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Use of Linear Correlation between *in Vitro* And *in Vivo* Tests for Evaluating Efficiency of Some Fungicides in Controlling Net Blotch of Barley (*Hordeum Vulgare*)

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ABSTRACT

Drechslera teres, the causal agent of net blotch of barley (*H. vulgare* L.), is a seed-borne and foliar pathogen. In the present study, six fungicides (Epoxiconazole, Azoxystrobin, Iprodion, Propiconazole, Thiophanate methyl and Mancozeb) were tested *in vitro* for their inhibitory effects against *D. teres*. Fungicidal toxicity was expressed as a percentage inhibition of mycelial growth. Inhibition concentration, which reduced 50% of mycelial growth (Ic_{50}) was calculated for each tested active ingredient. Among tested fungicides triazoles (Epoxiconazole and Propiconazole) were the most effective with Ic_{50} values of 0.254 and 0.263 mg/L respectively. In contrast, Thiophanate methyl showed the greatest Ic_{50} values was 334.584 mg/L compare with the other fungicides. These fungicides were evaluated in the field at two locations; Kafre El-Hamam and Sakha Agricultural Research stations in Sharkia and Kafre El.Sheikh governorates during 2014/2015 growing season. The first three fungicides were used as seed dressing alone (single application) or with one foliar spray (double application), and the second three fungicides were used as one or two sprays. Experiments carried out in planta under field conditions showed variation between fungicides in disease control, 1000 kernel weight (TKW), and grain yield/plot. The variable performance of the tested fungicides could be attributed the different active ingredient, application method, and the changing environmental conditions from one location to another. Triazoles fungicides also, showed greater efficacy than other fungicides. The correlation between both assays *in vitro* and *in vivo* showed that I_{C50} could be used as a complementary and not as an alternative to field trials to evaluate the performance of the tested fungicides.

Keywords: Barley, *Drechslera teres*, fungicides, *Hordeum vulgare*, yield components.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal grain crop, which ranks fifth globally among all crops in dry matter production (Baik and Ullrich, 2008). It is mainly used as human food and in malt industries, but there is a growing interest in it for animal feed (Bergh *et al*., 1999).

In Egypt barley is the main crop grown in a large scale in the North Costal Region and also in the newly reclaimed lands with saline soils and shortage of fresh water. The total harvested area was 57.000 ha in the eighties, while it increased to 135.000 ha in 2008/2009. Barley yields have also increased gradually over the past three decades from 2.92 $t.h^{-1}$ in the eighties to 3.63 $t.h^{-1}$ in 2008/2009 (El-Banna *et al*., 2011).

Barley is susceptible to many fungal diseases such as net blotch, which has been considered as one of the main causes of crop loses (Oerke *et al*., 1994).

The disease is associated with high loses (70-80%) in humid regions (Cherif *et al,* 1994). Net blotch is a seed-borne and a foliar disease of barley (Douglas and Gordon, 1985) caused by the fungus *Drechslera teres* (Sacc.) Shoem. (Syn. *Helminthosporium teres* Sacc.), the conidial state of *Pyrenophora teres* (Died) *Drechslera*.

The disease occurs in two forms; net form of net blotch (NFNB, *D. teres* f. *teres*) and spot form of net blotch (SFNB, *D. teres* f. *maculata*) (Hollaway and Mclean, 2008).

The optimum conditions for infection are from 10 to 30 hours of high humidity and a temperature regime from 15-25°C (Sato and Takeda, 1990).

In Egypt, net blotch is considered one of the widely-distributed disease in barley field and is responsible for 5-10% reduction in yield, as well as lowering the quality of grains especially in lower Egypt (Abedl-Hak *et al*., 1969). El-Nashar (1990) reported that disease was wide-spread in lower Egypt, common in Middle Egypt, while not detected in Upper Egypt.

Net blotch controlled by resistant cultivars, crop rotation and application of fungicides as seed treatments or foliar sprays (Telles Neto *et al*., 2007).

Foliar application or seed dressing treatments are considered the most important techniques applied for the chemical control strategy against barley net blotch disease (Rennie and Cockerell, 1993). Whitehead (2004) reported that seed dressing and foliar spraying of fungicides were considered more effective with the current chemical fungicides available for controlling net blotch such as strobilurins, triazoles, benzimidazole, chlorothalonil, morpholine, chlorophenyl, anilinopyrimidine, guanidine, carboxamide and dithiocarbamates.

Determination of fungicide efficacy, or estimation of resistance level, can be measured by calculating an Ic_{50} (concentration which inhibits growth by 50%). In this regard, Campbell and Crous (2002) used radial mycelial growth to evaluate the sensitivity of both net type and spot type, of *Pyrenophora teres* to trimenol, bromucanzole, flusilazole, propiconazole and tebuconazole. Flusilazole was shown to be the strongest inhibitor of fungal growth with a mean Ic_{50} of 0.71 mg/L.

In vitro assays may give an indication of the performance of fungicide, but may not reflect performance *in vivo*. Therefore, it is necessary to ascertain fungicides performance either in field trials or in controlled environment greenhouses.

Weppler and Hollaway (2004) reported that the environmental conditions and timing of application greatly influence fungicide efficacy. Soovali and Koppel (2010) concluded that the fungicide treatments had a strong impact on the control of infection of *D. teres* and increased kernel yield in variable disease infection conditions.

The risk of losing the efficiency of a fungicide against certain pathogens is greatly increased if the same active ingredient is used repeatedly over years. Therefore, the objectives of this study were aimed:

1. To evaluate the efficiency of different groups of six fungicides to inhibit growth of *D. teres in vitro*.
2. To evaluate the efficiency of these fungicides under field conditions by using different application methods.
3. To determine any correlation between both assays.

MATERIALS AND METHODS

1. Plant materials:

The grains of Giza 2000 the most susceptible cultivar to net blotch disease of barley obtained from Barley Research Section, Field Crop Institute, Agricultural Research Center (ARC), Giza, Egypt was used in field trials.

2. Fungal isolate:

The isolate of *Drechslera teres* which was identified as pathotype 19-22 the most common one in Egypt, was kindly supplied by Dr. Faten Kamel El-Nashar, Barley Dis. Res. Dept., Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.

3. Fungicides:

The six fungicides used *in vitro* and *in vivo* tests were, Epoxiconazole, Azoxystrobin, Iprodion, Propiconazole, Thiophanate methyl and Mancozeb (Table 1).

