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Monitoring of Tricyclazole and Isoprothiolane Residues and their Effects on Blast Disease, Yield and its Components, Grain Quality and Chemical Components of Rice

Rabab M. Elamawi[1]; Fatma A. Mostafa[2] and R. A. S. El-Shafey[3]

[1]Rice Pathology Department, Plant Pathology Research Institute, Agricultural Research Center, Sakha, Kafrelsheikh, 33717, Egypt

[2]Integrated Pest Management Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

[3]Rice Research and Training Center, Field Crop Research Institute, Agricultural Research Center, Sakha, Kafrelsheikh, 33717, Egypt

***Corresponding author: rabab.elamawi@yahoo.com**

ABSTRACT

The systemic fungicides tricyclazole (Beam 75% WP) and isoprothiolane (Fuji-one 40% EC) are widely used to control *Pyricularia oryzae* infection causing rice blast disease in Egypt. A field experiment was conducted at Rice Research and Training Center farm, Sakha, Kafrelsheikh, Egypt during 2015 and 2016 seasons on susceptible cultivar Sakha 101. The impact of tricyclazole (0.5 mg/L) and isoprothiolane (2 ml/L) on *P. oryzae* infection, yield and its components, grain quality and chemical components of rice were evaluated. Meanwhile, the fungicides residue dynamics on rice straw, husk and milled rice were determined using Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method combined with HPLC-MS/MS detection after 30 and 60 days post-harvest. The results indicated that tricyclazole and isoprothiolane were significantly possessed a high level of controlling blast disease whereas, both fungicides reduced disease severity, areas under disease progress curve (AUDPC), and rate of leaf blast progress (r-value) disease parameters in both seasons. Residue analysis of tricyclazole in rice straw (1.06 mg/kg) was higher than the residue in the husk and milled rice (0.19 and 0.14 mg/kg, respectively) after 30 days post-harvest. The residues of tricyclazole in the husk and milled rice were lower than the Maximum Residue Limit (MRL). Also, isoprothiolane residue analysis in milled rice was 0.58 mg/kg after 60 days post-harvest and below the MRL. Also, most of the yield and yield components were significantly increased by the application of both fungicides over the untreated plots. Tricyclazole and isoprothiolane treatments enhanced the grain chemical components concentration as carbohydrates, fats, ash, and fiber. Finally, the investigation revealed that tricyclazole and isoprothiolane were effective against rice blast disease and considered safe fungicides according to the European Food Safety Authority. Based on our residue analysis results and Egyptian rice consumption, we suggested that determine our own Egyptian MRL. Whereas, our total rice consumption reaching 4 million ton/year in 2016, it was considered fourth fold than Europe consumption (1 million ton/year).

Keywords: Rice, Tricyclazole, Isoprothiolane, *P. oryzae*, Grain yield, Quality, Fungicides residue

INTRODUCTION

Rice blast disease is one of the most destructive and widespread diseases, caused by *Magnaporthe oryzae* B. Couch (anamorph: *Pyricularia oryzae* Cavara) (Couch and Kohn 2002). This disease can potentially cause a significant yield and grain quality losses in many rice growing countries. Thus, it is the main constraint to global food security and agricultural trade (Ou, 1985). The pathogen can infect rice plants from seedling up to maturity stage. Rice blast fungus attacks and causes symptom at almost all plant parts i.e. leaves, leaf collars, stems, nodes, panicles, and grain. Application of fungicides, growing resistant cultivars, recommended agricultural practices as fertilizers and irrigation are used to control the rice blast disease (Ou, 1985).

Under Egyptian conditions, the systemic fungicides; tricyclazole and isoprothiolane have been authorized and extensively used to control this disease since the early of the 1990s. Due to the release of new resistant and high yielding Sakha 101 and Sakha 104 rice cultivars, the total rice growing area increased gradually from 15% in 1987 to 98% in 2003 season. The total treated area with fungicides decreased from 30% of total rice area in 1987 to only 1% in 2003 season. Meanwhile, during 2004, the total area of susceptible cultivars to blast disease sharply increased from 2 to 50%, when a sudden breakdown of blast resistance for both cultivars (Sakha 101 and 104) presented due to evolution of new virulent blast races IG-1 and IB-45, respectively (Sehly *et al*., 2008 and El-Shafey *et al*., 2015). Therefore, the treated area with tricyclazole and isoprothiolane increased up to 4 % by 2015 season (RRTC, 2015).

Tricyclazole (5-methyl-1, 2, 4-triazolo [3,4-b] benzothiazole), a systemic fungicide with a mode of action as melanin biosynthesis inhibitors, was developed more than 35 years ago (Froyd *et al*., 1976 and Inoue *et al*., 1987). Tricyclazole can persist for as long as 11 months in field agricultural soils under different climatic conditions (Jeong *et al*., 2012). The previous studies of Kumar *et al*., (2017) revealed that moisture regime, organic matter, pH of the water and atmospheric CO_2 level have a significant effect on dissipation of tricyclazole from the soil. Tricyclazole is classified as a moderately hazardous pesticide. Its toxicity for mammals is low, but its toxicity for aquatic organisms is considerable. The Maximum Residue Limit (MRL) of tricyclazole in brown rice determined at 1 mg/kg according to the European Food Safety Authority (2013).

The second widely used fungicides for controlling rice blast and brown spot diseases under the Egyptian conditions, in addition, it reduces the population of planthopper in rice (Uchida *et al*., 1983) is isoprothiolane. Isoprothiolane (di-isopropyl 1, 3-dithiolan-2-vinylmalonate) is a member of the phosphorothioate fungicides that was early synthesized in the late 1970s (Yoshida, *et al*., 1984). It is believed that the fungicide interferes with transmethylation in the biosynthesis of phosphatidylcholine, a major membrane lipid in eukaryotic cells, which plays an important role in signal transduction (Exton, 1994). It acts on the fungus at the penetration and growth stages of the infecting hyphae rather than at conidial germination and appressorium formation (Exton, 1994).

