29	4.34	2.91 d	5.3	147.5 b	5.8	100.3 cd
Toprex (Top$_{0.5}$)	7.08	4.59	3.64 ab	4.1	160.3 a	5.1	104.2cd
Top$_{0.5}$ + Ortiva	7.78	5.07	3.99 a	3.9	161.2 a	5.8	105.6 bcd
Top$_{0.35}$ + Ortiva	7.32	4.94	3.54 bc	4.5	160.3 a	4.9	115.7 abc
Top$_{0.35}$ + Mo + Ort.	6.68	4.71	3.76 ab	3.3	158.3 a	6.0	108.6 bcd
Ortiva	6.96	4.49	3.33 c	4.6	154.6 ab	6.9	97.9 d
Folicur + Proline	6.77	4.73	3.45 bc	3.6	160.4 a	4.8	125.4 a
Caramba + Cantus	6.95	4.78	3.79 ab	3.5	159.4 a	4.9	121.8 ab
Fun. (LSD$_{0.05}$)	**ns**	**ns**	**0.36**	**-**	**7.2**	**-**	**14.6**
NK Fair	7.01	4.70	3.72 a	3.5	169.2 a	4.4	125.3 a
Elektra	7.20	4.71	3.39 b	4.8	146.3 b	6.6	93.4 b
Cv. (LSD$_{0.05}$)	**ns**	**ns**	**0.18**	**-**	**3.6**	**-**	**7.28**
Fun. x Cv. (LSD$_{0.05}$)	**ns**	**ns**	**ns**	**-**	**10.2**	**-**	**ns**

Top$_{0.5}$ = Toprex @ 0.5 L·ha^{-1}, Top0.35 = Toprex @ 0.35 L·ha^{-1}, Mod = Moddus, Ort = Ortiva, Fun. = Fungicides, ns = non-significant. *a > b > c > c > d.

1000-grain weight (TGW) is an important yield component because large seed is more valuable. Application of fungicides altered TGW significantly at both stations. Heavy-lodged plants of untreated plots attained the lowest TGW at both stations (Table 3). Due to lodging, growing conditions are unfavorable for seed filling because of reduced light, which has a negative impact on photosynthesis [16–18]. Severe lodging interferes with the transport of nutrients and moisture from the soil and, thus, with storage in developing seeds. Incomplete filling results in small seeds with lower oil and protein content and weight. TGW was lower at RH than that of Giessen. This can be explained by severe lodging at RH compared with Giessen.

At both stations, performance of Caramba in combination with Cantus was consistent to enhance TGW, while application of Toprex by itself reduced TGW among fungicidal treatments. Berry and Spink (2009) [19] also reported that Caramba (metconazole) application in winter rapeseed enhanced TGW by increasing leaf area index and developing optimum size of the crop canopy, which helped to reduce lodging. Growth regulator Moddus (trinexapac) treatment attained maximum value of TGW and minimal lodging at Giessen, while at RH the same treatment plots were lodged [17–20]. This was caused by a thunderstorm at the time of maturity. Our results demonstrate a negative relationship between lodging and TGW.

Combined application of triazole fungicides (Folicur + Proline) significantly increased TGW at both stations (Table 2). At the same time this treatment decreased number of pods per main stem and increased number of seeds per stem. The reduction in pod number caused assimilates to accumulate in less pods, leading to higher TGW than that of the control. In both experiments, we found a direct relation of LAI with TGW [21]. Double-applied treatments attained the highest LAI and also maximum TGW than that of the control treatments. Reduction of TGW in the case of cv. NK Fair was associated with higher plant height in comparison with cv. Elektra. Shorter plants received equal and maximum light throughout the canopy and, consequently, large seeds were produced [8].

Table 3. Effect of fungicides and growth regulator on TGW, seed yield, and oil content of two cultivars of winter rapeseed at GI and RH.