Table 1. Fungicides tested for their efficiency in controlling net blotch

No.	Common name	Trade name	Supplier	Formulation	Recommended dose
1	Epoxiconazole	Opus	BASF	12.5% Sc*	400 ml/fed 2 ml/kg
2	Azoxystrobin	Escudo	Rotam Agrochemical - Egypt	25% Sc	45ml/100L 1ml/Kg
3	Iprodion	Rophlex	Safety Egypt	50% WP**	150g/100L 3g/Kg
4	Propiconazole	Tilt	Syngenta	25% EC***	25ml/100L
5	Thiophanate methyl	Mega Top	Starchem Industrial Chemicals	70% WP	65g/100L
6	Mancozeb	Anadol	Chemitra	80% WP	250g/100L

***Sc = Suspension concentrate** ****WP = Wettable powder.** *****EC = Emulsifiable concentrate.**

4. *In vitro* test:

This test was designed to assess the inhibitory activity of different groups of fungicides on the growth of *Drechslera teres*. Preliminary 6 concentrations (0, 1, 10, 20, 40, 80 and 160 mg/L) from all fungicides were tested on linear growth of *D. teres* to choose the range of effective concentrations of each fungicide.

The effective concentrations of each active ingredient were tested as follows: 0.05, 0.1, 0.25, 0.4, 0.55 and 0.7 mg/L for Epoxiconazole and Propiconazole; 0, 1, 2, 10, 20, 40, 80 and 160 mg/L for Azoxystrobin; 0.2, 0.4, 0.6, 0.8, 1, 10, 20, 40 and 80 mg/L for Iprodione; 240, 280, 320, 360, 400 and 440 mg/L for Thiophanate methyl and 2, 4, 8, 10, 20, 40, 80 and 120 mg/L for Mancozeb.

The technique described by Robberts *et al*., (1996) was followed. PDA at 45°C was amended with different concentrations of fungicides and poured in perti dishes and inoculated with circular mycelial plug (5 mm diameter) from 7-day-old cultures of *D. teres*. Untreated control was made in the same way where the same amount of fungicide was replaced by sterile distilled water. Three replicates were used for each treatment. Plates were then incubated at 22±2°C. The growth of the fungus was monitored daily until the fungus in untreated control plates reached to 9 cm in two perpendicular diameters, then the colony diameters in treated plates were determined by averaging two perpendicular measurements. Percentage growth inhibition was calculated using the equation:

$$\mathbf{I\ (\%) = D_0\text{-}D_r/D_0 \times 100,\ where:}$$

D_0= Average diameter of fungal colony of control.

D_r= Average diameter of fungal colonies in the presence of fungicide.

5. Ic_{50} determination:

The concentration of fungicides which reduced the mycelial growth of *D. teres* by 50% was calculating using probit analysis (Finny, 1971). The percentage of inhibition were transformed into probit values.

6. Field trials:

This test was carried out under field conditions to evaluate the efficacy of the six fungicides at the recommended rates (Table 1) in controlling net blotch using the susceptible barley cv. Giza 2000. Field experiments were conducted at two locations, *i.e*. Kafre El-Hamam and Sakha, Experimental Res. Stations belong to Sharkia and Kafre El.Sheikh governorates respectivley, during 2014/2015 season. The experiments were carried out in a randomized complete block design (RCBD) with three replicates of 3m×3.5m (1/400 feddan) and each plot included 6 rows. The experiment was surrounded by a border plant of highly susceptible genotypes.

Under field condition the six fungicides were divided into two groups each containing three fungicides and applied as following:

A. As seed dressing only or seed dressing + one foliar spray at growth stage GS* 30. This group included the active ingredient *i.e*. Epoxiconazole, Azoxystrobin and Iprodion.
B. As one foliar spray at (GS 30) or two foliar sprays at (30 and 39 †GS). This group included the active ingredient *i.e.* Propiconazole, Thiophanate methyl and Mancozeb.

Plants free from fungicides were used as a control treatment.

7. Disease assessment:

The reaction of barley plants to net blotch disease was recorded at start of flowering stage, (Larg, 1954), according to double-digit scale 00-99.

The first digit gives the relative height of disease (Sarri and Prescott, 1975) while the second digit shows the disease severity as percentage of leaf area affected in terms of 0-9, where: 0=0%, 2=20%, 3=30% and so on (Eyal *et al*., 1987).

The efficacy of the treatment was determined according to the equation adopted by Rewal and Jhooty (1985) in which:

*** GS 30 = leaf-sheaths strongly erected (Zadoks *et al*., 1974).**
† GS 39 = last leaf just available.

Efficacy% = C-T/C × 100

Where: C = % infection in the control

T = % infection in the treatment.

8. Yield assessment:

At maturity stage (no green tissues are present) plants of each treatment were harvested and following parameters were determined:

1. Grain yield per plot (Kg/plot).
2. Thousand kernel weight (TKW).

9. Statistical analysis:

The experimental design of all laboratory and field studies was randomized complete block design with three replications. Analysis of varians (ANOVA) of the date were performed with SPSS. Least significant differences (L.S.D at 5%) was used to compare treatment means. Correlation and regression analysis were performed with the same statistical package according to (Snedecor, 1957).

RESULTS

1. *In vitro* test:

Inhibitory response:

To evaluate the inhibitory activity of different groups of six fungicides, *i.e.* Epoxiconazole, Azoxystrobin, Iprodion, Propiconazole, Thiophanat methyl and Mancozeb, six preliminary concentrations from all fungicides were tested on linear growth of *D. teres* to choose the range of effective concentrations of each one Fig. (1). Data in Table (2) and Fig. (2) showed the different concentrations of each fungicide and their efficiencies on linear growth of *D. teres*. Based on linear growth measured, inhibitory percentage were calculated.