European Food Safety Authority (EFSA) indicated an adequate analytical method to determine isoprothiolane residues in rice with the LOQ of 0.01 mg/kg by using QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) (European Food Safety Authority, 2012). Residue levels of isoprothiolane in brown rice samples collected 7 days after the application was below the MRLs of 1.0 mg/kg as set by Chinese authorities (Zhang *et al*., 2016).

As a result for a long-term using or a wide application of the two fungicides tricyclazole and isoprothiolane for blast control and due to limited information is available concerning the level of residues of both fungicides in rice grain after harvesting in Egypt. In this study, the prevention of rice blast disease by tricyclazole and isoprothiolane was evaluated on the rice crop. In addition, The QuEChERS method followed by HPLC-MS/MS for quantification was used to monitor the residue level of fungicides in rice straw and grain, which was expected to acquire a complete knowledge of its residual behavior whilst provide its safe use guidance in the agricultural crop.

MATERIALS AND METHODS

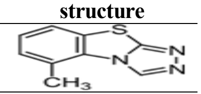
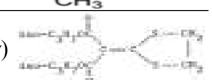
Field experiment:

An experiment was conducted to evaluate the efficacy of the two fungicides tricyclazole and isoprothiolane in controlling rice blast disease and to investigate the fungicide residues in rice, and assess their effect on grain quality and chemical components. Susceptible rice cultivar; Sakha 101 was planted at Rice Research and Training Center Farm, Sakha, Kafrel-Shiekh Governorate during 2015 and 2016 seasons. Nursery beds were seeded during the first week of May. The permanent field was transplanted one month later with 25-30 day old seedlings at the spacing of 20 x 20 cm. The experiment design was a randomized complete block (RCB) with three replicates. The plot size was 3x3.5 m. The nitrogen fertilizer was added as Urea (46.0 % N) according to the recommendation package of Sakha 101 cultivar. A flat partition (1m) was used between the plots to avoid contamination with other fungicides.

Application of tricyclazole and isoprothiolane fungicides under field conditions:

Characterizations of tricyclazole (Beam 75%WP) and isoprothiolane (Fuji one 40% EC) were listed in Table 1. Tricyclazole at the recommended rates of 0.5 g/L was sprayed twice; the first at the appearance of the disease and the second one at panicle initiation. On the other hand, isoprothiolane was applied at the recommended rates of 2 ml/L and sprayed three times; at the disease appearance, 15 days after the first application and at the panicle initiation.

Table 1. Characterizations of both fungicides tricyclazole and isoprothiolane ccording to EFSA, (2012 and 2013)

No.	Compounds Trade name	Common name	Molecular Weight (g/mol)	Post Harvest index Value	LC_{50} mg/L (EFSA, 2012 and 2013)	Toxicological class	Chemical structure
1	Beam 75 % WP	Tricyclazole	189.236	35 days	LC_{50} for rats is >1.96 mg/L for 4 hours	II (Moderately)	
2	Fuji-One 40% EC	Isoprothiolane	290.40	14 days	LC_{50} for rats is > 2.32 mg/L for 96 hours	II (Moderately)	

Assessment of epidemiological parameters:

The leaf blast disease developments were observed periodically at 30, 40, 50, 60, 70 and 80 Days Post-Transplanting (DPT) during the crop season. The epidemiological parameters i.e. Disease Severity (DS), area under disease progress curve (AUDPC) and rate of leaf blast disease increase (r-value) were estimates. Leaf blast was scored on 0-9 scale of International Standard Evaluation System of Rice (IRRI, 1996). Disease severity was estimated by counting the total number of type (4) or more of blast lesions/100 leaves. Panicle infection was estimated by collecting twenty-five panicles from each plot before harvesting, and the severity of panicle infection was calculated by using the formula adopted by Townsend and Huberger (1943) as follow:

$$DS(\%) = \frac{\text{Sum}(n x y)}{10N} \times 100$$

Where:

DS= Disease Severity of panicle blast infection; n= Number of infected panicles in each category, y= Numerical values of each category and 10 = Constant (highest numerical values), N= Total number of panicles.

The area under disease progress curve (AUDPC) was estimated to compare relative levels of blast disease progress for each treatment; data of leaf blast severity were used to calculate the area under disease progress curve (AUDPC) with 10-days intervals using the following equation adopted by Pandey *et al*., (1989) as follow:

$$\mathbf{AUDPC = D\,[½\,(Y1 + Yk) + Y2\ + Y3\ \ Yk\text{-}1]}$$

Where:

D = day intervals among blast reading; Y 1 = first blast disease score; Yk = last disease score.

The rate of leaf blast disease progress (r-value) was estimated to determine the disease progression rate in relation with time during the growing season. It was calculated from the different blast scores as a severity of leaf blast infection at the time of leaf blast lesions appearance and ten days intervals. The r-value was calculated using the following formula adopted by Van der Plank, (1963);

$$\mathbf{r\text{-}value = 1/t_2\text{-}t_1(\log_e X_2/1\text{-}X_2 - \log_e X_1/1\text{-}X_1),}$$

Where:

X_1= Blast disease severity at date t_1 ; X_2 = disease severity at date t_2 ; t_2 -t_1 = the interval in days among these dates.

Analysis of yield and yield components:

The studied characters (i.e., plant height, the number of tillers/hill, no. of panicles/hill, panicle length, panicle weight, 1000-grain weight, no. of filled grain, no. of unfilled grain per panicle and grain yield were estimated.