Treatments	Giessen			Rauischholzhausen		
	TGW (g)	Seed Yield (dt·ha^{-1})	Oil (%)	TGW (g)	Seed Yield (dt·ha^{-1})	Oil (%)
Control	4.48 *b	52.0 d	43.5	3.77 d	53.9	42.7 b
Toprex ($Top_{0.5}$)	4.59 ab	57.5 bc	43.3	3.89 cd	53.9	42.3 b
$Top_{0.5}$ + Ortiva	4.71 a	62.1 a	43.9	4.11 abc	55.5	43.2 ab
$Top_{0.35}$ + Ortiva	4.63 a	57.9 b	45.0	4.08 abc	58.2	42.8 b
$Top_{0.35}$ + Moddus + Ortiva	4.71 a	59.3 ab	43.9	3.92 cd	54.3	43.1 ab
Ortiva	4.61 a	55.1 cd	43.7	3.95 bcd	56.5	43.2 ab
Folicur + Proline	4.67 a	57.6 bc	43.6	4.17 ab	57.2	42.2 b
Caramba + Cantus	4.60 ab	62.1 a	44.1	4.26 a	58.0	44.2 a
Fun. ($LSD_{0.05}$)	0.12	3.60	ns	0.22	ns	1.16
NK Fair	4.46 b	58.0	45.2 a	3.73 b	53.8 b	43.5 a
Elektra	4.79 a	57.9	42.6 b	4.31 a	58.1 a	42.5 b
Cv. ($LSD_{0.05}$)	0.06	ns	0.73	0.11	0.54	0.58
Fun. x Cv. ($LSD_{0.05}$)	ns	ns	ns	ns	ns	1.63

$Top_{0.5}$ = Toprex @ 0.5 L·ha^{-1}, $Top_{0.35}$ = Toprex @ 0.35 L·ha^{-1}, ns = non-significant.*a > b > c > c > d.

Seed yield and its formation process depend on genetic, environmental, and agronomic factors, including growth regulation and the interaction between them. In this experiment, growth-regulating fungicides altered seed yield significantly at Giessen, while it was unaffected at RH (Table 3). Results showed that TGW was positively correlated with seed yield at both stations. Control treatment exhibited lowest seed yield with minimum TGW at both stations. This yield loss was associated with reduced LAI compared to other treatments. Assimilate production in plants is reduced if the LAI is below the optimum required to capture all light transmitted beyond the flower layer. Maximum seed yield was recorded for the combined application of Caramba and Cantus, corresponding to the highest number of pods and seeds per main stem. $Top_{0.5}$in combination with Ortiva also attained high seed yield through improving LAI and TGW at Giessen. Tuncturk and Ciftci (2007) [22] also reported that number of seeds per pod, 1000-seed weight, and number of seeds per pod have a direct positive effect on seed yield.

In our study, seed yield was strongly related to the severity of lodging. Ortiva alone-applied plants recorded the lowest seed yield and were susceptible to maximum lodging among fungicidal treatments at Giessen. The increase in individual seed weight was negligible among triazoles. Previous studies found that yield improvements in response to triazole fungicides uniconazole and paclobutrazol were unrelated to changes in individual seed weight [8,14]. Higher LAI in the case of $Top_{0.5}$ + Ortivawas likely a result of more rapid stem elongation and the partitioning of a greater proportion of assimilate to above-ground growth as a result of maximum seed yield at Giessen. It seems plausible that reducing LAI and plant height resulted in a stronger stem. These effects may explain a large reduction in lodging from combined application of Folicur and Proline at both stations.

The value of rapeseed linked to its seed oil content was influenced significantly by the application of fungicides at RH (Table 3). At both stations it was observed that application of only triazole fungicides ($Top_{0.5}$and Folicur + Proline) reduced oil content in comparison with control and other treatments [6,16]. Our results are contradicted by MertTürk *et al.* (2008) [23] and Butkute *et al.* (2006) [24], who worked with triazole fungicides Harvesan and Folicur, respectively, and reported that oil content of rapeseed increased significantly after triazole application in comparison with the control. Application of Ortiva and Cantus in combination with triazole fungicides enhanced oil content by extending the seed formation phase which led to increased oil accumulation in the seeds. Including Caramba improved yield associated parameters (number of pods and seeds per main stem) and seed yield, and also resulted in higher oil content.