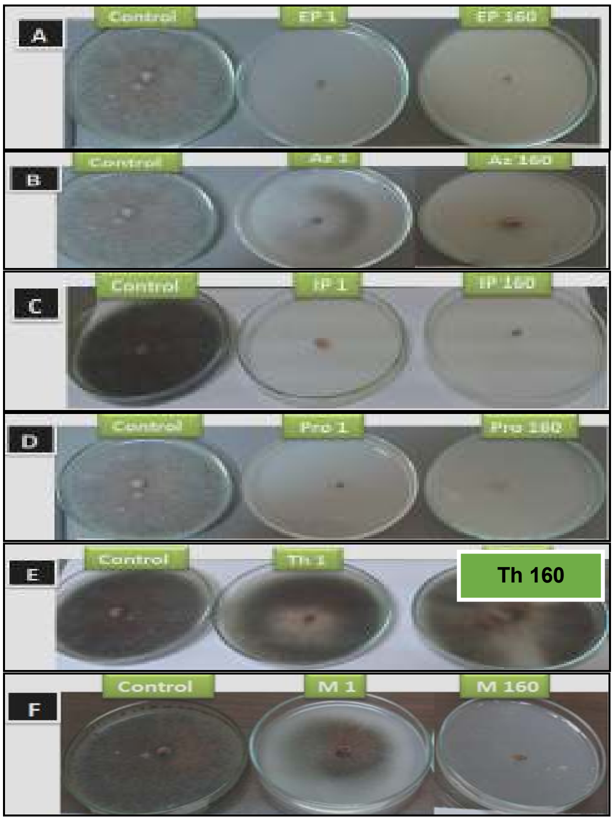

Fig. 1. *In vitro* preliminary test of the effect of six fungicides on linear growth of *D. teres*. The tested fungicides were (A) Epoxiconazole,(B)Azoxystrobin,(C)Iprodion,(D) Propiconazole, (E) Thiophanat methyl and (F) Mancozeb. Each fungicide was evaluated in six concentrations from (1-160 mg/L).

Table 2. Inhibitory effects of six fungicides at different concentrations on the linear growth of *D. teres* on PDA medium and values of Ic_{50}.

No.	Fungicide	Concentration mg/L	Linear growth (cm)	Inhibition %	Ic_{50}* (mg/L)
1	Epoxiconazole	0.05	7.97	11.43	0.254
		0.25	5.80	36.83	
		0.40	3.63	59.60	
		0.55	2.07	77.00	
		0.70	1.03	85.50	
2	Azoxystobin	1.0	7.0	14.40	19.045
		10	6.1	32.20	
		40	4.0	55.53	
		80	2.1	76.23	
		160	1.43	84.00	
3	Iprodion	0.2	7.77	13.66	1.582
		0.6	5.97	33.67	
		10	2.53	71.81	
		40	0.73	91.83	
		80	0.00	100	
4	Propiconazole	0.05	7.8	13.30	0.263
		0.25	5.7	36.26	
		0.40	4.0	55.50	
		0.55	2.0	77.73	
		0.70	1.37	84.76	
5	Thiophanate-methyle	240	8.27	8.13	334.584
		280	7.10	21.06	
		360	3.33	62.93	
		400	1.83	79.60	
		440	0.80	91.10	
6	Mancozeb	2.0	7.80	13.30	13.718
		10	4.73	47.33	
		40	2.47	72.56	
		80	1.98	77.73	
		120	0.80	91.10	
7	Control	S.D.W**	9.00	0.0	-

