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Research Article

Collateral Effects of Some Fungicides Commonly Used on Wheat Plants

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Abstract:

The present investigation was conducted at the experimental farm and laboratory of Etay El-Baroud Agriculture Research Station, Agricultural Research Center (ARC) during two growing seasons 2021 and 2022. The recommended and half recommended dosages of some foliar fungicides: Tilt® 25% EC (propiconazole), Nasrzol® 25% EC (propiconazole), and Montoro® 30% EC (difenoconazole) were examined on growth, yield and yield quality as well as grains viability of the commercial wheat cultivar Giza 171 compared with the control (untreated). The obtained results showed that Wheat grains that treated with full dosage of Nasrzol® had the lowest water absorption ratios after 5 and 24 hr, while the highest grains germination was recorded in wheat plants that treated with 15 cm3/fed. of Nasrzol®. The obtained results stated that all the tested fungicides superior the control in increase tillers number/m2 and plant height during both seasons, except half recommended dosages of Tilt® and Nasrzol® in plant height. The highest numbers of spike/ m2, spike lengths, grain yield/ m2, straw yield/ m2 and biological yield/ m2 were recorded in the plants that treated with the recommended dosage of Nasrzol® or full dosage of Tilt®. The highest carbohydrates percentage in the wheat grains and in straw was recorded in the plants that treated with full dosage of Tilt® (25 cm3/fed.), followed by the plants that treated with full dosage of Nasrzol® during both seasons. While, the highest protein percentage in the wheat grains and in straw was noticed in the plants of control group, followed by the plants that treated with half dosage of Tilt® during both seasons. The present study recommends the farmers to follow up labeling items for pesticides before use and take care about their awareness about their good practices and optimum conditions for planting processes for providing healthy crops

1. Introduction

Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. It is ranked the second after corn (FAOSTAT, 2022). Winter wheat is the major source for carbohydrate in human feeding (USDA-ERS, 2022). The total world harvested area of wheat was 234.59 million hectares, with total production of dry grains reached 814.23 million tons. In the same year, the total harvested area of wheat in Egypt was 1.57 million hectares with total production reached 11.29 million tons (FAOSTAT, 2022). Winter wheat is attack by several fungal pathogens during their growing season that which can cause significant loss in the grain yield. Some of the most important fungal diseases: tan spot *(Pyrenophora triticirepentis), Stagonospor anodorum* blotch (*Parastagono sporanodorum*), leaf rust (*Puccinia triticina*), stripe rust (*Puccinia stritiformis f. sp. tritici*), powdery mildew *(Blumeriagraminis f. sp. tritici*) and fusarium head blight (FHB, *Fusarium graminearum*) (Bockus et al., 2010). The losses in bread wheat yield caused by fungal diseases vary according to the type of pathogen. For example, wheat powdery mildew could cause yield losses up to 45%, if the infection started early with favorable environmental conditions for disease incidence and development during the growing season (Hong et al., 2018). Fungal pathogens of wheat Survive instead of live on crop residues. Hence, inoculum is always present in wheat producing areas. This is partly due to the agricultural practices such as no-till and the cultivation of unapproved wheat grains without fungicide treatment (Dill-Macky and Jones, 2000) as well as a lack of attention to weed control, which is an intermediate host for many fungal diseases. One of the most common and effective fungal disease controls strategies in winter wheat is the use of foliar fungicides (Lackerman et al., 2011).

Wheat has a greater potential to receive fungicide treatment than maize or soybean (Byamukama et al., 2016). Many fungicides are available for protection against fungal pathogens, especially on flag leaves, the major contributor to the amount of grain yield (Dimmock and Gooding, 2002). Fungicides are most often used at flowering to manage Fusarium head blight, however, applications at tillering and flag leaf growth stages are not uncommon. Several factors affect the profitability of using fungicides. These include cultivar susceptibility, disease stress, weather conditions, and grain prices. Wegulo et al. (2011) found that fungicides reduced *Fusarium* and deoxynivalenol more in moderately resistant cultivars than in susceptible cultivars. However, De Wolf et al. (2012) showed that cultivars susceptible to leaf disease have a greater likelihood of positive response to fungicide application than resistant cultivars at low, medium, or high levels of disease stress. Another factor affecting yield response to fungicide application is the level of weather-induced disease stress. Weather, specifically rainfall and temperature, drives the development of fungal disease epidemics (Wiik and Ewaldz, 2009; Wegulo et al., 2011). Precipitation is the most important factor for disease development (Thompson et al., 2014). Rainfall affects the extent of disease development in two ways: the period of leaf wetness for infection initiation (Rowlandson et al., 2015) and in the spread of pathogens through rain spray (Madden, 1997). Precipitation can also reduce the residual potency of fungicides depending on the time and frequency of fungicide application (Pigati et al., 2010). Grain prices influence the profitability of fungicide use in wheat by influencing the minimum number of bags required to offset the cost of purchasing and using the fungicide (Lopez et al., 2015). In addition to the main role played by fungicides in disease control, they may play another important role through their effect directly or indirectly on the yield and quality, in all variants of fungicide use. Also, total protein and carbohydrates increased significantly in all fungicide-applicant variants (Gaile et al., 2023). Motta-Romero et al. (2021) found that total protein and carbohydrates in the wheat grains increase in the presence of fungicides, with respect to untreated one. In the same line, Iwaniuk et al. (2022) evaluated the effects of triazole fungicides on concentrations of 20 amino acids (AAs) and on yield parameters of the wheat. Application of fungicides reduced AA concentrations. Synthetic fungicide is the most effective for increasing wet gluten, total protein and carbohydrates. Although chemical control of fungal diseases is important, in many cases it may also be responsible for damage to the physiological quality of many seeds, resulting in reduced water uptake of the seeds and low germination rates (Camilo, 2017; Liu et al., 2021). Therefore, the current study aims to evaluate the effect of some fungicides commonly used in Egypt on morphological, yield and quality of wheat plants in a field trails. Also, evaluate their effect on water absorption and grains germination under laboratory conditions.