Determination of rice grain quality characters and chemical components:

a. Rice grain quality characters: Hulling, milling, head rice percentages, amylose content % and gelatinization temperature (G.T) were analyzed according to the Standard Evaluation System for rice (IRRI, 1996).

b. Grain chemical components were estimated by using Near-infrared reflectance spectroscopy (NIRS spectra): FOSS NIRS^{TM} DS2500 F (FOSS, Hillerod, Denmark) instrument was used to determine the chemical components; protein, carbohydrate, moisture, fiber, fat and ash contents in treated and untreated rice grain. Samples were placed in a cup and then scanned in the diffused reflectance mode. Each sample was measured in two independent subsamples and the average spectrum was used for chemometric analysis using DS2500 IU software (FOSS) (Peng *et al*., 2015). Data were stored at every 2 nm interval in the wavelength range from 400 to 2,498 nm (Li *et al*., 2016).

Analysis of tricyclazole and isoprothiolane fungicides residues using QuEChERS method:

The QuEChERS method followed by HPLC-MS/MS for quantification was developed and spread rapidly worldwide for determination of pesticide residues in foods by Anastassiades and Lehotay (2003). Fungicides residues were established at Central Laboratory of Residue Analysis of Pesticides and Heavy metals in Food, Agricultural Research Center, Dokii, Giza, according to European Committee for Standardization/Technical Committee 275, Foods of plant origin 15662. The QuEChERS method which combines an innovative extraction method with the separation of the compounds in the extracts by gas and liquid chromatography for detection and identification of the individual pesticides by mass spectrometry was used.

The rice samples were prepared as follows; paddy rice samples were subjected to primary milling operation to remove husks. The straw samples were cut into small pieces and air-dried. Straw, husk and milled rice grain were ground and used for residue analysis. QuEChERS method combined with HPLC-MS/MS detection methods were used to determine the pesticide residues using acetonitrile extraction/partitioning. Water was added to get a total of approximately 10g of samples before the initial extraction. Then, magnesium sulfate, sodium chloride and buffering citrate salts (pH 5 to 5.5) were added. The mixture was shaken intensively and centrifuged for phase separation. An aliquot of the organic phase was cleaned-up by Dispersive Solid Phase Extraction (D-SPE) employing bulk sorbents as well as magnesium sulfate for the removal of residual water. Following cleanup with amino-sorbents (e.g. primary secondary amine sorbent, PSA) extracts were acidified by adding a small amount of formic acid to improve the storage stability of certain base-sensitive pesticides. The final extract can be directly employed for GC- and LC-based determinative analysis. Quantification was performed using an internal standard, which was added directly before injection in GC-NPD & ECD and GC-MSD systems. The limit of quantitation started at 0.001 mg/kg and up depending on the pesticide type and detection module. The measurement uncertainty expressed as expanded uncertainty and in terms of relative standard deviation (at 95 % confidence level) is lower than the default value set by the EU (± 50 %).

Statistical analysis: The data was subjected to Analysis of Variance (ANOVA) in addition the correlation and regression using SPSS v.16 statistical program package software. Treatment means were compared using Least Significance Difference (LSD) values at P $\leq$ 0.05. Differences among treatments were tested by Ducan's Multiple Range Test (DMRT).

RESULTS

Effect of the fungicides on rice blast disease development during the vegetative growth stage:

Evaluation of the effect of both fungicides on leaf blast disease progress on rice plants during 2015 and 2016 growing seasons is given in Figure 1. In general, the application of two fungicides tricyclazole and isoprothiolane progressively reduced blast disease severity till the end of the vegetative growth stage compared with the control treatment (untreated plants). The obtained results indicated that tricyclazole application significantly decreased leaf blast severity to only 9.4%, followed by isoprothiolane (20.5%) as compared with untreated rice plants (23.5%) at 10 days after first spraying (Fig. 1). Leaf blast severity did not exceed up to 10.0 % and 19.0% with tricyclazole and isoprothiolane applications, respectively by the end of 2015 season (Fig. 1A) while, it was 6.10 and 12.33, respectively in the second season; 2016 (Fig. 1B). In contrast, leaf blast severity reached its maximum levels in untreated rice plants, as it was 65.0 and 58.10 % in the vegetative stage in 2015 and 2016 seasons, respectively.

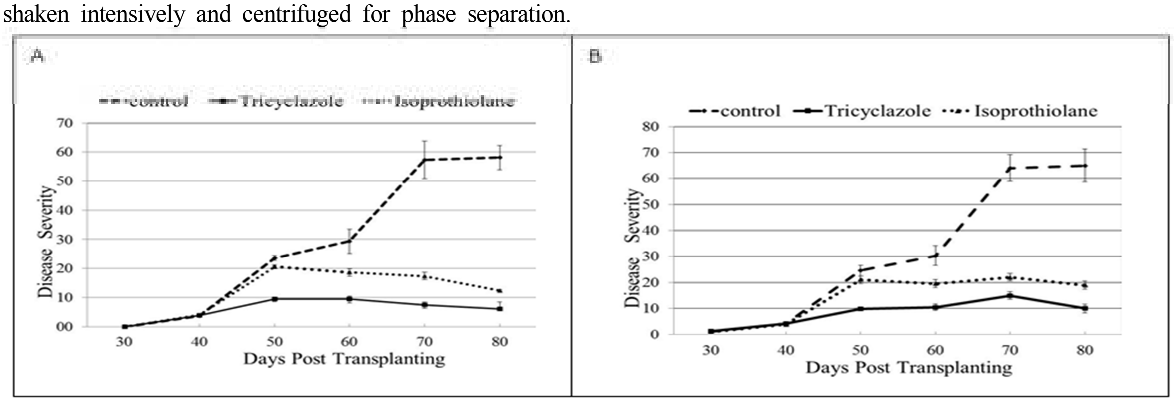


Figure 1. Development of leaf blast disease in the treated Sakha 101 rice cultivar with tricyclazole and isoprothiolane fungicides as compared with untreated plants during 2015 (A) and 2016 (B) seasons.