***ICc0: Effective inhibitory concentration which reduced the mycelial growth of *D. teres* by 50%.**

**** S.D.W: Sterilized distilled water. L.S.D at p ≤0.05, Fungicides = 1.83, concentration = 4.75**

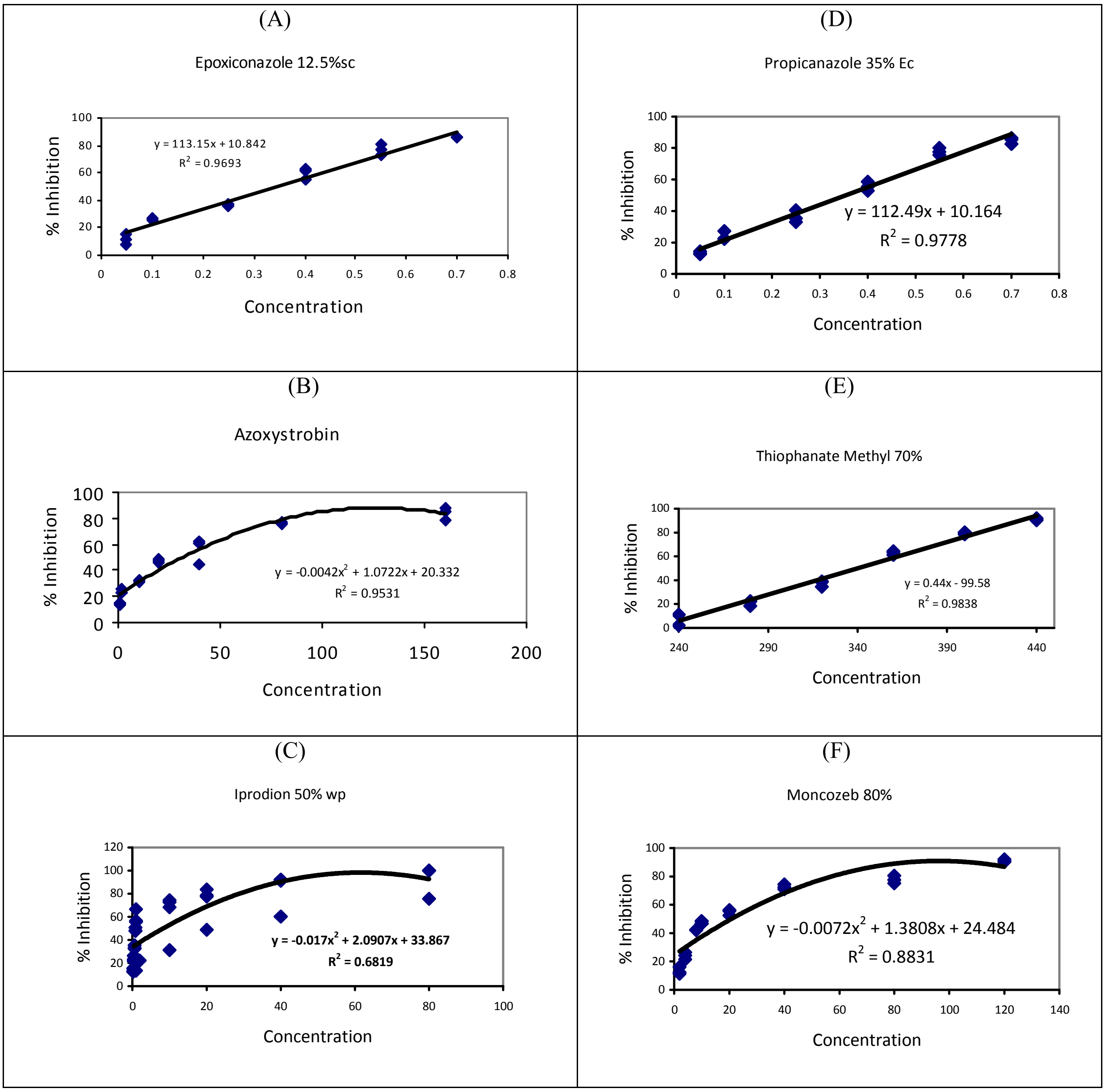

Fig 2. Regression equations that describe the effects of concentrations (mg12) of fungicides (x)i-e(A) Epoxiconazole (B) Azoxistropin (C) Iprodion (D) propiconazole (E) Thiphanante (F) Mancozeb on inhibition (%) in linear growth (Y) of *Drechslera teres*.

The obtained data in Table (2) proved that the six fungicides affected significantly ($p \leq 0.05$) the linear growth of *D. teres*, and also indicated that Epoxiconazole and Propiconazole (group of triazole) had the greatest efficient against growth of *D. tesres* compared with the control. These fungicides inhibited the linear growth of *D. teres* when the media was amended with concentration less than 1 mg/L. It is worthy to note that there was a gradual increase of inhibition by increasing concentration of the tested fungicides. Data in Table (2) also showed the highest inhibitions (<91%) were observed when the culture was amended with 40, 120 and 440 mg/L of Iprodion, Mancozeb and Thiophanate methyl respectively.

Ic_{50} determination:

Ic_{50} was calculated according to the linear relation between inhibitory probit and concentration logarithm (Table 2), and showed that, lower Ic_{50} values had higher fungicidal activity. Epoxiconazole and Propiconazole were the most effective fungicides following by Iprodion. In contrast, Thiophanate methyl showed the lowest effect inhibitory mycelial growth of *D. teres in vitro*. The effect of tested fungicides differed and could be arranged according to the Ic_{50} values in the following descending order, Epoxixonazole, Propiconazole, Iprodion, Mancozeb, Azoxystrobin and Thiophanate methyl.

2.Field experiment:

The first group:

Effect of fungicidal treatment on the disease severity:

This experiment included three fungicides, *i.e.* Epoxiconazole, Azoxystrobine and Iprodion. Data in Table (3) indicated that, highly significant difference ($P \leq 0.05$) in disease severity were observed between all fungicides and control (without fungicide) when applied as seed dressing (one application) or seed dressing + one foliar spray (two application).

Fungicides application reduced mean of net blotch severity compared with control, from 60.77% to 7.92, 11.25 and 12.92% for Epoxiconazole, Iprodion and Azoxystrobin, respectively at Kafre El-Hamam station.

The same trend was observed at Sakha station with reduction from 64.66% to 7.15, 7.94 and 10.84%. Epoxiconazole application gave the highest disease control followed by Iprodion and Azoxystrobin.

Data in Table (3) also proved that there is no significant difference between different applications.

Table 3. Effect of fungicides applied as seed dressing/one time spray on net blotch severity under field conditions at two locations, Kafre El-Hamam and Sakha stations during 2014/2015 growing season

No.	Fungicides	Kafre El-Hamam station					Sakha station				
		Disease severity %			Efficacy %		Disease severity %			Efficacy %	
		A*	B**	Mean	A	B	A	B	Mean	A	B
1	Epoxiconazole	9.00	6.83	7.92	85.19	88.76	8.82	5.48	7.15	86.18	91.63
2	Azoxystrobin	13.83	12.00	12.92	77.24	80.25	11.60	10.07	10.84	81.83	84.63
3	Iprodion	12.50	10.00	11.25	79.43	83.54	9.58	6.30	7.94	85.00	90.38
4	Control	60.77	60.77	60.77	-	-	63.83	65.50	64.66	-	-
	Mean	24.03	22.40	23.22	-	-	23.46	21.48	22.65		

A* = seed dressing (single application) **B**= seed dressing + one foliar spray (double application)**
LSD (p ≤0.05) for
Fungicides =3.76 **Fungicides = 3.09**
Applications = N.S **Applications = N.S**
FXA= N.S **FXA= N.S**

Effect of fungicidal treatment on thousand kernel weight (TKW):

Interaction between fungicides and applications was significant in both stations for 1000 kw (Table 4). 1000 kw increased due to fungicide application ranged from 15.40-33.03% at seed dressing (one application) and from 24.06-46.82% at seed dressing + one foliar spray (two application) over non-fungicide treatment at Kafre El-Hamam station (Table 4). Likewise, at Sakha station, increment of 1000 kw ranged from 24.56-41.91% and 29.24-58.09% at seed dressing and at seed dressing plus one foliar spray respectively. All fungicides significantly improved 1000 kw as one or double applications over non-fungicide treatment at two locations. As a general observation, double application was highly significant than single application. Epoxiconazole gave the highest value of 1000 kw following by Iprodion and Azoxystrobin, were ranking 55.62, 51.50 and 48.25 (g) for single application and 61.40, 53.40 and 51.88 (g) for double application respectively, comparing with control 41.81 (g) at Kafre El-Hamam station. At Sakha station, their performances at the same trend for 1000 k w, where Epoxiconazole, Iprodion and Azoxysrtobin were recorded 55.23, 51.29 and 48.48 (g) for single application and 61.48, 57.12 and50.26 (g) for double application respectively, comparing with control which recorded 38.92 and 38.89 (g).

Effect of fungicidal treatment on grain yield/plot (kg):

Data in Table (5) showed that the difference between fungicides and application were significant at (P≤5%) in both locations, but interaction between them were absent for Kafre El-Hamam station. So, there were high significance difference were observed between the fungicides comparing the control and the double application were the best in increasing the grain yield/plot over the control. Grain yield/plot was improved by 40.87 to 111.43% at Kafre El-Hamam station and by 56.73 to 149.35% at Sakha station over non-fungicide treatment. Epoxiconazole gave the highest grain yield was 6.98 and7.56 kg at Sakha and Kafre El-Hamam respectively, while Azoxystrobin occupied the last rank, which recorded 5.12 and 5.52 kg and the increase over control was 72.58 and 44.68% at Sakha and Kafre El-Hamam stations respectively. Epoxiconazole application gave the highest net blotch control and improved the yield components in two stations.