In the literature it was explained that oil content of rapeseed is influenced by air temperature, especially after flowering. This was confirmed with our results in which oil content of seed samples from RH was 1% lower due to its higher air temperature (9.7 °C) than that of Giessen experimental station (8.5 °C). Hassan *et al.* (2007) [25] also reported that an increase of 1 °C temperature caused a loss of 1.2% of oil in the rapeseed. Interactions between fungicide and cultivar regarding oil content of rapeseed were significant at Giessen, which showed that cultivars responded differently in oil content after application of fungicides.

Fungicides at RH influenced protein content of seed, whereas no clear variations were observed at the other location (Table 4). Butkute *et al.* (2006) [24] reported that the application of Folicur on rapeseed enhanced protein content significantly over the control. These results agreed with our findings from RH. Among the fungicidal treatments, Ortiva application improved protein content significantly in comparison to control at RH. Jenkyn *et al.* (2000) [26] also reported that application of azoxystrobin enhanced protein content in the grains of wheat. An inverse relationship was observed between oil and protein in both experiments. Higher oil and lower protein content was observed at the Giessen experimental station compared to RH experimental station, which might be due to temperature differences. These findings are consistent with those of Pritchard *et al.* (2000) [27], who recorded increased protein with decreased oil content, and concluded that wetter and cooler spring weather would favor higher oil accumulation, but lower proteins.

Significant interactions were recorded among cultivars and fungicides regarding oil and protein content at RH. Cultivars differed markedly for protein and oil content at both stations and responded differently to fungicide application. NK Fair was a late maturing cultivar and its LAI increased significantly at BBCH 80 compared to *cv.* Elektra. These results agreed with the findings of Dimov and Möller (2010) [28] that tested modern winter oilseed rape cultivars including *cv.* NK Fair and *cv.* Elektra in field experiments under typical German growing conditions.

In the present study, concentration of free fatty acids (FFA) in the oil of rapeseed varied from 0.12 to 0.16%, which was lower than that reported by May *et al.* (1993) [29] who obtained 0.41 to 0.54% FFA in the oil of rapeseed after application of fungicides. Our experimental data demonstrated that concentration of FFA was significantly influenced by the application of fungicides at RH, while FFA was unaffected at Giessen (Table 4). May *et al.* (1993) [29] reported that FFA was unaffected after application of fungicides, while FFA was significantly influenced by agronomic practices, including low seeding rates, increased nitrogen fertilization, and delayed planting in Ontario grown spring rapeseed. Concentration of FFA correlated with the intensity of lodging. Maximum FFA was recorded from heavily lodged Ortiva alone-treated plants at both stations.

Table 4. Effect of fungicides and growth regulator on protein content, free fatty acids (FFA) and peroxides value (PV) of two cultivars of winter rapeseed at GI and RH.

Treatments	Giessen			Rauischholzhausen		
	Protein (%)	FFA (%)	PV (meq·kg^{-1})	Protein (%)	FFA (%)	PV (meq·kg^{-1})
Control	21.8	0.14	3.18 *b	22.2 c	0.15 ab	2.46 a
Toprex ($Top_{0.5}$)	22.1	0.12	3.54 a	23.1 ab	0.15ab	2.34 bc
$Top_{0.5}$ + Ortiva	22.6	0.12	2.61 c	22.9 bc	0.15 ab	2.44 ab
$Top_{0.35}$ + Ortiva	21.7	0.14	2.31 d	22.9 bc	0.14 b	2.50 a
$Top_{0.35}$ + Moddus + Ortiva	21.5	0.13	2.36 d	23.9 a	0.15 ab	2.10 e
Ortiva	21.9	0.15	2.50 cd	24.1 a	0.16 a	2.43 ab
Folicur + Proline	21.8	0.14	2.26 d	23.9 a	0.15 ab	2.26 cd
Caramba + Cantus	22.0	0.14	2.39 cd	22.8 bc	0.15 ab	2.22 d
Fun. ($LSD_{0.05}$)	ns	ns	0.24	0.93	0.01	0.11
NK Fair	22.7 a	0.13	2.84	24.1 a	0.14 b	2.19 b
Elektra	21.1 b	0.13	2.45	22.1 b	0.16 a	2.49 a
Cv. ($LSD_{0.05}$)	0.70	ns	0.12	0.47	0.01	0.05
Fun. x Cv. ($LSD_{0.05}$)	ns	0.03	0.34	1.32	0.01	ns

$Top_{0.5}$ = Toprex @ 0.5 L·ha^{-1}, $Top_{0.35}$ = Toprex @ 0.35 L·ha^{-1}, ns = non-significant. *a > b > c > c > d.

