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Article

Triazole fungicides residues and their inhibitory effect on some trichothecenes mycotoxins excretion in wheat grains

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Abstract: Wheat is one of the global strategic crops and ranks third in terms of cereals production. Wheat crop is exposed to many fungal infections during the cultivation stages, some of which have the ability to secrete numbers of toxic secondary metabolites that threaten the quality of grains, consumer health, producer economics and global trade exchange. Fifty-four random samples were collected from wheat which originated from different countries. The samples included 14 of soft wheat to study the extent of their contamination with Deoxynivalenol (DON) and T-2 toxin by auto-ELISA technology and r-biopharm Microtiter plate. All samples were contaminated with DON toxin except one sample, and the contamination average ranged between 40.7 and 1018.8 µg kg^{-1}. The highest contamination rates were in Lithuanian and the lowest in Indian wheat. Meanwhile the highest average level of T-2 toxin contamination was in Lithuanian wheat grains with a rate of 377.4µg kg^{-1} and the lowest in Polish wheat with an average of 115.3µg kg^{-1}. GC-MS/MS and multiple reaction monitoring mode (MRM), was used to detect 15 triazole derivatives in collected samples, which may be used to combat fungal diseases on wheat during the growing season. Only 9 derivatives were found; Simeconazole, Penconazole, Hexaconazole, Cyproconazole, Diniconazole, Tebuconazole, Metconazole, Fenbuconazole and Difenoconazole. These derivatives varied according to the origin of wheat samples as well as their concentration whereas, another 6 derivatives were not detected in all samples. A direct inverse relationship was found between the DON concentration in the samples and the residues of Simeconazole, Penconazole, Diniconazole, Tebuconazole, Metconazole, Fenbuconazole and Difenoconazole and the T-2 toxin showed the same relationship except for Tebuconazole. The safe and rational use of some triazole derivatives may be a new approach and a promising strategy not only to reduce plant diseases and their problems, but also to get rid of some mycotoxins as grains contaminates.

Keywords: DON; T-2; Wheat; triazoles; GC-MS/MS; ELISA; MRM

1. Introduction

The wheat is one of the strategic crops in the world to feed both humans and animals. The edible wheat was classified to common wheat (*Triticum aestivum*), club wheat (*T. compactum*), durum wheat (*T. durum*) and many other species [1]. The wheat is the third most important cereal crop after maize and rice in the food security basket, and the global wheat production reached to 762.2 metric tons in 2019/2020 [2, 3].

Wheat plants mostly are attacked by several phyto-pathogenic fungi during cultivation, harvesting and storage, which is associated closely with reduction in production and quality. It may extend to consumer's health as well as economic value of crop [4,5]. Fusarium pathogens is the most important seed borne fungi which have been correlated with wheat seedling blight and root rot. However, different species of Fusarium were

found in both husks and grains by ratio 3:1, respectively [6,7]. *Fusarium graminearum*, *F. avenaceum*, *F. culmorum*, *F. poae* were associated with wheat kernels and other cereals grains, meanwhile, *F. cerealis*, *F. equiseti*, *F. sporotrichoides*, *F. tricinctum* were less frequent [8]. The most species of Fusarium fungi had ability to secret various types of mycotoxins as Fumonisins, Zearalenone and Trichothecenes [9]. Trichothecenes have over 150 toxins produced by various fungi and are considered incurring general cytotoxic effects, had ability to inhibit protein synthesis at ribosomes during all three stages of protein synthesis [10].

There are multiple protocols for fungal disease management but the fungicides are still at the forefront of these treatments and have the ability to control pathogenic fungi and their undesirable effects. Fungicide groups differ in active ingredients and most of them reduce the growth and prevent sporulation as triazoles. The mode of action of triazoles depends on preventing production of sterols which is are considered the main components of fungal cell membranes [11,12]. Triazoles group is one of the largest groups of fungicides and the Bayer Company produced first of the triazoles in 1973. The newer triazoles are intrinsically more active than the previous ones and their effectiveness is related back to their original ED 50 values. Triazoles moiety group is one of the most important systemic fungicides that includes a variety of compounds and contains 1,2,4-triazole moiety, which control a wide range of fungal diseases on wide range of crops especially grains [13]. The study aims to investigate the level of deoxynivalenol and T-2 toxins that are contaminate imported wheat grains, along with the remaining residue of various derivatives of triazole fungicides, which is widely used over the world to reduce the fungal diseases that attack the wheat crop during the growing season. Also, the possibility of predicting generally or indicating the form of the correlational relationship between them, which may be one of the effective directions for reducing mycotoxins that affect the quality of agricultural products, especially grains, during post-harvest and storage periods.