Table 4. Effect of three fungicides applied as seed dressing/one time spray on 1000 kernel weight (g) under field conditions at two locations, Kafre El-Hamam and Sakha stations during 2014/2015 growing season

No.	Fungicides	Kafre El-Hamam station					Sakha station				
		1000 kernel weight (g)			Increasing over control %		1000 kernel weight (g)			Increasing over control %	
		A*	B**	Mean	A	B	A	B	Mean	A	B
1	Epoxiconazole	55.62	61.40	58.51	33.03	46.82	55.23	61.48	58.35	41.91	58.09
2	Azoxystrobin	48.25	51.88	50.07	15.40	24.06	48.48	50.26	49.37	24.56	29.24
3	Iprodion	51.50	53.40	52.45	23.18	27.69	51.29	57.12	54.21	31.78	46.88
4	Control	41.81	41.81	41.81	-	-	38.92	38.89	38.90	-	-
	Mean	49.30	52.13				48.48	51.94			

A* = seed dressing (single application) **B**= seed dressing + one foliar spray (double application)**
LSD (p ≤0.05) for
Fungicides =1.24 **Fungicides = 0.56**
Applications = 0.87 **Applications = 0.39**
FXA= 1.74 **FXA= 0.79**

The second group:

Effect of fungicidal application on the disease severity:

This group included the active ingredient Propiconazole, Thiophanate methyl and Mancozeb. Two applications were made, i.e. one spray or two sprays.

Data in Table (6) showed that the high significant difference in disease severity between fungicides and control (without fungicide) when applied as one or two foliar sprays at Kafre El-Hamam station, but no significant difference at Sakha station were recorded between applications.

Propiconazole followed by Mancozeb and Thiophanate methyl reduced net blotch severity over non-fungicide treatment from 59.59 to 6.96, 11.05 and 11.67% respectively with efficacy 91.16, 86.67 and 84.85% respectively, at Kafre El-Hamam station. Likewise, Sakha station, fungicide application reduced mean net blotch severity over non-fungicide treatment, from 64.66% to 6.42, 13.04 and 14.27% with efficacy 91.65, 82.10 and 79.10 respectively.

Effect of fungicidal application on thousand kernel weight (TKW):

Data in Table (7) proved that the differences between fungicides × application interaction were significant at ($P \leq 5\%$) in both experimental stations for 1000 kw. Propiconazole gave the highest value of 1000 kw following by Thiophanate methyl and Mancozeb, were ranking 61.03, 53.57 and 51.39 (g) respectively, comparing with control 41.80 (g) at Kafre El-Hamam station. Likewise, at Sakha station, Propiconazole following by Thiophanate methyl and Mancozeb were recorded 55.32, 50.35 and 46.36 (g) respectively, comparing with control which recorded 39.20(g). As a general observation, all fungicides significantly improved 1000 kw over non-fungicide treatment ranged from 12.82-39.98% and 15.79-32.03% at one spray and 33.06-52.01% and 20.77-50.19% at two sprays at two stations, Kafre El-Hamam and Sakha respectively.

Table 5. Effect of fungicides applied as seed dressing/one time spray on yield/plot (kg) under field conditions at two locations, Kafre El-Hamam and Sakha stations during 2014/2015 growing season

No.	Fungicides	Kafre El-Hamam station					Sakha station				
		Yield/ plot (kg)			Increasing %		Yield/ plot (kg)			Increasing %	
		A*	B**	Mean	A	B	A	B	Mean	A	B
1	Epoxiconazole	6.98	8.14	7.56	79.43	111.43	6.23	7.73	6.98	99.68	149.35
2	Azoxystrobin	5.48	5.57	5.52	40.87	44.68	4.89	5.35	5.12	56.73	72.58
3	Iprodion	5.69	6.31	6.00	46.27	63.90	5.23	6.32	5.78	67.63	103.87
4	Control	3.89	3.85	3.87	-	-	3.12	3.10	3.11	-	-
	Mean	5.51	5.97				4.86	5.63			

A* = seed dressing (single application) **B**= seed dressing + one foliar spray (double application)**

LSD ($p \leq 0.05$) for

Fungicides =0.660 **Fungicides = 0.07**

Applications = 0.47 **Applications = 0.05**

FXA= N.S **FXA= 0.10**

Table 6. Effect of fungicides applied as one / or two foliar sprays on net blotch severity under field conditions at two locations, Kafre El-Hamam and Sakha stations during 2014/2015 growing season

No.	Fungicides	Kafre El-Hamam station					Sakha station				
		Disease severity %			Efficacy %		Disease severity %			Efficacy %	
		A*	B**	Mean	A	B	A	B	Mean	A	B
1	Propiconazole	8.67	5.25	6.96	85.49	91.16	7.37	5.47	6.42	88.30	91.65
2	Thiophahate methyl	14.33	9.00	11.67	76.02	84.85	14.86	13.68	14.27	76.73	79.10
3	Mancozeb	14.17	7.92	11.05	76.32	86.67	14.35	11.72	13.04	77.53	82.10
4	Control	59.75	59.42	59.59	-	-	63.85	65.47	64.66	-	-
	Mean	24.23	20.40	22.32	-	-	25.11	24.09	24.60	-	-

A* = one spray **B**= two spray**

LSD ($p \leq 0.05$) for

Fungicides =3.20 **Fungicides = 1.93**

Applications = 2.26 **Applications = N.S**

FXA= N.S **FXA= N.S**

Table 7. Effect of fungicides applied as one / or two foliar sprays on 1000 kernel weight (g) under field conditions at two locations, Kafre El-Hamam and Sakha stations during 2014/2015 growing season

No.	Fungicides	Kafre El-Hamam station					Sakha station				
		1000 kernel weight (g)			Increasing %		1000 kernel weight (g)			Increasing %	
		A*	B**	Mean	A	B	A	B	Mean	A	B
1	Propiconazole	58.51	63.54	61.03	39.98	52.01	51.77	58.86	55.32	32.03	50.19
2	Thiophahate methyl	48.90	58.24	53.57	16.99	39.33	45.50	55.21	50.35	16.04	40.88
3	Mancozeb	47.16	55.62	51.39	12.82	33.06	45.40	47.33	46.36	15.79	20.77
4	Control	41.80	41.80	41.80	-	-	39.21	39.19	39.20	-	-
	Mean	49.09	54.80		-	-	45.47	50.15			

A* = one spray **B**= two spray**

LSD ($p \leq 0.05$) for

Fungicides =1.23 **Fungicides = 0.48**

Applications = 0.87 **Applications = 0.34**

FXA= 1.74 **FXA= 0.68**

Effect of fungicidal application on yield/plot (kg):

The differences between fungicides × applications interaction was significant at (P≤5%) in both experimental station (Table 8).