2. Materials and Methods

2.1. Examined pesticides

Recommended and half-recommended dosages of some foliar fungicides: Tilt® 25% EC (propiconazole), Nasrzol® 25% EC (propiconazole), and Montoro® 30% EC (difenoconazole) were used. They were obtained from Central Agricultural Pesticides laboratory (CAPL), Agricultural Research Center (ARC), Egypt.

2.2. Lay out of experiments

For this proposal, a field experiment was layout in randomized complete block system with three replicates in the experimental farm of Etay El-Baroud agriculture research station during 2021 and 2022 seasons. The experimental soil physical and chemical properties are presented in Table (1) While some agro-climatic factors of the experiment area during 2020/2021 and 2021/2022 growing seasons are presented in Table (2). During both seasons of the study, wheat grains were sown in 20th Nov. In plots, each plot was 4 meters long and 3 meters apart. Wheat grains were sown in five rows per each plot within 60 cm between rows and 10 cm between hills. Wheat plants were, received all recommended practices of irrigation, fertilization, and weed control. During the two seasons, all tested fungicides were applied as foliar spraying three times, Starting at the beginning of flag leaf development and repeated two times with 15 days' intervals between the following spraying.

Table 1. Physico-chemical properties of the experimental soil during both seasons.

Properties	Season	
	2021	**2022**
Particle size distribution		
Clay %	60.41	59.61
Slit %	32.5	31.8
Sand %	7.09	8.59
Texture	Clay	
$CaCO_3$ %	3.15	2.45
Soluble cations (meq/L)		
Ca^{++}	6.12	5.10
Mg^{++}	3.54	2.61
K^{+}	1.56	1.64
Na^{+}	8.17	6.89
Soluble Anions (meq/L)		
Cl^{-}	10.11	8.42
HCO_3^{--}	0.85	0.70
SO_4^{--}	8.43	7.02
Available nutrient (mg/kg)		
K^{+}	74.11	68.34
P	2.66	2.34
N	41.78	40.09
OM%	0.68	0.54
pH	7.70	7.75
E.C (dS/m)	1.93	1.88
SAR	3.73	3.53

-OM= organic matter, E.C= electric conductivity, and SAR =sodium adsorbed ratio.

2.3. Data recorded

2.3.1. Grain viability test

Hundred grains of the wheat (either control or treatments) were soaked in water for 30 min and lifted to dry under laboratory condition for six hr. After full dry, the grains were divided into two equal divisions in Petri dishes for assessment.

2.3.2. Water absorption ratio

Treated wheat grains were pre-weighted and soaked in water for 24 hr, then post-weighted again to determine water absorption ratio as follows:

$$\textbf{Water absorption ratio} = \frac{Post\ weight\ (g) - pre\ weight\ (g)}{Post\ weight\ (g)} x\ 100$$

2.3.3. Grain germination (%)

Fifteen treated wheat grains from each treatment were germinated artificially under laboratory conditions and the percentage of germination was calculated as follows:

$$\textbf{Germination}\ (\%) = \frac{Number\ of\ full\ germinated\ grains}{Number\ of\ grains\ in\ Petri\ dish}\ x\ 100$$

2.3.4. Growth traits

The growth parameters of the plans: number of tillers/m^2, plant height (cm), leaf contents of chlorophyll a, chlorophyll b, and carotenoids were measured.

2.3.5. Grain yield and its components traits

The specific parameters of the grains: number of spike/m^2, spike length (cm), 1000-grains weight (g), grain yield/m^2 (g), straw yield/m^2 (g), and biological yield/m^2 (g) were measured.

2.3.6. Grain quality traits

The biochemical parameters of the grains were determined for grain content of total carbohydrates (%) and total protein (%).

2.4. Estimate of leave′s contents of chlorophyll and carotenoids.

Leave′s contents of chlorophyll and carotenoids were done chromatically according to method of Guthyyie (1928). There shall be grinding (0.5 g of fresh leaves) with a pinch of sodium carbonate+20 g pure sand and then wetted with small amount of acetone. Five ml of acetone were added to the previous mixture, and filtered. The remain was washed repeatedly with acetone and filtered until transparent color and does not come down any color. The solution was completed to one liter every ten ml. Therefore, the solution was measured at wavelengths: 663, 644, and 440 nm for chlorophyll a, b, and carotenoids, respectively. Their levels were estimated through the equations:-

$$Ch\ A(mg/100\ g\ sample) = \frac{(10.3xch\ A\ read) - (0.918x\ ch\ B\ read)x\,\frac{final\ volume}{sample\ weigt}x100}{1000} \quad [1]$$

$$Ch\ B(mg/100\ g\ sample) = \frac{(19.7xch\ B\ read) - (3.87x\ ch\ A\ read)x\,\frac{final\ volume}{sample\ weigt}x100}{1000} \quad [2]$$

$$Carotene(mg/100\ g\ sample) = \frac{(4.695x\text{Carotene}\ read)x\,\frac{final\ volume}{sample\ weigt}x100}{1000 - 0.268x\ \Sigma ch\ (A+B)} \quad [3]$$

2.4.1. Determination of carbohydrates percentage in seed.

According to Dubois et al. (1956) method, 0.01 g dry weight of seed's powder was mixed with 3 ml of 10% NaOH, boiled for 10 min, and filtered. Half ml of the extract was mixed with the same volume of 5% phenol reagent. An aliquot (2.5 ml) of sulphic acid (H_2SO_4) was added slowly on the surface of the tube, and allowed to stand at room temperature for 30 min. The developed color was measured at 490 nm. Glucose was used as a standard.

$$Carbo.(\%) = \frac{F\ (mg)x\ Extract\ volume\ (ml)}{Aliquot\ (ml)x\ sample\ weight\ (g)}\ x\ 100 \quad [4]$$

Where: F is mg carbohydrate obtained from the graph of glucose

2.4.2. Determination of total protein

After digestion of 0.001 g seed′s powder with 10% NaOH and centrifugation at 3000 rpm, an aliquot (50 µl) was taken for total protein assay according to method of Lowery et al. (1951). Bovine serum albumin (BSA) was used as a standard and the concentration of protein was measured colorimeterically at 750 nm.

$$Protein(\%) = \frac{F\ (mg)x\ Extract\ volume\ (ml)}{Aliquot\ (ml)x\ sample\ weight\ (g)}\ x\ 100 \quad [5]$$

Where: F is mg protein obtained from the graph of BSA.