In this study, PV was altered significantly by application of fungicides at both stations. Growth regulator Moddus, and triazole fungicides Folicur and Caramba, treatments reduced PV significantly compared to the control at both stations (Table 4). This may be due to minimal damage of seeds during harvesting from these treatments. These treatments protected plants from severe lodging compared to the control treatment. Lodging caused mechanical damage to seeds during harvesting [15], and damaged seeds led to increased PV. Severely lodged *cv.* Elektra also attained significantly higher PV compared with *cv.* NK Fair at RH.

In this study, it was observed that fungicidal treatments like Caramba + Cantus, which produced maximum oil content also attained higher oleic acid, which agreed with the findings of MertTürk *et al.* (2008) [22], who reported that application of triazole fungicide Harvesan increased oil content, as well as oleic acid compared with control. Linoleic and linolenic acids were significantly affected by application of fungicides. These unsaturated fatty acids slightly increased in concentration by the application of triazole fungicides $Top_{0.5}$ alone at Giessen and Folicur + Proline at RH (Table 5). Results demonstrated that increased oleic acid related to the decreased linoleic acid. This relation of fatty acids can be explained by the activity of the enzyme FAD2 that converts oleic acid to linoleic acid which is in turn, converted to linolenic acid by FAD3.

Table 5. Effect of fungicides and growth regulator on unsaturated fatty acids of two cultivars of winter rapeseed at GI and RH.

Treatments	Giessen			Rauischholzhausen		
	C18:1	**C18:2**	**C18:3**	**C18:1**	**C18:2**	**C18:3**
Control	61.3	19.5 ab	9.42 b	59.0	19.8 a	10.0 cd
Toprex ($Top_{0.5}$)	60.3	19.6 a	9.80 a	59.8	19.3 bc	9.7 e
$Top_{0.5}$ + Ortiva	60.9	19.3 abc	9.55 ab	60.0	19.2 c	9.8 cde
$Top_{0.35}$ + Ortiva	61.6	18.7 d	9.52 ab	60.1	19.0 c	9.8 de
$Top_{0.35}$ + Moddus + Ortiva	61.1	19.0 bcd	9.50 b	59.5	19.1 c	9.8 de
Ortiva	61.3	18.7 d	9.26 b	60.3	19.7 ab	10.4 b
Folicur + Proline	60.7	18.9 cd	9.36 b	59.9	19.9 a	10.7 a
Caramba + Cantus	61.0	18.8 cd	9.42 b	60.8	19.1 c	10.1 bc
Fun. ($LSD_{0.05}$)	ns	0.49	0.29	ns	0.31	0.26
NK Fair	62.4* a	18.5	9.26 b	60.1 a	19.3	10.0
Elektra	59.7 b	19.6	9.70 a	59.8 b	19.5	10.1
Cv. ($LSD_{0.05}$)	0.54	0.25	0.15	0.54	ns	ns
Fun. × Cv. ($LSD_{0.05}$)	ns	ns	ns	ns	0.45	0.37

$Top_{0.5}$ = Toprex @ 0.5 $L \cdot ha^{-1}$, $Top_{0.35}$ = Toprex @ 0.35 $L \cdot ha^{-1}$, ns = non-significant; C18:1 = Oleic acid, C18:2 = Linoleic acid, C18:3 = Linolenic acid. *a > b > c > d > e.