Yield/plot increment due to fungicide applications ranged from 34.63-76.49% and 30.79-77.7% as one spray and ranking 62.83-122.51% and 40.71-134.29% as two sprays at Kafre El-Hamam and Sakha stations respectively, over non-fungicide application. Propiconazole gave the highest grain yield/plot following by Thiophanate methyl and Mancozeb, were ranking 7.67, 6.32 and 5.71 (kg) respectively, over non-fungicide application at Kafre El-Hamam station. At Sakha station, Propiconazole followed by Mancozeb and Thiophanate methyl were recorded 6.46, 4.33 and 4.29 (kg) over non-fungicide applications.

As a general observation, all fungicides belong to triazole group showed high efficacy against net blotch disease and increasing 1000 kw and yield/plot with different applications and different locations.

Table 8. Effect of fungicides applied as one or two foliar sprays on yield/plot (kg) under field conditions at two locations, Kafre El-Hamam and Sakha stations during 2014/2015 growing season.

No.	Fungicides	Kafre El-Hamam station					Sakha station				
		Yield/ plot (kg)			Increasing %		Yield/ plot (kg)			Increasing %	
		A*	B**	Mean	A	B	A	B	Mean	A	B
1	Propiconazole	6.83	8.50	7.67	76.49	122.51	5.60	7.31	6.46	77.7	134.29
2	Thiophanat methyl	5.39	7.24	6.32	39.28	89.52	4.19	4.39	4.29	33.02	40.71
3	Mancozeb	5.21	6.22	5.71	34.63	62.83	4.12	4.53	4.33	30.79	45.19
4	Control	3.87	3.82	3.85	-	-	3.15	3.12	3.14	-	-
	Mean	5.33	6.45				4.27	4.84			

A* = one spray **B**= two spray**

LSD (p ≤0.05) for

Fungicides =0.21 **Fungicides = 0.07**

Applications = 0.15 **Applications = 0.04**

FXA= 0.30 **FXA= 0.09**

Correlation between Ic_{50} (*in vitro*) and efficacy of fungicide in field:

Statistical analysis was carried out to ascertain if there are correlation between Ic_{50} values and fungicides performance in field. Table (9) show that correlation between Ic_{50} and fungicidal efficiency under field conditions was non-significant r = 0.585 (P= 0.222). When Thiophanate methyl fungicide was excluded from the analysis r value increased to 0.899 (P = 0.038). These data showed that the five fungicides followed the same pattern and perfectly matched the *in vitro* data. In contrast, Thiophanate methyl fungicide which exhibit low performance *in vitro* (Ic_{50} 334.584 mg/L), but its performance in field (79.18%) was reasonable. The regression equation show in Fig. (3) indicates Ic_{50} could be accounted for 0.81 of the total variation in fungicidal efficiency under field conditions.

Table 9. Correlation between Ic_{50} values mg/L for the six fungicides tested *in vitro* and mean of fungicides efficacy % of net blotch disease under four different environmental.

No.	Fungicides	Ic_{50} values [a] mg/L (x)	Mean of fungicides efficacy % (y) [b]	Correlation* n = 6	Correlation** n = 5
1	Epoxiconazole	0.254	87.94		
2	Propiconazole	0.263	89.15		
3	Iprodion	1.582	84.59	r = 0.585	r = 0.899
4	Mancozeb	13.718	80.66	P = 0.222	P = 0.038
5	Azoxystrobin	19.054	80.99	(ns)	significant
6	Thiophahate methyl	334.584	79.18		

a: Efficacy of fungicides *in vitro*.

b: Efficacy of fungicides over all location and application methods in the field.

*** Correlation between Ic_{50} *in vitro* and efficacy of fungicides in the field of six fungicides, (n = 6) and correlation coefficient (r) was non-significant.**

**** Correlation between Ic_{50} *in vitro* and efficacy of fungicides in the field of five fungicides when the outlier Thiophanate methyl was excluded from the analysis, (n=5) and correlation coefficient (r) was significant.**

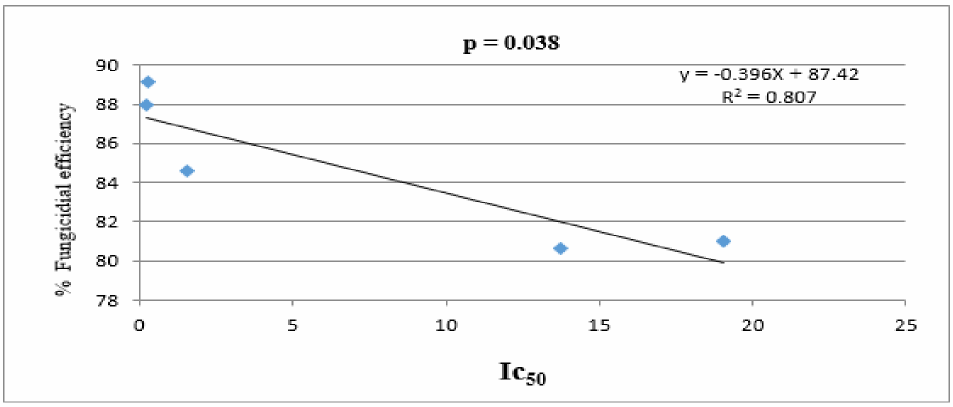

Fig. 3. Regression equation that describes the relationship between Ic_{50} (x) and fungicidal efficiency under field conditions (Y)