2.5. Statistical analysis

All data were subjected to the analyses of variance (One-way ANOVA) separated in randomized block design RCBD design followed by compared means with LSD at level probability5% according to Gomez and Gomez (1984) using Costat **(2005)** computer software package.

3. Results and Discussion

3.1. Effect on water absorption ratio and germination percentage

The obtained results in Table (2) and (3) clear that water absorption ratio and germination percentage of the wheat grains significantly affected by all used fungicides during the two seasons.

3.1.1.Water absorption ratio

Data in Table (2) reveal that water absorption ratio in the wheat grains significantly differed under the different fungicides after 1, 5 and 24 hr from grains soaking. The highest water absorption ratio (44.77 and 42.47%) were recorded after 1 hr of soaking in wheat grains that treated with half-recommended dosage of Tilt® (12.5 cm^3/fed.) in the 1st and 2nd seasons, respectively. In contrast, the lowest water absorption ratio was showed after 1 hr (26.28%) in wheat grains that treated with half-dosage of Nasrzol® (15 cm^3/fed.) during the

1st season, with respect to the control (37.42%) during the 2nd season. Wheat grains that treated with full dosage of Tilt® recorded highly water absorption ratios after 5 hr (57.51 and 61.13%) and 24 hr (81.66 and 82.53%), with respect to their controls after 5 hr (56.81 and 61.92%) and 24 hr (80.67 and 83.59%). On the other hand, the wheat grains that treated with full dosage of Nasrzol® had the lowest water absorption ratios after 5 hr (47.51 and 46.28%) and 24 hr (67.47 and 62.48%) in 1st and 2nd seasons, respectively.

Fungicides almost increase seed tissue permeability and make it easy to penetrate. So, water will move inside seed easy. In this study, all fungicides used increased seed absorption with water compared with the control. In the same line, Solorzano and Malvick (2011) found that fungicides: azoxystrobin and fludioxonil increased the permeability of seed coats to water, which accelerates seed germination.

Gogna et al. (2015) revealed that the significant reduction of germination in fungicides-treated wheat seeds was due of their inability to mobilize stored starch in the absence of endophytes and fungicides. Also, they lead to damage and blockage of the tissues responsible for transporting and absorb the seed with water, which reduces the chances of seed germination. In contrast, Lugtenberg et al. (2016) found that seed treatment with fungicides could lead to similar loss or disruption of the seed microbiome including the endophytes and reduced seed water absorption compromising seed germination and early seedling development. Moreover, Camilo (2017) reported that seed dressing with insecticides and fungicides protects the seeds; however, it may also be responsible for damage to the physiological quality of many seeds. Within these damages occurring immediately after coating treatment or after storage caused reducing seed water absorption and low germination rates. Liu et al. (2021) reported that triconazole had low fungicidal activity and significantly reduced water absorption ratio and inhibited the germination of the wheat seeds.

3.1.2. Grains germination percentages

The results presented in Table (3) show a significant reduction in the wheat grains germination under all tested fungicides during the two seasons. Wheat grains germination as higher under half-recommended dosages of all used fungicides than the recommended dosages during the period of the study. The highest grains germination (82.58 and 82.96%) were recorded in the control group, followed by wheat treated with 15 cm^3/fed. of Nasrzol® (81.75 and 82.30%), then wheat treated with 12.5 cm^3/fed. of Tilt® (79.55 and 81.92%) in the 1st and 2nd seasons, respectively. In contrast, the lowest grains germination (74.81 and 73.51%) were showed in wheat grains that treated with full dosage of Nasrzol® (30 cm^3/fed.) during both seasons, respectively.

Table 2. Effect of some fungicides on water absorption ratio of wheat grain during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm^3/fed.)	Absorption (%)					
		1 hr		5hr		24 hr	
		1st Season	2nd Season	1st season	2nd Season	1st season	2nd Season
Tilt® 25% EC	25	38.98^b	39.42^b	57.51^a	61.13^a	81.66^a	82.53^a
Nasrzol® (5% EC	30	37.84^b	38.56^{bc}	47.51^b	46.28^e	67.47^b	62.48^e
Montoro® 30% EC	40	39.01^b	39.50^b	55.57^a	56.60^c	78.90^a	76.41^c
Tilt® 25% EC	12.5	44.77^a	42.47^a	57.48^a	58.28^b	81.62^a	78.67^b
Nasrzol® 25% EC	15	26.28^d	41.45^a	50.79^b	51.88^d	72.13^b	70.04^d
Montoro® 30% EC	20	32.49^c	39.28^b	48.25^b	53.36^d	68.52^b	72.04^d
Control	-	39.63^b	37.42^c	56.81^a	61.92^a	80.67^a	83.59^a
F test		***	***	***	***	***	***
P value		**0.0005**	**0.0005**	**0.0004**	**0.0003**	**0.0005**	**0.0004**
LSD 5%		**3.59**	**1.68**	**4.33**	**1.65**	**6.15**	**2.24**

Our findings cleared significant reduction in the wheat grain germination under all tested fungicides. The significant reduction of germination of fungicides-treated wheat seeds was due of the above-described factors affecting water absorption (Gogna et al., 2015). In addition, Camilo (2017) reported that seed coating of insecticides and fungicides protects the seeds; however, it may also be responsible for damage to the physiological quality of many seeds, with these damages occurring immediately after coating treatment or after storage caused reduced seed water absorption and lower germination rates. In the same way, Liu et al. (2021) reported that triticonazole had low fungicidal activity and significantly reduced water absorption ratio and inhibited the germination of wheat seeds.

On the other side, Entz et al. (1990) found no significant effect of Tilt® on the wheat germination at 5 ˚C. In addition, Solorzano and Malvick (2011) found that germination of seeds was higher with azoxystrobin and fludioxonil than the non-treated control. Fungicides increase the permeability of seed coats to water, which accelerates seed germination. While Lugtenberg et al. (2016) found that seed treatment with fungicides could lead to similar loss or disruption of the seed microbiome including the endophytes and reducing seed water absorption compromising seed germination and early seedling development.