2. Materials and Methods

2.1. Samples collection

Fifty-four samples, 2 kg each, of wheat imported by Saudi Arabia from the countries of different origin from the harvest season of 2017 /2018, were collected. Random representative samples were prepared according to the procedure described in SANCO/12571/2013 document [14]. Samples were kept in freezing at-20ºC upon arrival to laboratory; samples were finely homogenized, soaked in liquid nitrogen and grinded prior to analysis process.

Table 1. Random representative sample origin, wheat type, code and number of collected samples.

Nr.	Origin Country	Wheat type	Code	No. of Samples
1	American	Soft	USA(S)	3
2	Canadian	Hard	Canada(H)	10
3	German	Hard	Germany(H)	6
4	German	Soft	Germany(S)	2
5	Australian	Hard	Australia(H)	3
6	French	Soft	French(S)	6
7	Lithuanian	Hard	Lithuania(H)	4
8	Polish	Hard	Polish(H)	13
9	Indian	Soft	India(S)	3
10	Brazilian	Hard	Brasilia(H)	4
	Total	-	-	54

*Samples origin have been given a serial code to facilitate data analysis and the source of samples was e from Saudi Grains Organization (SAGO).

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2.2. **Recovery experiments and method validation**

Wheat samples free from pesticides were used in validation studies and matrix-matched calibration standards preparation. Validation method was performed at three fortification levels 0.50, 0.05 and 0.005 mg/Kg with three replicates for each, and results as shown in Table 2. Working standard solutions at 1000 mg/L containing all the pesticides used for the validation method was prepared in acetonitrile. Six matrix-matched calibration standards at 3, 5, 10, 50, 100 and 250 ng/g were prepared at five replicates for each. Values of the limit of detection (LOD) and limit of quantitation (LOQ) of the analytical method used were estimated from the following equations as clarified in ICH (2005) [15].

LOD = 3.3 SD/b LOQ = 10 SD/b

LOD: limit of detection, LOQ: limit of quantitation, SD: The residual standard deviation of the regression line or standard deviation of y-intercepts of the regression line.

Table 2. Separation and validation data of studied fungicides.

Compound name	Time segment	Rt	Ion transitions		C.E	Recovery %			RSD% Ave.	R^2	MRL ppm	LOQ ppm	LOD ppm
						0.5	0.05	0.005					
Simeconazole	1	13.272	121	101.1	10	86.2	87.3	71.86	8-14	0.992	0.01*	0.01	0.003
			121	75.1	25								
Tetraconazole	1	16.328	170.9	136	10	95.3	92.88	91.97	5-9	0.998	0.1	0.01	0.003
			336	217.9	20								
Penconazole	2	17.478	248	192.1	15	105.1	92.45	91.55	7-11	0.995	0.05*	0.006	0.002
			248	157.1	25								
Hexaconazole	2	19.986	256	82.1	10	96.6	102.83	96.7	9-15	0.994	0.01*	0.006	0.002
			231	175	10								
Azaconazole	2	21.012	217	173.1	15	85.2	86.13	78.88	9-12	0.988	0.01*	0.01	0.003
			219	175	15								
Cyproconazole	3	21.369	139	111	15	98.2	98.89	94.37	10-15	0.995	0.1	0.01	0.003
			222	125.1	15								
Diniconazole	3	22.031	267.9	232.1	10	107.3	108.52	96.57	10-17	0.995	0.01*	0.006	0.002
			269.9	232	10								
Etaconazole	3	22.102	173	145	15	110.1	107.2	96.11	11-16	0.998	0.01*	0.01	0.003
			173	109	30								
Propiconazole	4	23.631	172.9	145	15	104.5	91.3	90.32	8-16	0.994	0.05*	0.006	0.002
			172.9	109	30								
Tebuconazole	4	23.933	125	89	15	98.4	93.2	87.51	8-10	0.993	0.1	0.01	0.003
			250	125	20								
Epoxiconazole	4	24.521	192	138.1	10	99.18	100.88	87.43	12-16	0.998	0.6	0.01	0.003
			192	111	25								
Bromuconazole	5	24.939	173	145	15	85.00	87.84	89.24	13-15	0.985	0.2	0.012	0.004
			173	109	30								
Metconazole	5	25.569	125	89	20	91.5	92.5	90.92	6-8	0.996	0.15	0.012	0.004
			125	99	20								
Fenbuconazole	6	28.694	128.9	102.1	15	91.8	88.54	92.33	6-11	0.999	0.1	0.01	0.003
			197.9	129	5								
Difenoconazole	6	31.769	322.8	264.8	15	94.5	93.57	93.74	12-16	0.998	0.1	0.01	0.003
			264.9	202	20								