DISCUSSION

In Egypt, there is no recommended fungicides to control net blotch of barley caused by *Drechslera teres*. Therefore, the present study was planning to evaluate the efficacy of six fungicides with different active ingredient in inhibiting linear growth of *D. teres* (*in vitro*) and evaluate their performance under field conditions (*in vivo*) in controlling net blotch when they were applied as seed treatment and foliar spray. The *in vitro* results of present study proved the effectiveness of tested fungicides in reducing linear growth of *D. teres*. Treatment of Epoxiconazole and Propiconazole showed an inhibitory effect on mycelial growth at very low concentration (0.05 mg/L). These two fungicides belong to triazole group which reported by Blandino *et al*. (2012) to be the most active molecules against *Fusarium* sp. These types of fungicides interfere with the metabolism of fungal pathogens, mainly by inhibition of ergosterol biosynthesis (Hewitt, 1998) and often cause striking morphological malformations of cell wall. Ramirez *et al*. (2004) reported that none of the isolates of *F. graminearum* were able to grow in the presence of any active triazole substance at concentration > 15 mg/L. Results of presented study proved that Iprodion had inhibitory effect on linear growth at concentration began from 0.2 mg/L, while in the case of Azoxystrobin, Mancozeb and Thiophanate methyl the inhibitory concentration started from 1, 2 and 240 mg/L respectively. Nesci *et al*. (2003) reported that chemical fungicides might inhibit the function of several enzymes by the oxidized compounds or/and by more non-specific interactions with the proteins. In the present study, data showed that the difference in fungal growth inhibitory capacities between the tested fungicides (indicated by Ic_{50}) was highly significant ($P\leq0.05$) except between Epoxiconazole, Propiconazole and Iprodion. The inhibitory effect of the tested fungicides could be arranged according to the Ic_{50} values in the following descending order; Epoxiconazole, and Propiconazole showed very strong inhibition with Ic_{50} 0.254 and 0.263 mg/L respectively. The second group with an IC_{50} 1.582 mg/L included Iprodion. The third group included, Mancozeb and Azoxystrobin with IC_{50} 13.718 and 19.054 mg/L respectively. Thiophanate methyl was far less effective one with an Ic50 334.584 mg/L. Previous authors referred to resistance of *D. teres* isolates to triazoles expressed in Ic_{50} values, for instance, Campbell and Crous (2002) reported a strong resistance shown by both net and spot type isolate to Triadimenol and lower resistance to other triazoles.

To evaluate the efficiency of the same six fungicides on net blotch severity and yield components, field experiments were carried out at different environmental conditions under natural infection at Kafre El-Hamam and Sakha experimental stations during 2014/2015 growing season. The effectiveness of synthetic fungicides, their expected suppressing role against *D. teres* were achieved by using Epoxiconazole, Azoxystrobin and Iprodion as seed dressing (one application) or seed dressing + one foliar spray (two applications) and Propiconazole, Thiophanate methyl and Mancozeb as one or two foliar sprays, it could be arranged in descending manner as follows; Epoxiconazole, Iprodion and Azoxystrobin (the first group) and Propiconazole, Mancozeb and Thiophanate methyl (the second group). As regard to, the effect of fungicides on yield components, thousand kernel weight (TKW) and grain yield/plot, the results proved that there is a significant difference between fungicides application and the control in one or double application.

Disease severity in this work was variable between two locations, where net blotch was moderate in Kafre El-Hamam and high in Sakha location, this may have been due to combination of two factors, the cumulative reduction of infection among plants during a season when condition for net blotch were not always optimal and failure of conidia to infect new susceptible tissue on the upper portions of plants. It was in full agreement with Bekele (2005) who found that net blotch epidemic was influenced by seasonal weather conditions, as well as variety and effectiveness of fungicide.

As regard to the effect of methods of fungicides application, results the present study showed that, there were no significant difference in disease severity when using fungicides as seed treatment (one application) or seed treatment + one foliar spray (double application), this may have been due to the efficacy of the fungicidal seed treatment which had some effect on fall net blotch severity. In this respect, Reis and Casa (2007) reported that the main sources of *D. teres* inoculum are seed and crop residue, control measures should be directed to the sources such as seed treatment and along with crop rotation. Reis *et al*. (2012) found that when seed treatment is not capable of preventing seed transmission to above-ground plant parts, it is considered ineffective. In addition, Reis and Casa (2007) found that seed treatment effectiveness depends on chemical fungitoxicity, fungal sensitivity and seed coverage quality. In contrast, when fungicides as one or two foliar sprays, data showed that there were significant differences in reducing disease severity when fungicides were used as two foliar sprays than that one foliar spray at Kafre El-Hamam station, but no significant difference was noticed at Sakha station, this finding are in agreement with Kith *et al*., (2008), who found that, applying a fungicide spray is necessary in medium to high rainfall regions, the choice of a single or two sprays strategy depend on the environment in which the crop is growing. Likewise, variability in results may have been due to various fungal sensitivity to fungicides at the two locations. Scott *et al*. (1992) reported that two applications of Propiconazole were required to reliably control spot-type, where only one application was required for net-type control in the Western Capa Province of South Africa. This was probably due to the spot type of *D. teres* was the predominant type (83%) in the Western Capa. Also, Louw *et al*. (1996) indicated that the spot type of *D. teres* was the predominant type in the Western Capa Province. This was probably due to the introduction of the new dominant Australian cultivar clipper, which was susceptible to spot type but resistant to net type. El-Nashar (1990) found that spot-type isolates were not common in Egypt, where the net-type of *D. teres* was the predominant type. In this respect, Badr *et al*. (2015) reported that of sixty-three isolates of *D. teres* collected from barley growing areas in Egypt, sixty-two isolates produced typical net blotch symptoms and only one isolate produced spot type lesions. They also found that all Egyptian barley cultivars are susceptible to *D. teres* pathotypes. Therefore, this study evaluated an attractive control measures depending in intensive application of fungicides.

In the present study, there was differential fungicidal efficacy, where the efficacy of fungicides Epoxiconazole, Azoxystrobin and Iprodion higher in Sakha than Kafre El-Hamam station. In contrast, the efficacy of fungicides, Propiconazole, Thiophanate methyl and Mancozeb were higher in Kafre El-Hamam station than Sakha station, this may have been due to the various degrees of sensitivity of populations of *D. teres* to treatment with fungicides (different active ingredient), this was in agreement with Shaw (1989) who reported that sexual recombination and quantitative inheritance of resistance could change the distribution of resistant phenotypes. This would be mean that progeny with various degrees of resistance would be produced. Peever and Milgroom (1992) were able to show that by crossing isolates of *D. teres* triadimenol resistance in the progeny was controlled by a major gene, as well as three to five other minor genes. Peever and Milgroom (1993) reported that *D. teres* displays cross-resistance from some demethylation inhibitors (DMIs) and therefore, genes resistant to other DMIs may mask the actual sensitivity to net blotch isolates toward bromuconazole.