Table 3. Effect of some fungicides on germination percentage of the wheat grain during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm³/fed.)	Germination (%)	
		1st season	2nd Season
Tilt® (25% EC)	25	75.09d	75.77c
Nasrzol® (25% EC)	30	74.81d	73.51c
Montoro® (30% EC)	40	76.80c	76.49bc
Tilt® (25% EC)	12.5	79.55b	81.92a
Nasrzol® (25% EC)	15	81.75a	82.30a
Montoro® (30% EC)	20	79.14b	79.76ab
Control	-	82.58a	82.96a
F test		***	***
P value		**0.0002**	**0.0003**
LSD 5%		**1.47**	**3.50**

3.2. Effect on wheat growth traits during 2021/2022 and 2022/2023 seasons.

The present data in Table (4) confirmed that the wheat growth traits (tillers number/m^2 and plant height) significantly differed under all tested fungicides during both seasons.

3.2.1. Number of tillers/m^2

The obtained results in Table (4) state that all tested fungicides superior the control in increasing tillers number/m^2 during both seasons. Full dosage of the three tested fungicides exceeded half of recommended dosage in increase number of tillers/m^{2}during the two seasons of this study. Wheat plants that sprayed with the recommended dosage of Nasrzol® (30 cm^3/fed.) recorded the highest number of tillers/m^2 (274.33 and 287.00), followed by wheat plants that sprayed with full dosage of Tilt® (25 cm^3/fed.) (271.67 and 265.00) without any significant differents between the two fungicides during both seasons, respectively. On the other side, wheat plants under the control recorded the lowest number of tillers/m^2 (199.00 and 195.33) during 1st and 2nd seasons, respectively.

All tested fungicides superior the control in increase wheat growth as tillers number/plant. Some fungicides such as triazoles accelerate chloroplast differentiation, and chlorophyll production, enlarged chloroplasts, while also protecting the integrity of chlorophyll and these functions improve plant height and branches number with significant increase of total dry mater (Fletcher et al., 2000). Also, triazole compounds increase the level of cytokinine, which might stimulate chlorophyll biosynthesis and this led to significant increase in plant height and branches numbers (Jaleel et al., 2008). Also, Petit et al. (2012) reported that fungicides are widely used to control pests in the crop plants. However, it has been reported that these pesticides may have negative effects on the crop physiology, especially on photosynthesis. An alteration in the photosynthesis might lead to a reduction in photo assimilate production, resulting in a decrease in both growth and yield of the crop plants. For example, a contact fungicide such as copper inhibits photosynthesis by destroying chloroplasts, affecting photosystem II activity and chlorophyll biosynthesis. Systemic fungicides such as benzimidazoles, an ilides, and pyrimidine are also phytotoxic, whereas azoles stimulate photosynthesis. Our findings are in the same line with those reported by Entz et al. (1990) who found significant effect of Tilt® on barley growth (plant height, tillers number and plant fresh and dry weights). Moreover, Karkanis et al. (2018) evaluated the efficacy and compatibility of herbicides with fungicides in durum wheat and reported that there was a higher tiller and spike number under chemical fungicides compared to the control.

3.2.2. Plant height (cm)

Data in Table 5 indicate that plant height significantly differed under full or the dose of all tested fungicides during both seasons. All the tested fungicides, except half-recommended dosages of Tilt® and Nasrzol® superior the control in increase plant height during both seasons. Wheat plants that sprayed with half-recommended dosage of Montoro® (20 cm^3/fed) had the tallest plants (96.67 and 94.97 cm), followed by wheat plants that sprayed with full dosage of Tilt® (25 cm^3/fed.) (96.17 and 95.73 cm) without any significant different between the two fungicides during both seasons, respectively. On the other side, wheat plants under half dosage of Tilt® recorded the lowest plant height (88.50 and 87.87 cm) in the 1st and 2nd seasons, respectively. No significant differences were observed for half dosage of Nasrzol® during the 2nd season and the control during 1st season. The impact of synthetic fungicides on the plant height were previously investigated. Entz et al. (1990) found significant effect of Tilt® on barley growth (plant height, tillers number and plant fresh and dry weights). Also, Fletcher et al. (2000) and Jaleel et al. (2008) showed that triazole compounds increased the level of cytokinin, which might stimulate chlorophyll biosynthesis and this led to significant increase in plant height and branches numbers. Koehle et al. (2002) found that growth of plants increased in wheat plants sprayed with pyraclostrobin compared with unsprayed plants. While, Palmer et al. (2003) showed that spray fungicides did not influence shoot growth and leaf area development. Moreover, Byamukama et al. (2019) proved that the positive effect of fungicide application for yield increase is better manifested if there are sufficient moisture conditions at the time most favorable for disease development during the vegetation season. The highest growth was indicated by prothioconazole and tebuconazole applications.

Table 4. Effect of some fungicides on the wheat growth traits during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm^3/fed.)	No. tillers/m^2		Plant height (cm)	
		1st season	2nd season	1st season	2nd season
Tilt® (25% EC)	25	271.67a	265.00a	96.17a	95.73a
Nasrzol® (25% EC)	30	274.33a	287.00a	96.33a	92.63a
Montoro® (30% EC)	40	255.00b	258.00ab	92.00bc	91.17ab
Tilt® (25% EC)	12.5	228.33c	205.33c	88.50c	87.87bc
Nasrzol® (25% EC)	15	207.00d	201.67c	93.33ab	85.07c
Montoro® (30% EC)	20	232.33c	226.33bc	96.67a	94.97a
Control	-	199.00d	195.33c	92.00bc	92.77a
F test		***	***	**	**
P value		**0.0002**	**0.0003**	**0.0042**	**0.0038**
LSD 5%		**14.26**	**33.97**	**3.80**	**4.74**

3.3. Effect on the wheat leaf pigments

Results in Tables (5, 6, and 7) show that wheat leaves contents of chlorophyll a and b as well as carotenoids significantly affected by the different tested fungicides during both seasons.