We concluded that positive effects on plant growth were observed when triazole and strobilurin fungicides were applied. Moreover, it was shown that application of growth-regulating fungicides can effectively control overlarge canopies in order to reduce lodging and achieve optimally-sized seeds. Positive yield effects were achieved after combined application of triazole at BBCH 53 and strobilurin fungicides at BBCH 65, compared with their sole applications. Quality parameters of rape oil, including oil content, fatty acid profile, free fatty acids, and peroxide value can be influenced by application of fungicides, but are also dependent on weather conditions and cultivar effects.

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Authors Contribution

Muhammad Ijaz and Bernd Honermeier designed the study. Muhammad Ijaz prepared the material. Bernd Honermeier helped in conducting field experiments. Muhammad Ijaz, Bernd Honermeier, and Khalid Mahmood, analyzed data through statistically analysis. Muhammad Ijaz,Khalid Mahmood and Bernd Honermeier wrote the manuscript. Bernd Honermeier supervised the project.

Conflicts of Interest

The authors declare no conflict of interest.

List of Abbreviations

cm	centimeter
Cv.	Cultivar
dt	decitones
FAO	Food and Agriculture Organization (of the United Nations)
FID	Flame Ionization Detector
FFA	Free Fatty Acids
GC	Gas Chromatography
GI	Giessen
GSL	Glucosinolates
Fun	Fungicides
g	gram
ha	hectare
K	Potassium
kg	kilogram
LAI	Leaf Area Index
LSD	Least Significant Difference
mequ	Milliequvalent
ns	Non-Significant
p	Probability
PH	Plant Height
PIAF	Planning Information Analysis Program for Field Trials
PV	Peroxides value
RCBD	Randomized Complete Block Design
RH	Rauischholzhausen
TGW	1000-grain weight

References

1. Kumar, B.; Sing, Y.; Ram, H.; Sarlach, R.S. Enhancing seed yield and quality of Egyptian Clover (*Trifolium alexandrinum* L.) with foliar application of bio-regulators. *Field Crop Res.* **2013**, *146*, 25–30.
2. Venancio, W.S.; Rodrigues, M.A.T.; Begliomini, E.; Souza, N.L.D. Physiological effects of strobilurin fungicides on plants. *Cienc. Exatas Terra Cienc. Agr. Eng.* **2003**, *9*, 59–68.
3. Ruske, R.E.; Gooding, M.J.; Jones, S.A. The effects of adding picoxystrobin, azoxystrobin and nitrogen to a triazole programme on disease control, flag leaf senescence, yield and grain quality of winter wheat. *Crop Prot.* **2003**, *22*, 975–987.
4. Zhang, Y.J.; Zhang, X.; Zhou, M.G.; Chen, C.J.; Wang, J.X.; Wang, H.C.; Zhang, H. Effect of fungicides JS399-19, azoxystrobin, tebuconazole, and carbendazim on the physiological and biochemical indices and grain yield of winter wheat. *Pestic. Biochem. Physiol.* **2010**, *98*, 151–157.
5. Graebe, J.E. Gibberellin biosynthesis and control. *Ann. Rev. Plant Physiol.* **1987**, *38*, 419–465.
6. Setia, R.C.; Bhathal, G.; Setia, N. Influence of paclobutrazol on growth and yield of *Brassica carinata* A. *Br. Plant Growth Regul.* **1995**, *16*, 121–127.