All fungicidal treatment for which our data are presented caused a decrease in net blotch severity and increasing the yield components, in this respect Jayasena *et al*. (2002) evaluated ten fungicides as single application to control spot-type of net blotch of barley caused by *D. teres* f. *maculate* at three locations during 1999-2000. They reported that under moderate disease severity, yield losses ranged from 17-19% depending on location and under high disease severity, yield loss reached 32%. They also found that pyraclestrobin, Propiconazole and mixture of Propiconazole with Iprodion were the most effective in controlling disease improving yield and grain quality. Our results showed that pronounced impacts of grain yield and 1000 kernel weight were obtained in Kafre El-Hamam compared to Sakha station, this results confirmed with Abed El-Tawab (2016), found that grain yield parameters were negatively affected and clear reduced in Sakha station compared to in El-Gemmiza, it might due to disorders of climatic condition, heavy rainfall occurred in Sakha at planting time course. It was agreement with Mathre (1997), who stated that the environmental conditions were considered from the most important factors affecting growth of the plants and infection with certain pathogen. It was in full agreement with Yau and Ryan (2013), who found correlation (r =0.70) between rainfall and grain yield of barley and others legumes plants. Our data were agreement with those obtained by Abdel-Fattah (2015), he found that the effect of the five fungicides Propiconazole + Difernoconazole (Montro), Pyraclostobin + Boscalid (Bellis), Epoxiconazole (Opus), Pyraclostrobin + Metrim (Cabriotop) and Metalaxyl + Mancozeb on net blotch severity and yield components were evaluated under natural infection at Sakha experimental station during 2013 and 2014 seasons. The effectiveness on net blotch severity could arranged in descending as a previous respectively, when applied as one or two sprays, as regard to the effect of fungicides on yield components thousand kernel weight (TKW) and grain weight/plot. These results are agreement with our finding.

At the present study, the comparison of the in-planta activities shown by the tested fungicides with that obtained *in vitro*, indicated that fungicides performance *in vitro* did not always reflect the one seen in plant. Where the most of fungicides showed high *in vitro* activity against *D. teres*, it was associated with high in planta efficacy. But in contrast, Thiophanate methyl fungicide had lower activity *in vitro* against *D. teres*, but reasonable activity in plant, this may be due to Thiophanate methyl transformed to the more active molecule carbendazim after application (Baude *et al*., 1973). In this regard. Triadimefon, an early systemic triazole foliar fungicide, that acts by inhibitory steroid demethylation and was used against many plant pathogens (Roberts and Huston, 1999), it is enzymatically reduced in plants and fungi to Triadimenol, a more fungi-active metabolite (Kenneke *et al*., 2008). On the other hand, recently Kurt *et al*. (2011) found that mycelial growth was completely inhibited *in vitro* by three naturally occurring fungitoxic compounds (methyl, allyl and benzyl isothiocyanate), however in planta assay, only allyl and isothiocyanate showed a similarly high level activity.

The results of regression analysis suggested that Ic_{50} was of practical value because it can be used to predict the performance on fungicide under field conditions, which saves time. However, the confirmation of this results may require more experiments, include a diverse collection of fungicide. One should keep in mind that laboratory tests Ic_{50} should be used complementary to field tests and not as an alternative.

Finally, it can be concluded that some triazole fungicides were the most active group *in vitro* and in field against net blotch of barley, when it was used as seed dressing or as foliar spray.

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إستعمال الارتباط الخطي بين اختبارات المعمل والحقل لتقييم كفاءة بعض المبيدات الفطرية في مكافحة التبقع الشبكي في الشعير

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يعدّ الفطر دريشليرا تيريز المسبب للتبقع الشبكي في الشعير من الفطريات التي تنتقل عن طريق الحبوب والمجموع الخضري. في هذه الدراسة اختبرت فعالية 6 مبيدات فطرية (ايبوكسي كونازول، ازوكسي ستروبين، ابرديون، بروباكونازول، ثيوفانات ميثايل ومانكوزيب) تحت ظروف المعمل لمعرفة تأثيرها المثبط على نمو الفطر دريشليرا تيريز عن طريق قياس النسبة المئوية لتثبيط نمو الميسيليوم. تم حساب التركيز المثبط لحوالي 50% من النمو الميسيلومي (Ic_{50}) لكل مادة فعالة تحت الاختبار. حيث كانت المادة الفعالة الترايزول للمبيدات الفطرية (ايبوكسي كونازول وبرباكونازول) كانت الاكثر فعالية حيث كانت قيمة Ic_{50} على التوالي 0.254 و 0.263 مليجرام لكل لتر. وفي المقابل كانت قيمة Ic_{50} للمبيد ثيوفانات ميثايل اعلى قيمة من جميع المبيدات وهي 334.584 مليجرام لكل لتر، وذلك بالمقارنة بجميع المبيدات المختبرة. تم تقييم هذه المبيدات تحت ظروف الحقل في محطتي بحوث كفر الحمام وسخا بمحافظتي الشرقية وكفر الشيخ خلال موسم 2015/2014، باستعمال الثلاث مبيدات الاولى كمعاملة الحبوب فقط (معاملة واحدة) او مع رشة واحدة (معاملتين)، وتم معاملة الثلاث مبيدات الثانية كرشة واحدة او رشتين. أظهرت المبيدات تحت ظروف الحقل تباين في مكافحة المرض ووزن الالف حبة ووزن محصول القطعة التجريبية، ويرجع ذلك التباين الى اختلاف المادة الفعالة وطريقة الاستخدام وكذلك الظروف البيئية. أظهرت أيضاً مجموعة التريزولات كفاءة اعلى بالمقارنة بباقي المبيدات. تدل العلاقة الارتباطية المعنوية بين قياس كفاءة المبيدات في كل من المعمل والحقل على انه يمكن اعتبار قيمة Ic_{50} مكملة لاختبارات الحقل وليست بديلاً عنها وذلك عند تقييم اداء المبيدات في مقاومة المرض.