The results shown in Table (5) clear that leaves content of chlorophyll a were higher under all tested fungicides than the control during both seasons. Leaves content of chlorophyll a were higher under full dosage of the three tested fungicides than half-recommended dosage during this study. Leaves content of chlorophyll a gradually increased with the increase of plant age during both seasons. Wheat plants that sprayed with the recommended dosage of Tilt® (25 cm^3/fed.) recorded the highest leave's content of chlorophyll a after one week (34.60 and 35.66 mg/100 g f. w), two weeks (38.75 and 39.94 mg/100 g f. w) and three weeks (48.44 and 49.92 mg/100 g f. w) during both seasons, respectively, followed by wheat plants that sprayed with full dosage of Nasrzol® then plants that treated with full dosage of Montoro® during the two seasons. In contrast, wheat plants under the control recorded the lowest leave's content of chlorophyll a after one week (24.17 and 25.86 mg/100 g f. w), two weeks (27.07 and 28.97 mg/100 g f. w) and three weeks (33.84 and 36.21 mg/100 g f. w) during both seasons, respectively. The positive effect of the applied fungicides on leaf pigments was found before in through numerous studies as those reported by Gao et al. (1988) who found that triazole treatments increased chlorophyll content in the leaves of the wheat. Also, Sujatha et al. (1999) cleared that photosynthesis net increased after triazole applications in rice seedlings. Bryson et al. (2000) reported that pyraclostrobin caused physiological changes in the treated plants such as increased leaf greenness; chlorophyll content, photosynthetic rates, and water use efficiency, as well as delayed senescence. In the same way, Fletcher et al. (2000) reported that triazoles accelerated chloroplast differentiation, and chlorophyll production, enlarged chloroplasts, while protected the integrity of chlorophyll. In contrast, fungicides may have negative effects on the crop physiology, especially on photosynthesis. An alteration in photosynthesis might lead to a reduction in photo assimilate production, resulting in a decrease in both growth and yield of crop plants. For example, a contact fungicide such as copper inhibits photosynthesis by destroying chloroplasts, affecting photosystem II activity and chlorophyll biosynthesis. Systemic fungicides such as benzimidazoles, anilides, and pyrimidine were also phytotoxic, whereas azoles stimulated photosynthesis (Petit et al., 2012).

Table 5. Effect of some fungicides on the wheat leaf content of chlorophyll a during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm^3/fed.)	Chlorophyll a					
		1st season			2nd season		
		1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
Tilt® (25% EC)	25	34.60a	38.75a	48.44a	35.66a	39.94a	49.92a
Nasrzol® (25% EC)	30	30.76b	34.45b	43.06b	30.19b	33.82b	42.27b
Montoro® (30% EC)	40	29.33c	32.84c	41.06c	30.35b	33.99b	42.49b
Tilt® (25% EC)	12.5	24.38f	27.30f	34.13f	26.15d	29.29d	36.61d
Nasrzol® (25% EC)	15	26.15e	29.29e	36.61e	29.18c	32.69c	40.86c
Montoro® (30% EC)	20	27.40d	30.69d	38.36d	28.98c	32.46c	40.57c
Control	-	24.17f	27.07f	33.84f	25.86e	28.97e	36.21e
F test		***	***	***	***	***	***
P value		0.001	0.001	0.0008	0.001	0.0009	0.001
LSD 5%		0.34	0.38	0.48	0.25	0.28	0.35

Table 6. Effect of some fungicides on the wheat leaf content of chlorophyll b during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm³/fed)	Chlorophyll b					
		1st season			2nd season		
		1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
Tilt® (25% EC)	25	15.69f	14.91f	13.34f	16.25f	15.44f	13.81f
Nasrzol® (25% EC)	30	17.27e	16.40e	14.68e	16.93e	16.08e	14.39e
Montoro® (30% EC)	40	18.15d	17.25d	15.43d	18.81c	17.87c	15.99c
Tilt® (25% EC)	12.5	20.96b	19.91b	17.81b	20.34b	19.32b	17.29b
Nasrzol® (25% EC)	15	18.38d	17.46d	15.62d	18.14d	17.23d	15.42d
Montoro® (30% EC)	20	19.30c	18.34c	16.41c	18.83c	17.89c	16.01c
Control	-	22.27a	21.15a	18.93a	22.15a	21.04a	18.83a
F test		***	***	***	***	***	***
P value		0.0004	0.001	0.0009	0.001	0.001	0.001
LSD 5%		0.46	0.38	0.44	0.29	0.28	0.25

The obtained data in Table (6) indicate that leaves content chlorophyll b was leaser under all tested fungicides than the control during both seasons. Leave's content of chlorophyll b was leaser under full dosage of the three tested fungicides than half-recommended dosage during the two seasons of this study. Leaves content of chlorophyll b gradually decreased with the increase of plant age during both seasons. Wheat plants under the control recorded the highest leave's content of chlorophyll b after one week (22.27 and 22.15 mg/100 g f. w), two weeks (21.15 and 21.04 mg/100 g f. w) and three weeks (18.93 and 18.83 mg/100g f. w) during both seasons, respectively, followed by wheat plants that sprayed with half-recommended dosage of Tilt®, then plants that treated with half-recommended dosage of Montoro®during the two seasons. On the other hand, wheat plants that treated with full dosage of Tilt® recorded the lowest leaves content of chlorophyll b after one week (15.69 and 16.25 mg/100 g f. w), two weeks (14.91 and 15.44 mg/100 g f. w) and three weeks (13.34 and 13.81 mg/100 g f. w) during both seasons, respectively.