(*) Indicates lower limit of analytical determination as mentioned by EU; Rt: Retention time; C.E: Collision cell energy (volt); RSD Ave: relative standard deviation average; R^2: correlation coefficient of regression line; MRL: Maximum residue level (reported by European commission); LOQ: Limit of quantitation; LOD: Limit of detection.

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2.3. Extraction and cleaning-up method

Buffered QuEChERS procedure was used in extraction and cleaning up of fungicide residues [16]. As wheat is a dry matrix and a little bit high in fat, steps mentioned by Mastovska et al., (2010) were used to raise method sensitivity and output [17]. Five grams of fine ground wheat sample in 50 ml Teflon centrifuge tube, 10 ml of cold D.I. water was added, shaken carefully and let it to swell and settle for 20 min. Ten ml of 1% HAc (glacial) in MeCN (V/V), were added and mixed well. TPP (Tri Phenyl Phosphate) as internal standard (IS) at rate of (200) ng/g sample was added and mixed well. Dispersive clean-up was done by adding six grams of activated anhydrous MgSO4 and 1.5 grams of anhydrous NaOAc together and hand shaking was started vigorously as fast as possible for one min, then it was shaken with orbital shaker for 60 min. Tubes were centrifuged at 5000 rpm for 10 min. Five ml of the upper layer were precisely transferred in 15 ml centrifuge tube and kept in deep-freezer for 30 min., then 750 mg activated anhydrous MgSO4, 250 mg PSA and 250 mg C18 were added to the cold extract and were mixed well for one min. Tubes were centrifuged at 5000 rpm for 5 min. Four ml were transferred from cleaned extract, were evaporated with Turbovap evaporator under N2 at 40^{o}C till lowest volume (0.2-0.3 ml), then volume was adjusted to one ml with toluene.

2.4. Triazoles determination

GC-MS/MS system of Agilent model 7890B-7000C were operated in multiple reaction monitoring mode (MRM) with two ion transitions to obtain the maximum sensitivity for detection of the target molecules, mass transitions used are presented in Table 2. HP-5MS capillary column (30 m × 250 µm × 0.25 µm) from J&W Scientific was used for pesticide residue analysis, multi-mode inlet at 280 °C, splitless mode. Oven programmed at 150 °C for two min, ramped at three °C min^{-1} to 200 °C then ramped to 280 °C at eight °C min-1 and then held for 10 min., carrier gas Helium (He) at flow rate one ml min-1, retention time (Rt) as shown in Table 2. MS operated in electron impact ionization mode (EI), transfer line, MS source, quad1 and quad2 temperatures were 280, 300, 180 and 180 °C respectively. Helium (He) quenching gas and N2 collision gas at 2.25 and 1.5 ml min-1 respectively. System was back flushed at 300 °C at 50 psi for five min. and method retention time was locked to chlorpyrifos-methyl at 13.093 min, Rt of TTP used as IS was 24.162 min.

2.5. Trichothecenes determination

Trichothecenes toxins estimated according to Tima et al., (2016) [18]. 5 gram of ground samples were individually ~~was~~ weighted in suitable container with 25 ml of methanol 75% HPLC grade. The sample was shaken vigorously for 3 min and then the extract was filtrated through Whatman filter No.1. One ml of filtrate extract was diluted with 1 ml of distilled deionized water.