Effect of the fungicides on the epidemiological parameters of leaf and panicle blast disease:

The Disease severity (%) and efficiency of both fungicides for controlling leaf and panicle blast disease in rice plants were determined at 80 Days Post-Transplanting (DPT) under natural infection as in Table 2. The efficiency of tricyclazole and isoprothiolane for controlling leaf blast disease were (84.61 - 89.50%) and (70.76 - 78.77 %), respectively, in the two seasons; 2015 and 2016.

Areas under disease progress curve (AUDPC) of leaf blast showed that both fungicides significantly exhibited lower AUDPC values than those of the untreated rice plants. Meanwhile, tricyclazole application sharply decreased AUDPC value (102.51), than isoprothiolane (170.62), compared to untreated rice plants (459.31) in 2015 season. During 2016 season, the application of the two fungicides resulted in lower values in AUDPC than those in 2015 season, where tricyclazole and isoprothiolane showed 81.87 and 143.69, respectively, as compared to untreated rice plants as it was 424.38 (Table 2).

Regarding the rate of leaf blast disease development which expressed by r-value, tricyclazole and isoprothiolane application were exhibited lower r-value estimates in 2015 and 2016 seasons (Table 2) as compared to untreated rice plants. Tricyclazole and isoprothiolane treatments had the capacity to delay and slow down a blast disease increase in the pre-treated rice leaves, which resulted in lower estimates of r-value, expressed on these leaves. As, they were 0.04 and 0.06 in 2015 season and 0.01 and 0.03 in 2016 season, respectively. In contrast, r-value was higher with untreated rice plants (0.41 and 0.63) in both seasons. As indicated in the same Table 2 the obtained results revealed that both fungicides produced lower panicle blast severity than untreated rice plants, which it was (0.525 and 1.90) in 2015 season, and (0.451 and 1.570) in 2016 season with tricyclazole and isoprothiolane treatments, respectively. Meanwhile, the panicle disease severity reached to 5.00 and 4.56 in the control (untreated rice plants) during 2015 and 2016 seasons, respectively (Table 2). The efficiency of tricyclazole and isoprothiolane to control panicle blast disease were 89.30 and 62.00, respectively in 2015, and 90.13 and 65.11 in 2016 season, respectively (Table 2).

Table 2. Effect of fungicides on epidemiological parameters for leaf and panicle blast disease under natural infection in Sakha 101 rice cultivar in 2015 and 2016 seasons.

Treatment	season	Leaf Disease Severity at 80 DPT*	Efficacy %	AUDPC at 80 DPT*	r-Value	Panicle Disease Severity	Efficacy %
Control	2015	65.00a	-	459.31a	0.41a	5.00a	-
Tricyclazole		10.00c	84.61	102.51c	0.04c	0.52c	89.30
Isoprothiolane		19.00b	70.76	170.62b	0.06b	1.90b	62.00
F test		**		**		**	-
Control	2016	58.10a	-	424.38a	0.36a	4.56c	-
Tricyclazole		6.10c	89.50	81.87c	0.01c	0.45a	90.13
Isoprothiolane		12.33b	78.77	143.69b	0.03b	1.57b	65.11
F test		**		**	**	**	

DPT, days post-transplanting. AUDPC, area under disease progress curve. r-Value, rate of leaf blast increase.

Effect of tricyclazole and isoprothiolane fungicides on yield and yield components:

Observations on yield characters *viz.* plant height, tiller numbers, panicle length, 1000-grain weight and grain yield responded significantly following chemical treatments compared to non-treated plants as recorded in Table 3. Plant height was significantly higher for tricylazole treated plants in 2015 and 2016 seasons with values of 94.90 and 94.12 cm respectively, followed by isoprothiolane 91.10 and 90.89 cm, respectively. Different treatments had no significant effect on the number of tillers/hill and panicles/hill. However, panicle length significantly increased with tricyclazol application 23.50 and 24.14 cm, followed by isoprothiolane 22.01 and 23.01cm in 2015 and 2016 seasons, respectively as compared to untreated plants (21.20 and 22.00 cm) in both seasons respectively.

Application of both fungicides on 1000-grain weight, No. of filled grain/panicle, No. of unfilled grain/panicle and grain yield showed significant variations (Table 3). Tricyclazole application was highly effective in increasing 1000-grain weight, No. of filled grain/panicle and grain yield (29 g, 129 g and 4.77 t/fed), followed by isoprothiolane (27.66 g, 118.66 g and 4.39 t/fed) comparing to the untreated plants (25.67 g, 98.00 g and 3.76 t/fed) during 2015 season. The same trend appeared in the 2016 season. Thus, tricyclazole and isoprothiolane treatments provided an effective rice blast control in both seasons. Consequently, yield increases percentage was significantly higher when tricyclazole was applied from 26.86 and 24 in 2015 and 2016, respectively compared to isoprothiolane.

Effect of fungicides on rice grain quality:

Tricyclazole and isoprothiolane treatments significantly led to enhancement of rice grain-milling quality during both seasons. Tricyclazole treatment showed high hulling %, milling %, head rice % and gelatinization temperature (GT) % (79.79, 69.275, 65.790 and 6 %, respectively) in 2015 and (79.04, 69.495, 66.405 and 6 %, respectively) 2016 seasons as shown in Table 4. Rice grain hulling %, milling %, and head rice % were reduced with untreated rice plants (infected plants) in both seasons.