Table 7. Effect of some fungicides on the wheat leaf content of carotenoide during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm³/fed)	Carotenoide					
		1st season			2nd season		
		1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
Tilt® (25% EC)	25	7.73f	6.57f	4.64d	8.00d	6.80d	4.80d
Nasrzol® (25% EC)	30	8.51e	7.23e	5.10cd	8.34d	7.09d	5.00d
Montoro® (30% EC)	40	8.94d	7.60c	5.37bc	9.26c	7.87c	5.56c
Tilt® (25% EC)	12.5	10.32b	8.78b	6.19a	10.02b	8.52b	6.01b
Nasrzol® (25% EC)	15	9.05d	7.69d	5.43bc	8.94c	7.60c	5.36c
Montoro® (30% EC)	20	9.51c	8.08c	5.70b	9.28c	7.89c	5.57c
Control	-	10.97a	9.32a	6.58a	10.91a	9.28a	6.55a
F test		***	***	**	**	**	**
P value		0.0009	0.001	0.001	0.001	0.009	0.01
LSD 5%		0.32	0.38	0.45	0.42	0.37	0.32

The data in Table (7) confirm that leaves content carotenoide was lower under all tested fungicides than the control during both seasons. Leave's content of carotenoide was lower under full dosage of the three tested fungicides than half-recommended dosage during the two seasons of this study. Leave's content of carotenoids gradually decreased with the increase of the plant age during both seasons. Wheat plants under the control had the highest leave's content of carotenoids after one week (10.97 and 10.91 mg/100 g f. w), two weeks (9.32 and 9.28 mg/100 g f. w) and three weeks (6.58 and 6.55 mg/100 g f. w) during both seasons, respectively, followed by wheat plants that sprayed with half-recommended dosage of Tilt®, then plants that treated with half-recommended dosage of Montoro®during the two seasons. On the other hand, wheat plants that treated with full dosage of Tilt® recorded the lowest leaves content of carotenoids after one week (7.73 and 8.00 mg/100 g f. w), two weeks (6.57 and 6.80 mg/100 g f. w) and three weeks (4.64 and 4.80 mg/100 g f. w) during both seasons, respectively.

3.3. Effect on grains, straw yield and yield components traits

The results in Tables (8 and 9) indicate that spike number/m², spike length, grain yield/m², straw yield/m² and biological yield/m² significantly differed under all used fungicides during both seasons.

Data presented in Table (8) show that number of spike/m² significantly affected by the different fungicides during both seasons. All the used fungicides resulted in significant increase in spike number/m² compared to control. The recommended dosages of all tested fungicides superior half dosages increased spikes number/m^2. The highest number of spike/m^2 (260.62 and 272.65) was recorded in wheat plants that treated with the recommended dosage of Nasrzol® (30 cm^3/fed.), followed by wheat plants under full dosage of Tilt® (258.08 and 251.75), then wheat plants that treated with full dosage of Montoro® (242.25 and 245.10) during 1st and 2nd seasons, respectively. In contrast, the lowest number of spike/m^2 was showed in wheat plants under the controls (189.05 and 185.57) during both seasons, respectively.

Our findings revealed that all used fungicides resulted in significant increase on spike number/m^2 compared to control. Similar results were found by Entz et al. (1990) who found that Tilt® reduced the level of disease and significantly increased spike number in the wheat. Also, Kelley (2001) found that over a period of six years, the fungicide propiconazole significantly increased spike number of the winter wheat. In the same line, Ransom and McMullen (2008) showed that within an environment and averaged across the winter wheat cultivars, fungicides improved spike number. Gaba et al. (2016) found a significant spike number increase by applied morpholine and triazole treatments. While, a higher spike number under synthetic fungicides compared to the control (Karkanis et al., 2018; Motta-Romero et al., 2021; Iwaniuk et al., 2022; Gaile et al., 2023).

Data showed in Table (8) revealed that spike length significantly affected by all tested fungicides during both seasons. All tested fungicides, except half dosage of Tilt® (12.5 cm^3/fed.) led to significant increase in spike length compared to control. The highest spike lengths were recorded in wheat plants that treated with full dosage of Tilt® (13.50 and 11.87 cm) and wheat plants under half dosage of Nasrzol® (13.83 and 11.20 cm), then wheat plants that treated with half dosage of Montoro® (13.17 and 9.53 cm) during both seasons, respectively, without any significant difference among the three treatments during 1st season. In contrast, the shortest spike was found in wheat plants that treated with half dosage of Tilt® (10.50 and 7.40 cm) during both seasons, respectively.

In the present work, all used fungicides resulted in significant increase in spike length compared to control. Our results are in harmony with those obtained by Kelley (2001) who found that over a period of six years, fungicide, propiconazole significantly increased spike length of the winter wheat. In the same line, Ransom and McMullen (2008) showed that within an environment and averaged across the winter wheat cultivars, fungicides improved spike length. Gaba et al. (2016) found a significant spike length increase by applied morpholine and triazole treatments. While, numerous studies stated that there was higher spike length under synthetic fungicides compared to the control (Karkanis et al., 2018; Motta-Romero et al., 2021; Iwaniuk et al., 2022; Gaile et al., 2023).

The obtained results in Tables (8 and 9) reveal that 1000-grains weight did not affected significantly by the different tested fungicides when compared with the control during both seasons.

In the contrast of our findings, Ransom and McMullen (2008) showed that within an environment and averaged across the winter wheat cultivars, fungicides improved 1000-grains weight. Also, Gaba et al. (2016) found a significant 1000-grains weight increase by applied morpholine and triazole treatments. While, Karkanis et al. (2018) reported that there was higher 1000-grains weight under synthetic fungicides than the control. Moreover, Byamukama et al. (2019) proved that the positive effect of fungicide application for the yield increase where the highest 1000-grains weight was found under fungicides compared to non-treated. In the same way, 1000-grains weight of wheat was found to increase in the presence compared to the absence of fungicide (Motta-Romero et al. (2021; Iwaniuk et al., 2022; Gaile et al., 2023).

The obtained data in Table (9) show that grain yield/m^2 significantly differed under the different fungicides during both seasons. All tested fungicides had higher grain yield/m^2 than the control. Full dosages of all tested fungicides exceeded half dosages of fungicides increased the grain yield/m^2. The highest grain yield/m^2 (632.05 and 654.84 g) was recorded in the wheat plants that treated with full dosage of Tilt® (25 cm^3/fed.), followed by the wheat plants that treated with full dosage of Nasrzol® (544.34 and 533.66 g), then the wheat plants that treated with full dosage of Montoro® (520.53 and 539.30 g) during both seasons, respectively. On the other side, the lowest grain yield/m^2 was found in the wheat plants under the controls (391.45 and 305.91 g) during the two seasons, respectively.