Auto-ELISA (ChemWell, Awareness Technology Inc., USA) was used to conduct the procedures of DON toxin determination. 50 µl of standers? (50, 100, 200 and 400 µl L^{-1}) and prepared samples ~~was~~ were injected into separate wells of Microtiter plate No. R5902 (r-biopharm, Germany) accredited from AOAC. 50 µl of enzyme conjugate was added to the bottom of each well and then 50 µl of Anti DON toxin antibody solution was added to each well with gently mixing by shaking the plate and incubating ~~e~~ it for 10 min at 20-20°C. After incubation all the remaining liquid was removed from the wells and was refilled with 250µl distilled deionized water per well. The well was emptied again by removing all remaining liquid and this step was repeated two more times. 100 µl of substrate/chromogen was added to each well, mixed gently and incubated for 5 min at 20-20°C. Finally, 100 µl of stop solution was added to each well, mixed gently by shaking and measuring the absorbance at 450 nm. The measuring was done through 10 min of stop solution addition as well as the measuring range was ~~is~~ 0.2-6.0 ppm. Special software (RIDA®SOFT Win. Net) was used to evaluate the RIDASCREEN®Enzyme immunoassays. T-2 toxin estimation was similar with DON protocol with replacement the kit by No. R5302 (r-biopharm, Germany) which has a special Microtiter plate, enzyme conjugate, antibody solution and substrate/chromogen for T-2 toxin.

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3. Results

Fifty-four samples of wheat of different origins were analyzed for triazoles residues, which is considered one of the most important groups of fungicides used on wheat to control fungal infection. Validation method was performed at three fortification levels of 0.50, 0.05 and 0.005 mg/kg with three replicates for each, recovery values ranged from 85-110, 86-108 and 71-96 % respectively as shown in (Table 2) and average of RSD% ranged from 5-17%. Values of the limit of detection (LOD) and limit of quantitation (LOQ) were statistically calculated and ranged from 0.002-0.004 and 0.006-0.012 ppm respectively. MRL varied from one compound to another. The compounds that had no MRL done yet, such as Simeconazole, Hexaconazole, Azaconazole, Diniconazole and Etaconazole considered the 0.01 ppm level as the lowest determination and MRL level while for Penconazole and Propiconazole 0.05 ppm as the lowest determination and MRL level as reported by the European commission in Table (2).

All samples were analyzed in two replicates and final results as are shown in Table (3). RSD % of results ranged from 8-22%, and average of contaminated sample (What). Tetraconazole, Azaconazole, Etaconazole, Propiconazole, Epoxiconazole and Bromuoconazole were below the limit of quantitation in all samples, while Simeconazole, Penconazole, Hexaconazole, Cyproconazole, Diniconazole, Tebuconazole, Metconazole, Fenbuconazole and Difenoconazole were detected in 4, 27, 24, 10, 11, 24, 4, 7 and 4 samples of all the 54 samples analyzed. The most of analyzed samples did not exceed the MRL individually, but average may exceed it, that samples have been collected from different shipments during the season and it vary from shipment to another according to area variation and distribution at country of origin. Results exceeded the MRL as reported by EC for hexaconazole and diniconazole in samples of all origins but comparing them with Japanese MRL levels database by (The Japan Food Chemical Research Foundation), it was 0.1 ppm for hexaconazole, so it falls in safe limit.

The results of the Table (4) indicate that, DON was found in the most of tested wheat samples (53 samples) for both soft wheat samples collected from different origins like USA, Germany, France, India, and hard wheat samples collected from Canada, Germany, Australia, Lithuania, Poland and Brazil. The highest concentration of DON was found in Lithuanian wheat samples with an average value of 1018.8 μg kg^{-1}, followed by Canadian wheat with an average of 870.5 μg kg^{-1}. Whereas soft Indian wheat samples recorded the lowest DON contamination rate with an average of 40.7 μg kg^{-1} per 3 samples. T-2 toxin was also found in varying proportions in all the 54 wheat samples tested. The highest level of contamination was found in Lithuanian hard wheat with an average of 377.4 μg kg^{-1} for four samples followed by French soft wheat with an average of 132.4 μg kg^{-1} for six samples. The hard Polish wheat samples (13 samples) recorded the lowest level of contamination with T-2 toxin 115.3 μg kg^{-1}.

Figures from 1 to 9 indicate that, there are an inverse relationship between the concentration of different triazoles derivatives residues (Simeconazole, Penconazole, Diniconazole, Tebuconazole, Metconazole, Fenbuconazole, Difenoconazole) in the wheat tested and DON toxin contamination with different correlation value, while there was no correlation with both Hexaconazole and Cyproconazole. The same inverse correlation was noticeable between T-2 toxin and triazoles derivatives residues (Simeconazole, Penconazole, Diniconazole, Metconazole, Fenbuconazole, and Difenoconazole) but no such relationship existed with Hexaconazole and Cyproconazole and Tebuconazole.