Table 3. Effect of tricyclazole and isoprothiolane on yield and yield component for Sakha 101 rice cultivar under field conditions in 2015 and 2016 seasons

Treatment	seasons	plant height /cm	No. of tillers/ hill	No. of panicles/ hill	Panicle length/ cm	Panicle weight/ g	1000-grain weight/g	No. of filled grain /panicle	No. of unfilled grain /panicle	Grain yield (t/fed.)	Yield increase (%)
Control	2015	90.40b	30.67	30.19	21.20c	3.203b	25.67b	98.00b	91.33a	3.76c	-
Tricyclazole		94.90a	30.70	30.33	23.50a	4.027a	29.00a	129.00a	69.66b	4.77a	26.86
Isoprothiolane		91.10b	30.36	30.00	22.01b	3.473b	27.66b	118.66a	85.66ab	4.39b	16.75
F. test		*	NS	NS	**	*	*	*	*	**	
Control	2016	91.02b	29.10	28.79	22.00c	3.39c	26.21b	99.78b	90.45a	3.86c	-
Tricyclazole		94.12a	29.51	29.00	24.14a	4.20a	29.60a	130.12a	67.87b	4.82a	24.00
Isoprothiolane		90.89b	29.20	28.81	23.01b	3.91b	28.01b	123.58a	79.95b	4.41b	13.00
F. test		*	NS	NS	**	**	*	*	*	**	

Table 4. Effect of blast fungicides on rice grain-milling quality for Sakha 101 rice cultivar under field conditions

Treatments	Hulling (%)		Milling (%)		Head rice (%)		Amylose (%)		G.T *(%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	65.67c	64.72c	59.220c	54.660c	55.720c	53.005c	21.975a	22.070a	5b	5b
Tricyclazole	79.79a	79.04a	69.275a	69.495a	65.790a	66.405a	21.160b	21.035b	6a	6a
Isoprothiolane	78.15b	78.05b	68.435b	68.00b	62.095b	63.065b	21.125c	21.670b	5b	5b
F. test	**	**	**	**	**	**	**	*	*	*

*** Gelatinization Temperature**

Effect of fungicides on rice grain chemical components:

The near-infrared (NIR) spectrum was used to determine the chemical composition of treated rice grain after milling processes (Table 5). Application of tricyclazole enhanced the carbohydrate (82.75%), fat (0.66%), protein content (7.08%) and ash (0.99%). However, the application of isoprothiolane led to increase the concentration of carbohydrate (84.22%) and fiber (5.35%) in milled rice compared to untreated rice grain (79.80 and 5.13%, respectively). On the other hand, isoprothiolane reduced fat (0.27%), protein (6.61%) and ash (1.03%) comparing to the untreated rice plants. Meanwhile, the carbohydrate concentration for untreated grain (infected plants) reduced while fat and ash percentage increased. The protein content and fiber seem to be not affected by the leaf blast infection. There is a negative correlation between rice carbohydrate and protein content as the increase in rice carbohydrate is followed with decrease in protein content.

Table 5. Chemical component as a percentage of treated-rice grain using NIRS

Treatments	Moisture (%)	Carbohydrate (%)	Fat (%)	Protein (%)	Fibbers (%)	Ash (%)
Control	14.02a	79.80c	0.99a	6.94b	5.13b	1.25a
Tricyclazole	13.54b	82.75b	0.66b	7.08a	4.47c	0.99b
Isoprothiolane	13.45b	84.22a	0.27c	6.61c	5.35a	1.03b
F. test	*	**	**	**	**	*

Correlation analysis for blast disease parameters, yield components, grain yield, and grain quality:

Correlation coefficients between some blast disease parameters, grain yield, yield components and grain quality traits presented in Table 6. The analysis showed that leaf disease severity was positively correlated with some parameters as; severity of panicle blast (0.987*), AUDPC (0.987**) and unfilled grain (UFG) (0.852**), whereas, there were negative correlations with panicle length (-0.846*), panicle weight (-0.880*) and 1000-grain weight (-0.948**). Severity of panicle blast was positively correlated with UFG (0.916*), while it was negatively correlated with panicle weight (-0.913*) and 1000-grain weight (-0.979*). The grain quality traits were highly and negatively correlated with severity of leaf and panicle disease severity. AUDPC was negatively correlated with all tested traits. These results are in agreement with Torres and Teng (1993) indicated that both leaf and panicle blast disease severity had a significant negative effect on plant height compared to measured plant parameters. Panicle blast had more significant influence than leaf blast in increasing the percentage of unfilled grain that reduces grain yield. Both leaf blast and panicle blast had significant effects on yield. High leaf blast severity is almost followed by high panicle blast incidence; however, even when leaf blast severity was low, high panicle blast incidence could still be obtained, provided that favourable conditions for blast infection were considered with the susceptible cultivars.

Regression analysis of blast effects on rice yield losses:

The data of 2015 and 2016 seasons (Fig. 2) showed that the yield grain loss caused by leaf blast disease occurring in the field could be estimated by the equation ($Y = 4.8 + 0.02x$, R^2 linear= 0.921) in which X = percentage leaf blast disease severity at 80 DPT. The equation suggests that 1% leaf blast disease severity causes ~0.02% yield loss. Leaf blast severity level was negatively correlated with grain yield. On other hand, positive correlation was found between both application of fungicides triycyclzol and isoprothiolane and grain yield. Negative correlation between control (untreated plants) leaf disease severity were recorded as in Figure 2.