Fungicides such as pyraclostrobin caused physiological changes in the treated plants in increased leaf greenness, chlorophyll content, photosynthetic rates, and water use efficiency, as well as delayed senescence. These changes may contribute to the yield increases often observed following treatment with pyraclostrobin over those achieved by disease control with a triazole fungicide alone (Bryson et al., 2000). Our results are in the same trend with those of Entz et al. (1990) who showed that Tilt® reduced the level of disease and significantly increased grain yield. In addition, Bayles and Hilton (2000) found that Strobilurin application to wheat in the United Kingdom (UK) showed an average yield increase of 0.75 and 0.34 kg/ha, respectively, higher than disease control. Kelley (2001) and Viecelli et al. (2019) found that over a period of six years, the fungicide, propiconazole significantly increased the grain yield of the winter wheat. Pits et al. (2008) and Gaba et al. (2016) observed that seed yield significantly enhanced by the use of metconazole and tebuconazole in combination with azoxystrobin, which had positive effects on LAI and the certain yield. In fact, synthetic fungicide is the most effective for increasing grain yield (Ransom and McMullen, 2008; Karkanis et al., 2018; Byamukama et al., 2019; Motta-Romero et al., 2021; Iwaniuk et al., 2022; Gaile et al., 2023). At the same way, Wegulo et al. (2009) showed that up to 42% yield loss was prevented by applying foliar fungicides to the winter wheat.

The results illustrated in Table (9) cleared that straw yield/m^2 significantly affected by the different fungicides during the two seasons. Straw yield/m^2 of the wheat was higher under all used fungicides than the control. Also, straw yield/m^2 of the wheat was higher under full dosages of all tested fungicides than half dosages of them, except Tilt® during both seasons. The highest straw yield/m^2 (882.19 and 958.74 g) was recorded in the wheat plants that treated with full dosage of Nasrzol® (30 cm^3/fed.), followed by the wheat plants that treated with full dosage of Montoro® (805.47 and 802.30 g), then the wheat plants that treated with half dosage of Tilt® (795.53 and 759.42 g) during both seasons, respectively. On the other side, the lowest straw yield/m^2 was found in the wheat plants that treated with half dosage of Nasrzol® (612.80 and 598.94 g) during the two seasons, respectively. Similar results were reported previously by Entz et al. (1990) who showed that Tilt® reduced the level of disease and significantly increased straw and biological yield. Kelley (2001) found that over a period of six years, the fungicide propiconazole significantly increased the straw and biological yield of the winter wheat. Also, Gaba et al. (2016) found a significant grain yield, straw and biological yield increase by applied morpholine and triazole treatments. Moreover, the straw and biological yield increased significantly under fungicide applicationas stated in several investigations (Ransom and McMullen, 2008; Karkanis et al., 2018; Byamukama et al., 2019; Motta-Romero et al., 2021; Iwaniuk et al., 2022; Gaile et al., 2023).

Table 8. Effect of some fungicides on the spikes number/m^2, spike length and 1000-grains weight of the wheat during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm^3/fed)	Spike number/m^2		Spike length (cm)		1000-grain weight (g)	
		1st season	2nd season	1st Season	2nd season	1st season	2nd Season
Tilt® 25% EC	25	258.08^a	251.75^a	13.50^{ab}	11.87^a	61.27	53.85^{ab}
Nasrzol® 25% EC	30	260.62^a	272.65^a	12.17^{ad}	8.97^{cd}	58.43	53.66^{ab}
Montoro® 30% EC	40	242.25^b	245.10^{ab}	12.00^{bcd}	9.50^c	60.53	56.56^a
Tilt® 25% EC	12.5	216.92^c	195.07^c	10.50^d	7.40^d	57.57	52.72^{ab}
Nasrzol® 25% EC	15	196.65^d	191.58^c	13.83^a	11.20^{ab}	57.10	51.52^b
Montoro® 30% EC	20	220.72^c	215.02^{bc}	13.17^{abc}	9.53^{bc}	56.70	53.23^{ab}
Control	-	189.05^d	185.57^c	11.50^{cd}	8.07^{cd}	60.90	50.31^b
F test		***	***	*	***	**Ns**	**Ns**
P value		**0.0002**	**0.0003**	**0.0159**	**0.0010**	**0.712**	**0.129**
LSD 5%		**13.55**	**32.28**	**1.78**	**1.69**	**7.55**	**4.19**

Ns= Not significant

Table 9. Effect of some fungicides on the wheat grain, straw and biological yield/m^2 during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm^3/fed)	Grain yield/m^2 (g)		Straw yield/m^2 (g)		Biological yield/m^2 (g)	
		1st Season	2nd season	1st Season	2nd season	1st Season	2nd Season
Tilt® 25% EC	25	632.05^a	654.84^a	780.61^{bc}	723.16^{bc}	1412.67^a	1378.00^a
Nasrzol® 25% EC	30	544.34^{ab}	533.66^{ab}	882.19^a	958.74^a	1426.53^a	1492.40^a
Montoro® 30% EC	40	520.53^b	539.30^{ab}	805.47^b	802.30^b	1326.00^b	1341.60^{ab}
Tilt® 25% EC	12.5	391.80^c	308.31^c	795.53^b	759.42^b	1187.33^c	1067.73^c
Nasrzol® 25% EC	15	463.60^{bc}	449.73^b	612.80^d	598.94^c	1076.40^d	1048.67^c
Montoro® 30% EC	20	487.07^b	445.50^b	721.07^c	731.43^{bc}	1208.13^c	1176.93^{bc}
Control	-	391.45^c	305.91^c	643.35^d	709.82^{bc}	1034.80^d	1015.73^c
F test		***	***	***	**	***	***
P value		**0.0010**	**0.0007**	**0.0004**	**0.0048**	**0.0001**	**0.0003**
LSD 5%		**91.22**	**129.99**	**62.39**	**139.75**	**74.17**	**176.68**