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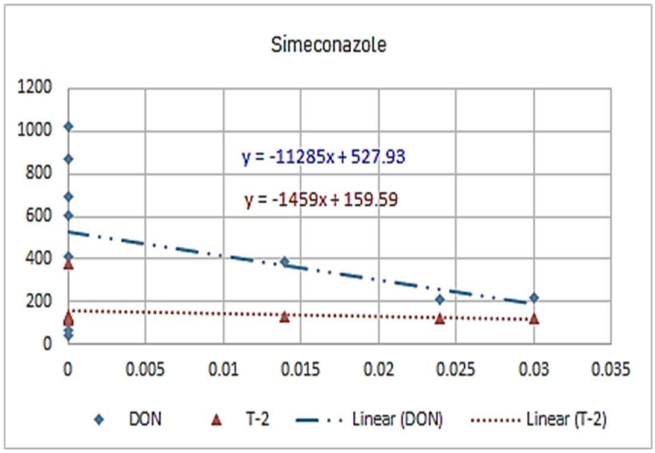

Fig 1. The relation between Simeconzole residue and DON and T-2 toxin in wheat samples

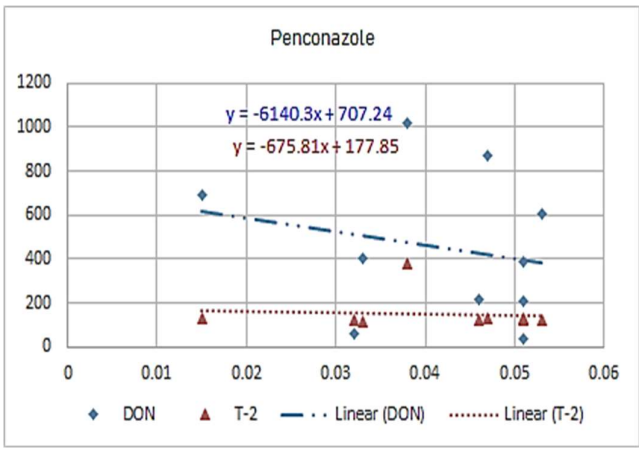

Fig 2. The relation between Penconazole residue and DON and T-2 toxin in wheat samples

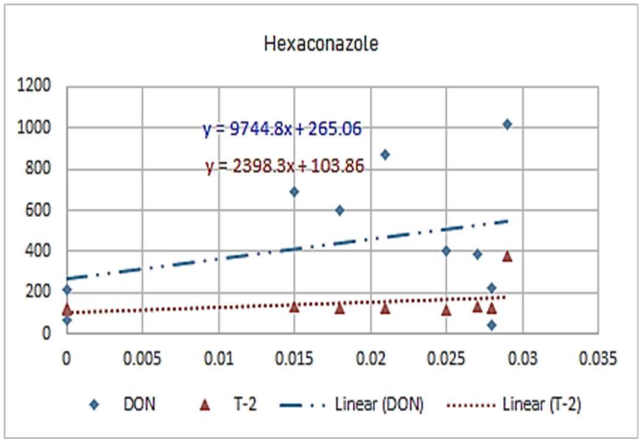

Fig 3. The relation between Hexaconazole residue and DON and T-2 toxin in wheat samples.

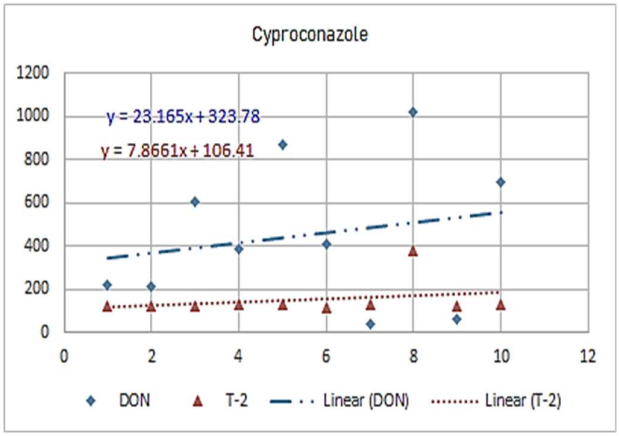

Fig 4. The relation between Cyproconazole residue and DON and T-2 toxin in wheat samples.