Table 6. Correlation matrix between rice leaf and panicle blast disease severity, rice grain yield and its components and grain quality of Sakha 101 cultivar

	LDS	PDS	GY	AUDPC	PL	PW	TGW	FG	UFG	Milling	HR
LDS	1.00	0.987**	-0.960**	0.987**	-0.846*	-0.880*	-0.948**	-0.989**	0.852*	-0.944**	-0.957**
PDS		1.00	-0.991**	0.068	-0.888*	-0.913*	-0.979**	-0.998**	0.916*	-0.933**	-0.974**
GY			1.00	-0.992**	0.913*	0.925**	0.993**	0.985**	-0.951**	0.903*	0.967**
AUDPC				1.00	-0.889*	-0.911*	-0.982**	-0.997**	0.915*	-0.937**	-0.976**
PL					1.00	0.991**	0.951**	0.892*	-0.961**	0.702	0.835*
PW						1.00	0.956**	0.923**	-0.965**	0.760	0.876*
TGW							1.00	0.976**	-0.964**	0.868*	0.949**
FG								1.00	-0.917*	0.939**	0.979**
UFG									1.00	-0.783	-0.906*
Milling										1.00	0.973**
HR											1.00

****. Correlation is significant at the 0.01 level (2-tailed),* Correlation is significant at the 0.05 level (2-tailed).**
LDS; Leaf Disease Severity. PDS; Panicle Disease severity.GY; Grain Yield. AUDPC; Area Under Disease Progress Curve. PL; Panicle Length. Panicles Weight TGW; 1000 Grain Weight. FG; Filled Grain. UFG; Un-Filled Grain.HR; Head Rice.

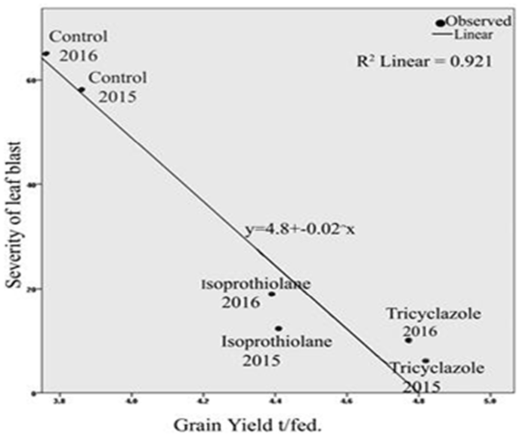


Figure 2. Relationship between leaf blast rating at 80 days post-transplanting and rice grain yield.

The residue of tricyclazole and isoprothiolane in straw, husk, and milled rice:

A modified QuEChERS method combined with HPLC-MS/MS detection for the analysis of fungicides residues in rice was used. The limit of quantitation (LOQ) was set to be the lowest fortification level at which acceptable recovery data were obtained as the European Committee. The tricyclazole and isoprothiolane residues in different samples after the harvest are listed in Table 7. The data showed that tricyclazole residues were detected in straw, husk, and milled rice at 30 and 60 DPH during 2015 season. The order of residues found in the sample matrices treated with tricyclazole is: straw > hull > milled rice, where the concentrations of this fungicide were 1.06, 0.19 and 0.14 mg/kg, respectively. Tricyclazole residues in milled rice, hull, and straw were also related to the storage period after post-harvest where the concentration was reduced and ranged from 0.40, 0.06 and 0.01 mg/kg, respectively at 60 DPH. The tricyclazole residue patterns in milled rice appear to be high among two storage periods 30 and 60 days where the concentration was 0.14 and 0.01 mg/kg, respectively. On the other hand, the residue pattern in the straw is consistent with those in the hull. It is worth noting that the residues in straw appear to be highest among two storage periods where the residues were 1.06 and 0.40 mg/kg, respectively. In 2016 season, tricyclazole was only detected in milled rice 60 DPH. The tricyclazole concentration was less than the LOQ. Concerning the residue of isoprothiolane in 2016 season, the concentration was detected only with treated-milled rice after only one storage period 60 day. The concentration of isoprothiolane was 0.58 mg/kg. The residue of both fungicides was not detected in untreated samples (straw, husk, and milled rice) in 2015 or 2016 seasons.

Other pesticide residues were detected during the using QuEChERS method combined with HPLC–MS/MS for extraction and analysis of the rice samples without the application of those pesticides during the growing seasons. The detected compounds (Sulfur, Malathion, and Chloropyrifos) were found in treated and untreated samples.

Table 7. Pesticide residues of tricyclazole and isoprothiolane as mg/kg in Sakha 101 rice cultivar at 30 and 60 days post-harvest in 2015 and 2016

Detected compound (mg/kg) in rice samples	2015 season						Detected compound at 60DPH 2016 season in milled rice
	30 DPH*			60 DPH			
	straw	husk	milled rice	straw	husk	milled rice	
1-Control							
Tricyclazole	n.d.	n.d	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	n.d.	n.d	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfur	0.57	0.41	0.38	0.16	0.12	0.11	n.d.
Chloropyrifos	0.01	n.d	<LOQ	<LOQ	<LOQ	<LOQ	n.d.
2- Tricyclazole							
Tricyclazole	1.06	0.19	0.14	0.40	0.06	0.01	<LOQ
Sulfur	0.67	0.35	0.27	0.08	<LOQ	<LOQ	n.d.
Chloropyrifos	0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.
3- Isoprothiolane							
Isoprothiolane	**	**	**	**	**	**	0.58