7. Rademacher, W. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 501–531.
8. Zhou, W.; Ye, Q. Physiological and yield effects of uniconazole on winter rape (*Brassica napus* L.). *Plant Growth Regul.* **1996**, *15*, 69–73.
9. Ijaz, M.; Honermeier, B. Effect of triazole and strobilurin fungicides on seed yield formation and grain quality of winter rapeseed (*Brassica napus* L.). *Field Crops Res.* **2012**, *130*, 80–86.
10. Child, R.D.; Evans, D.E.; Allen, J.; Arnold, G.M. Growth responses in oilseed rape (*Brassica napus* L.) to combined applications of the triazole chemicals triapenthenol and tebuconazole and interaction with gibberellin. *Plant Growth Regul.* **1993**, *13*, 203–212.
11. Jensen, W.B. The origin of the soxhlet extractor. *J. Chem. Educ*. **2007**, *84*, 913–914.
12. Sepännen, L.; Hiltunen, R. Analysis of fatty acids by gas chromatography and its relevance to research on health and nutrition. *Anal. Chim. Acta* **2002**, *465*, 39–62.
13. Zhou, W.; Leul, M. Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. *Plant Growth Regul.* **1998**, *26*, 41–47.
14. Armstrong, E.L.; Nicol, H.I. Reducing height and lodging in rapeseed with growth regulators. *Aust. J. Exp. Agric.* **1991**, *31*, 245–250.
15. Baylis, A.D.; Wright, T.J. The effects of lodging and a paclobutrazol-chlormequat chloride mixture on the yield and quality of oilseed rape. *Ann. Appl. Biol.* **1990**, *116*, 287–295.
16. Baylis, A.D.; Hutley-Bull, P.D. The effects of a paclobutrazol based growth regulator on the yield, quality and ease of management of oilseed rape. *Ann. Appl. Biol.* **1991**, *118*, 445–452.
17. Berding, N.; Hurney, A.P. Flowering and lodging, physiological-based traits affecting cane and sugar yield. *Field Crops Res.***2005**, *92*, 261–275.
18. Rolston, R.; Trethewey, J.; Chynoweth, R.; Mccloy, B. Trinexapac-ethyl delays lodging and increases seed yield in perennial ryegrass seed crops. *N. Zeal. J. Agric. Res*. **2010**, *53*, 403–406.
19. Berry, P.M.; Spink, J.H. Understanding the effect of a triazole with anti-gibberellin activity on the growth and yield of oilseed rape (*Brassica napus*). *J. Agric. Sci.* **2009**, *147*, 273–285.
20. Rajala, A.; Peltonen-Sainio, P.; Onnela, M.; Jackson, M. Effects of applying stem shortening plant growth regulators to leaves on root elongation by seedlings of wheat, oat and barley: Mediation by ethylene. *J. Plant Growth Regul.* **2002**, *38*, 51–59.
21. Faraji, A. Quantifying factors determining seed weight in open pollinated and hybrid oilseed rape (*Brassica napus* L.) cultivars. *Crop Breed. J.* **2011**, *1*, 41–54.
22. Tuncturk, M.; Ciftci, V. Relationships between yield and some yield components in rapeseed (*Brassica napus* ssp. *Oleifera* L.) cultivars by using correlation and path analysis. *Pak. J. Bot.***2007**, *39*, 81–84.
23. Mert-türk, F.; Gül, M.K.; Egesel, C.Ö. Nitrogen and fungicide applications against *Erysiphe cruciferarum* affect quality components of oilseed rape. *Mycopathol.* **2008**, *165*, 27–35.
24. Butkute, B.; Sidlauskas, G.; Brazauskiene, I. Seed yield and quality of winter oilseed rape as affected by nitrogen rates, sowing time and fungicide application. *Commun. Soil Sci. Plant Anal.* **2006**, *37*, 272–274.
25. Hassan, F.U.; Manaf, A.; Qadir, G.; Basra, S.M.A. Effects of sulphur on seed yield, oil, protein and glucosinolates of canola cultivars.*Int. J. Agric. Biol.* **2007**, *3*, 504–508.

26. Jenkyn, J.F.; Bateman, G.L.; Gutteridge, R.J.; Edwards, S.G. Effect of foliar sprays of azoxystrobin on take-all in wheat. *Ann. Appl. Biol.* **2000**, *137*, 99–106.
27. Prithchard, F.M.; Eagles, A.; Norton, R.M.; Salisbury, P.A.; Nicolas, M. Environmental effects on seed composition of Victorian canola. *Aust. J. Exp. Agric.* **2000**, *40*, 679–685.
28. Dimov, Z.; Möllers, C. Genetic variation for saturated fatty acid content in a collection of European winter oilseed rape material (*Brassica napus*). *Plant Breed*. **2010**, *129*, 82–86.
29. May, W.E.; Hume, D.J.; Hale, B.A. Effects of agronomic practices on free fatty acid levels in the oil of Ontario-grown spring canola. *Can. J. Plant Sci.* **1993**, *74*, 267–274.