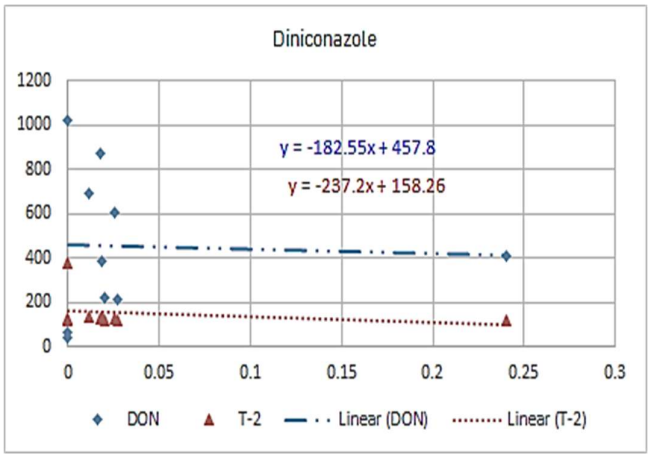

Fig 5. The relation between Diniconazole residue and DON and T-2 toxin in wheat samples

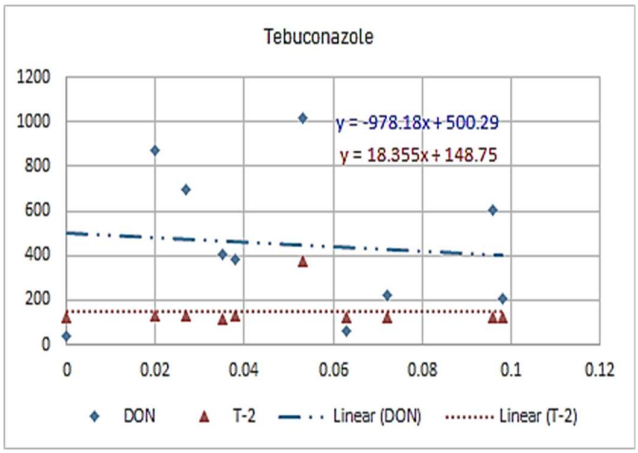

Fig 6. The relation between Tebuconazole residue and DON and T-2 toxin in wheat samples

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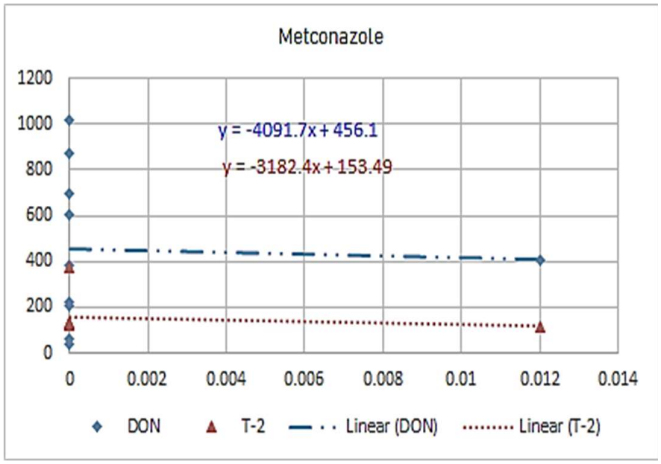

Fig 7. The relation between Metaconazole residue and DON and T-2 toxin in wheat samples.

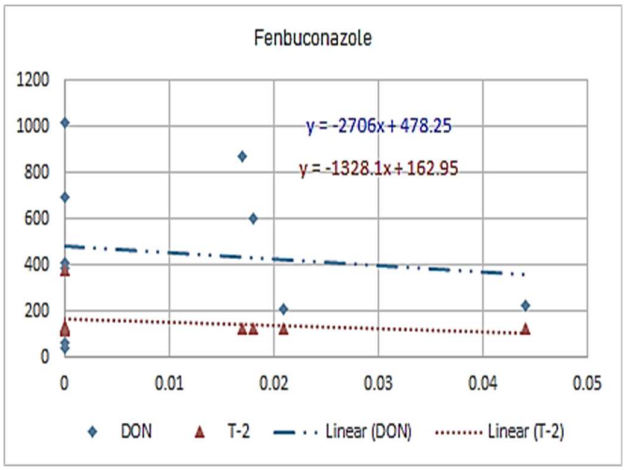

Fig 8. The relation between Fenbuconazole residue and DON and T-2 toxin in wheat samples.