***days post-harvest, **not tested, n.d. = not detected**

DISCUSSION

Control of rice blast still relies on fungicide applications, especially with susceptible cultivars. Tricyclazole and isoprothiolane achieved protection against rice blast disease based on the used three parameters (disease severity, AUDPC and r-value) at both growing seasons. The efficiency of tricyclozaole (table 2) to control rice leaves /panicles blast disease was better than the efficiency of isoprothiolane. Similar results have been reported by several researchers. Osman *et al*., (2004), Chen *et al*., (2013), Iqbal *et al*., (2014) and Pandey (2016), they reported that tricyclozole was most effective in decreased leaf blast severity as a foliar application on different susceptible rice cultivars. The reduction of leaf blast severity may be attributed to the reduction of secondary infection, consequently, this led to less production and lower virulence capacity of conidia produced on fungicide-treated lesions in the field, as mentioned by (Kurahashi, 2001 and Zhang and Zhou, 2004). In addition, Padovani *et al*., (2006) and Jeong *et al*., (2012) returned the long-term protection during the whole growth process for the reason that the stability and accumulation of tricyclozaole and isoprothiolane till 11 months post application in water-soil systems. Therefore, two applications of tricyclazole and three applications of isoprothiolane provide rice plants with long-term protection till the reproductive stage. Similar results were obtained by Prabhu *et al*., (2003) they mentioned that two applications of tricyclazole significantly reduced AUDPC value. Hence, the lower estimated AUDPC values in resistant genotypes indicate the slower and restricted progress of leaf blast development. Values of AUDPC increases with the increase in degree of leaf blast severity in susceptible genotypes (Gupta *et al*., 2016).

Our observations on the studied yield characters (plant height, tiller numbers, number of panicles and panicle length) responded significantly following fungicides treatments compared to non-treated plants (Table 4). These findings are in line with those of Iqbal *et al*., (2014) they studied the efficacy of different fungicidal sprays *viz.,* carbendazim, mancozeb and tricyclazole at three growth stages *viz.,* 50 % flowering, milk/dough stage and physiological maturity for rice blast disease control. They observed that tricyclazole significantly increased the number of tillers/hill and no. of panicles/hill as well as the number of filled grain/panicle in comparison to different treatments.

Rice grain quality is a great importance character for all people involved in producing, processing and consuming rice since it affects the nutritional and commercial value of grain. In this study, grain quality traits were improved by tricyclazole and isoprothiolane treatments. On the other hand, the negative effects of blast disease on grain quality seemed to exist. However, the effect of the disease on grain quality could be indirect through the influence of various physiological parameters during the grain filling process. In Egyptian rice genotypes, the hulling percentage ranged from 78.04 to 81.48 % while milling percentage ranged from 62.70 to 69.10 % and head rice percentage ranged from 51.70 to 62.20 according to Nessreen *et al*. (2014). In contrast with our results, Koutroubas *et al*., (2009) reported that grain quality traits were less affected by the rice blast disease infection.

Our results showed the grain chemical composition contents for moisture, protein, lipids, ash, crude fibers and carbohydrate measured using NIRS system. There were significant differences among chemical composition due to the different fungicides applications and untreated grain (infected plants). High accuracy of calibration and prediction models for different chemical components was ensured between chemical composition contents reference and predicted values for NIRS as mentioned by Xie *et al*., (2014), Bagchi *et al*., (2016), Sampaio *et al*., (2017) and Siriphollakul *et al*., (2017). Therefore, effective utilization of NIRS in high throughput screening of rice samples for their proximate composition content in a nondestructive way. NIRS has the advantage of being fast, economical, and environmentally safe, and allows for simultaneous determination of several traits with one scan.

In the Egyptian rice cultivars, the protein content ranges from 6.2 % to 8.3 %, lipids from 0.2 to 0.5 % in milled rice, crude fiber content ranges from 0.20 to 0.65 %. Nessreen *et al*., (2014) determined the chemical composition of the three genotypes Sakha 104, Egyptian hybrid1 and Giza 182 according to the method described by Association of Official Analytical Chemists official methods of analysis 1990, they recorded that the moisture content of milled rice grain of Egyptian rice varieties ranged between 14.10 % and 14.30 %. While total carbohydrate ranged from 90.65 to 91.30. The protein content of milled rice grain for Egyptian rice varieties ranged from 6.40 to 7.55. Total lipids content of milled rice grain ranged from 0.30 % to 0.75 %.

Rice blast may cause significant grain yield reduction without fungicide treatment which can result in 40 % grain yield losses when the disease severely occurs in susceptible cultivars (Groth, 2006). The present data suggested that two applications of tricyclazole or three applications of isoprothiolane are sustainable and more efficient in increasing grain yield of the blast susceptible Sakah 101 cultivar. Prabhu *et al*., (2003) and Chen *et al*., (2013) showed that the potentiality of tricyclazole in controlling leaf blast to increase rice yield characters and yield.

Linear regression of the data gave almost horizontal lines with low correlation coefficients ($Y = 0.02x + 4.8$, R^2 linear= 0.921) (Fig. 2). Similar behavior of yield grain loss was also funded by Koutroubas *et al*., (2009) who revealed that both leaf and neck blast were negatively correlated with grain yield. It was estimated that each unit increase in leaf blast rating and neck blast resulted in 5.97 and 0.23 g/plant as yield reduction, respectively.