The data illustrated in Table (9) indicate that all tested fungicides had higher biological yield/m^2 than the control. Full dosages of all tested fungicides exceeded half dosages of them in increase biological yield/m^2. The largest biological yield/m^2 (1426.53 and 1492.40 g) was recorded in the wheat plants that treated with full dosage of Nasrzol® (30 cm^3/fed.), followed by the wheat plants that treated with full dosage of Tilt® (1412.67 and 1378.00 g), then the wheat plants that treated with full dosage of Montoro® (1326.00 and 1341.60 g). On the other hand, the lowest biological yield/m^2 was recorded in the wheat plants under the controls (1034.80 and 1015.73 g) during 1st and 2nd seasons, respectively. Entz et al. (1990) showed that Tilt® reduced the level of disease and significantly increased the straw and biological yield. Kelley (2001) found that over a period of six years, the fungicide propiconazole significantly increased the straw and biological yield of the winter wheat.

3.4. Effect on total carbohydrate and protein

The results obtained in Tables (10 and 11) clear that the wheat grains and straw contents of total carbohydrate and protein significantly affected by the different fungicides used during the experiments.

The results in Table (10) confirm that the wheat grains and straw contents of total carbohydrates were higher under all used fungicides than the control. Carbohydrates percentages in the wheat grains and straw were higher under full dosages of all tested fungicides than half dosages of fungicides during the study. The highest carbohydrates percentages in wheat grains (62.78 and 61.24%) and in straw (81.31 and 83.80%) were recorded, with respect to full dosage of Tilt® (25cm^3/fed.), followed by wheat plants that treated with full dosage of Nasrzol®, then wheat plants that treated with half dosage of Montoro® during both seasons, respectively. In contrast, the low percentages of total carbohydrates in the wheat grains (42.42 and 41.41%) and in straw (56.80 and 60.78%) were recorded under the control during the two seasons, respectively. The effect of synthetic fungicides in the grain content of total carbohydrate were reported by Jenkyn et al. (2000), where application of azoxystrobin enhanced protein and carbohydrates content in the grains of the wheat. Butkute et al. (2006) reported that application of foliar on rapeseed enhanced protein and carbohydrates content significantly than control. Also, total protein and carbohydrates in wheat grains increased in the presence of fungicide higher than the untreated plants (Motta-Romero et al., 2021, Iwaniuk et al., 2022; Gaile et al., 2023).

The presented data in Table (11) show that the wheat grains and straw contents of total protein were lower under all used fungicides than the control. Total protein percentages in the wheat grains and straw were higher under half-recommended dosages of all tested fungicides than full dosages of them. The highest protein percentages in the wheat grains (15.91 and 15.82%) and in straw (9.79 and 10.15%) were recorded under the control, followed by the wheat plants that treated with half-dosage of Tilt®, then the wheat plants that treated with half-dosage of Montoro® during both seasons, respectively. In contrary, the low percentages of total carbohydrates in the wheat grains (11.21 and 11.61%) and in straw (6.62 and 6.12%) were recorded in the wheat plants that sprayed with the recommended dosage of Tilt® during the two seasons, respectively. The effect of synthetic fungicides in grain content of total protein were reported. Jenkyn et al. (2000) showed that application of azoxystrobin enhanced protein and carbohydrates content in the grains of the wheat. Butkute et al. (2006) reported that application of folicur on rapeseed enhanced protein and carbohydrates content significantly than control.

Table 10. Effect of some fungicides on the wheat grain and straw contents of total carbohydrate during 2021/2022 and 2022/2023 seasons.

Treatments	**Dose (cm^3/fed.)**	**Total carbohydrate (%)**			
		Grain		**Straw**	
		1st season	**2nd season**	**1st season**	**2nd season**
Tilt® 25% EC	25	62.78^{a}	61.24^{a}	81.31^{a}	83.80^{a}
Nasrzol® 25% EC	30	57.96^{ab}	57.59^{b}	72.28^{b}	70.95^{b}
Montoro® 30% EC	40	53.75^{bc}	55.07^{b}	68.91^{c}	71.33^{b}
Tilt® 25% EC	12.5	45.79^{de}	42.84^{d}	57.29^{f}	61.45^{d}
Nasrzol® 25% EC	15	48.51^{cde}	49.05^{c}	61.45^{e}	68.58^{c}
Montoro® 30% EC	20	49.52^{cd}	49.81^{c}	64.39^{d}	68.10^{c}
Control	-	42.42^{e}	41.41^{d}	56.80^{f}	60.78^{e}
F test		***	***	**	**
P value		**0.0004**	**0.0003**	**0.0002**	**0.0001**
LSD 5%		**6.92**	**3.22**	**0.80**	**0.59**

Table 11. Effect of some fungicides on the wheat grain and straw contents of total protein during 2021/2022 and 2022/2023 seasons.

Treatments	Dose (cm³/fed.)	Total protein (%)			
		Grain		Straw	
		1st season	2nd season	1st season	2nd season
Tilt® (25% EC)	25	11.21g	11.61f	6.62f	6.12f
Nasrzol® (25% EC)	30	12.33f	12.09e	5.90g	5.65g
Montoro® (30% EC)	40	12.97e	13.43c	7.18d	6.97d
Tilt® (25% EC)	12.5	14.97b	14.53b	8.09b	8.04b
Nasrzol® (25% EC)	15	13.13d	12.96d	6.96e	6.36e
Montoro® (30% EC)	20	13.79c	13.45c	7.63c	7.81c
Control	-	15.91a	15.82a	9.79a	10.15a
F test		***	***	***	***
P value		0.0001	0.0002	0.0003	0.0002
LSD 5%		0.21	0.24	0.18	0.16

4. Conclusion

From the present findings, it obtained that the commonly used fungicides on wheat may induce adverse effects on the crops at cases of bad use. Such practice influences on biological parameters of the plants, quality and yield. So, the farmers to follow up labeling items for pesticides before use and take care about their awareness about their good practices and optimum conditions for planting processes for providing healthy crops.

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