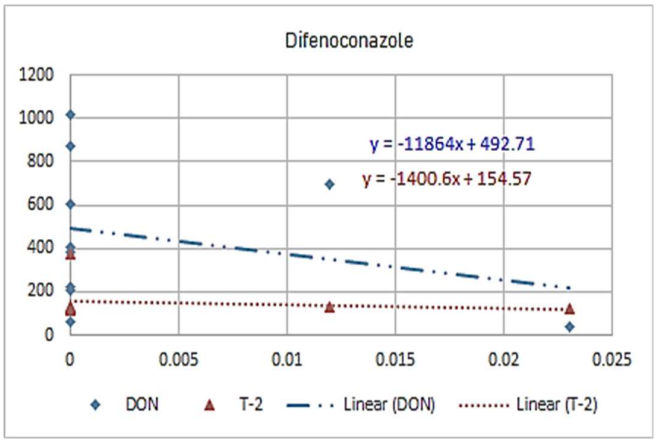

Fig 9. The relation between Difenoconazole residue and DON and T-2 toxin in wheat samples.

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Table 3. Determination of triazole derivatives residue (ppm) in different imported wheat types grains from different origin countries by GC-MS/MS.

Fungicide	Total detected sample	USA(S)		Canada(H)		Germany(H)		Germany(S)		Australia(H)		French(S)		Lithuania(H)		Polish(H)		India(S)		Brasilia(H)	
		Samples detected*	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %	samples detected	Avg %
Simeconazole	4	ND	---	ND	---	0.022 0.037	0.03 33.33	0.024	0.024 50.00	ND	---	0.014	0.014 16.66	ND	---	ND	---	ND	---	ND	---
Tetraconazole	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---
Penconazole	27	0.015	0.015 33.33	0.077 0.047 0.023 0.044	0.047 40.00	0.034 0.053 0.047 0.052	0.046 66.66	0.051	0.051 50.00	0.032	0.032 33.33	0.061 0.042	0.051 33.33	0.043 0.030 0.041 0.040	0.038 100.00	0.087 0.051 0.016 0.017 0.023 0.021 0.022	0.033 53.84	0.041	0.051 33.33	0.063 0.043	0.053 50.00
Hexaconazole	24	0.015	0.015 33.33	0.027 0.019 0.023 0.013 0.024	0.021 50.00	0.034 0.023 0.027 0.030	0.028 66.66	ND	---	ND	---	0.030 0.040 0.012	0.027 50.00	0.023 0.033 0.040 0.022	0.029 100.00	0.017 0.032 0.016 0.041 0.023	0.025 38.46	0.028	0.028 33.33	0.018	0.018 25.00
Azaconazole	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND		ND	---	ND	---	ND	---	ND	---
Cyproconazole	10	ND	---	0.042	0.042 10.00	0.077 0.154	0.115 33.33	0.046	0.046 50.00	ND	---	0.018 0.027 0.017	0.020 33.33	ND	---	0.027	0.027 7.69	0.025 0.042	0.033 66.66	ND	---
Diniconazole	11	0.012	0.012 33.33	0..022 0.014 0.018	0.018 30.00	0.026 0.014	0.020 33.33	0.027	0.027 50.00	ND	---	0.022 0.017	0.019 33.33	ND	---	0.024	0.024 7.69	ND	---	0.026	0.026 25.00
Etaconazole	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---
Propiconazole	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---
Tebuconazole	24	0.027 0.043 0.012	0.027 100.0	0.020 0.021 0.019	0.020 30.00	0.062 0.067 0.077 0.082	0.072 66.66	0.098	0.098 50.00	0.063	0.063 33.33	0.048 0.028 0.039	0.038 50.00	0.052 0.054	0.053 50.00	0.034 0.055 0.021 0.026 0.062 0.016	0.035 46.15	ND	---	0.096	0.096 25.00
Epoxiconazole	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---
Bromuconazole	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---
Metconazole	4	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	0.015 0.013 0.012 0.011	0.012 30.76	ND	---	ND	---

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Fenbuconazole	7	ND	---	0.017	0.017 10.00	0.041 0.047	0.044 33.33	0.021	0.021 50.00	ND	---	ND	---	ND	---	ND	---	ND	---	0.013 0.016 0.026	0.018 75.00
Difenoconazole	4	0.012 0.012	0.012 66.66	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	ND	---	0.022 0.024	0.023 66.66	ND	---

ND: Not Detected; Sample detected: Number of contaminated sample detected for each wheat group and fungicide; Total detected sample: Number of total contaminated samples for all wheat origins; Avg.: Average residue of detected samples in ppm; %: Detected contaminated samples percentage to No. of samples analyzed for each wheat origin.

Table 4. Determination of deoxynivalenol (DON) and T-2 toxins (ppb) in different imported wheat grains from different origin countries.

Mycotoxin	Total detected sample	Sample serial code																			
		USA(S)		Canada(H)		Germany(H)		Germany(S)		Australia(H)		French(S)		Lithuania(H)		Polish(H)		India(S)		Brasilia(H)	
		samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`	samples detected	x`
DON (ppb)	53	943.0 130.0 1008.0	693.7	1598.0 410.0 1123.0 840.0 912.0 639.0 626.0 1543.0 485.0 530.0	870.6	122.0 132.0 133.0 320.0 145.0 468.0	220.0	287.0 133.0	210.0	82.0 60.0 49.0	63.7	569.0 14.0 17.0 38.0 852.0 822.0	385.3	1806.0 1187.0 814.0 268.0	1018.8	388.0 501.0 825.0 661.0 314.0 460.0 658.0 347.0 230.0 250.0 379.0 71.0 212.0	407.4	24.0 34.0 64.0	40.7	1739.0 ND 36.0 34.0	603.0
T-2 (ppb)	54	139.8 131.2 120.1	130.4	117.5 124.0 138.2 128.4 127.1 140.4 135.6 131.6 109.7 122.9	127.5	112.4 129.1 121.4 107.4 131.1 130.6	122.0	118.5 123.0	120.8	123.5 122.3 112.9	119.6	138.2 145.5 140.5 125.0 123.6 121.7	132.4	1107.0 136.8 136.1 129.5	377.4	105.1 104.0 104.4 100.2 116.6 98.1 110.0 124.3 121.4 129.1 113.7 131.1 140.5	115.3	126.6 112.3 139.7	126.2	139.3 119 133.9 108.3	125.1

4. Discussion

Wheat crop is exposed to several fungal pathogens attack especially on the shoots and spikes, causing blight, spots, streaking, rust and smuts. The triazole compounds are considered one of the many available and recommended fungicides for controlling diseases in most wheat-growing areas and used on a large scale to combat fungal pathogens as Fusarium head blight, Septoria tritici blotch, leaf rust, wheat blast and these are in a harmony with recorded by (Machado et al., 2017; Dorigan et al., 2019) [19,20].

Fifty four samples of imported wheat grains were collected in Saudi Arabia, of which 14 samples of soft wheat and 40 samples of hard wheat were used for estimating qualitatively and quantitatively derivatives of triazole fungicide. The results showed that neither Tetraconazole, Azaconazole, Etaconazole, Propiconazole, Epoxiconazole nor Bromuconazole were detected in all the tested samples. Meanwhile, there were residues of nine of the triazole derivatives, namely Simeconazole, Penconazole, Hexaconazole, Cyproconazole, Diniconazole, Tebuconazole, Metaconazole, Fenbuconazole and Difenoconazole. The detected triazole derivatives varied in retention time (RT), Ion transitions, and Collision cell energy (C.E) as well as the % Recovery at levels of 0.5, 0.05, and 0.005. Meanwhile the ~~M~~ maximum residue level (MRL) ranged between 0.01 and 0.6 according to allowable European limits [21].

The wheat samples of different origins were all contaminated with DON toxin, except one sample of Brazilian origin. The contamination level of the tested samples with DON toxin varied and it ranged between 40.7 to 1018.8 μg kg^{-1}. The contamination average of DON toxin per different origins was less than the regulation limits, which are estimated at 1250 μg kg^{-1}, according to European Commission, 2006; but individually there were some of the samples the DON toxin of which were more than the limits, such as Canada, Lithuania and Brasilia [22]. The variation ~~of~~ in DON toxin contamination may have been due ~~return~~ to different factors such as environmental, agricultural treatments, and fungicides application during growing; which may have a role in reducing fungal infection and doing what is known as mycotoxins decontamination [23,24,25]. Also, the ~~tested~~ wheat samples tested were all contaminated with T-2 toxin. The contamination level with T-2 toxin varied and ranged between 98.1 to 1107.0 μg kg^{-1} whereas, the permissible EU limits are from 400 to 1000 for cereals [25].

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