Pesticides are highly regulated in most countries because of their potential impact on the environment and the health of the agricultural operator, and maximum residue levels are defined for food and feed to protect the health of the consumer (Nader *et al*., 2014). Differences in MRLs may cause serious disruptions to trade. In 2010, for instance, American and European laboratories detected the fungicide tricyclazole and isoprothiolane in various samples of Indian Pusa Basmati rice using QuEChERS method. Their results revealed that most Indian Basmati rice contained isoprothiolane above the EU default MRL of 0.01 mg/kg. This triggered recalls at the retail level and 2 rapid alerts in 2011. Indian Pusa Basmati was cut from the USA and EU markets. The damage caused to Indian rice exporters was serious. Meanwhile, isoprothiolane is approved for agricultural applications in most Asian and Latin American countries, but not in the EU or the USA. While the Japanese MRL is 10 mg/kg for brown rice, the EU MRL was set at 0.01 mg/kg before July 2012. The European Food Safety Association decided to increase the limit of MRL at 5 mg/kg (EFSA, 2012). In contrast, the USA began to block Indian Basmati rice as from 2011 because the fungicide tricyclazole was found. The fungicide is not approved for rice in the USA and consequently, zero tolerance with a MRL at the analytical limit of quantification of 0.01 mg/kg applies. In contrast, the MRL in the EU is at 1 mg/kg in brown rice.

The concentration or reduction of pesticides due to the milling process steps have to be taken into consideration under EU legislation (Regulation EC, 2005). Where, Isoprothiolane concentrations were reduced in a range between 31 and 82 % due to milling processes. Therefore, the fungicide is concentrated in the bran. For example, when brown rice with an isoprothiolane concentration of 0.071 mg/kg was milled, the pesticide was reduced to 0.028 mg/kg in the milled rice but concentrated to 0.32 mg/kg in the bran. This concentration effect has to be taken into consideration if the bran goes on to be used for feed or as a food additive (Regulation EC, 2005). Similar results were obtained with our experiments where the order of residues found in the sample matrices treated with tricyclazole is reduced from straw to hull to milled rice, where the concentrations of this fungicide were 1.06, 0.19 and 0.14 mg/kg, respectively at 30 DPH. Tricyclazole residues in milled rice, hull, and straw were also related to the storage period after post-harvest where the concentration was reduced and ranged from 0.40, 0.06 and 0.01 mg/kg, respectively at 60 DPH. The residue of tricyclazole in milled rice was considerably lower than the MRL (1 mg/kg in brown rice) according to the European Food Safety Authority (2013). Although, in 2008, tricyclazole has not been included in Annex I to Council Directive 91/414/EEC due to health concerns and toxicity, and hence the withdrawal of authorizations for plant protection products containing that substance is needed (EC-European Commission, 2008). Major adverse effects of tricyclazole observed were: decreased body weight gain and increased organ weights and others in the liver of mice (Food Safety Commission of Japan, 2014). In addition, Fattahi *et al*., (2015) there result indicated that tricyclazole toxin can impair testosterone secretion and the testicular structure, leaving an adversely effect on sperm production system.

The analysis residue of isoprothiolane was 0.58 mg/kg in milled rice at 60 days post-harvest which is considered lower than the presented MRL at 5 mg/kg in brown rice according to European Food Safety Authority (2012). In an agreement with our results, Bandyopadhyay *et al*., (2015) reported that tricyclazole residue in rice grain, husk, and soil was found at below detectable limit (0.02 mg/kg) in all the treated samples. Zhang *et al*., (2016) found that the concentrations of isoprothiolane in brown rice samples collected 7 days after the application was 0.844 and 0.644 mg/kg in 2011 and 2012 season, respectively. The residue of isoprothiolane was below the MRLs 1.0 mg/kg as set by Chinese authorities (Ministry of Agriculture). Therefore, they considered that the interval of 7 days between the application of the recommended dosage of the formulation and harvest was safe under Chinese conditions.

Concerning our status, further studies need to reduce the dosage of isoprothiolane below to 2 ml/L as a recommended dose under the Egyptian climate parameters, which could not be considered safe to human beings according to European Food Safety Authority. In Egypt, we don’t have our own MRL but we follow the EU MRL. Although, in Egypt, the average of rice Per capita consumption was 24.9 kg/person/year comparing for Europe (average 5.2 kg/person/year) according to OECD-FAO, (2017), which is much higher. Subsequently, it should determine our Egyptian MRL according to our consumption. Further, toxic pesticides with low MRLs should be avoided: this would also be beneficial for the health of the farmers, consumers and the environment.

Regarding the non-detection or below the LOQ of fungicide residues in rice materials, this may be related to the degradation and/or metabolism of the fungicide molecules by the plant (Sundravadana *et al*., 2007 and Phong *et al*., 2009). These authors attributed non-detected or below the LOQ to the fragmentation of the original molecular structure. Thus, their results highlight the need for more studies to identify and detect metabolites in rice grain. Hence, triazole group fungicides such as propiconazole, difenoconazole, tebuconazole and tricyclazole are metabolized within the plant and may undergo oxidation, reduction, hydrolysis, or the formation of other compounds, or even total degradation into simpler compounds.

CONCLUSIONS

Tricyclazole 75% WP at 0.5 g/L. was found to be most effective in controlling blast disease of paddy rice. Tricyclazole provided better protection than Isoprothiolane. This treatment improved rice grain yield and grain quality, in addition, enhanced the chemical composition as carbohydrate. The residue of tricyclazole was found lower than the MRL 1 mg/ kg in 2015 season and below LOQ in 2016, therefore, tricyclazole should rather be used preventively than curatively for effective rice blast control. These results could be utilized for the assessment of the environmental risk associated with the use of those pesticides in rice cultivation. Nevertheless, the NIR technique can be used to identify the effect of treatments on rice grain chemical components especially carbohydrates, fat and proteins. A modified QuEChERS method combined with HPLC-MS/MS detection can be effectively applied to routine analysis of residues for target pesticides in rice. Therefore, tricyclazole should be used for rice blast control and with low fungicidal residue analysis, as an important component of food safety.

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رصد الاثر المتبقي للمبيدين الفطريين ترايسكلازول و إيزوبروثيولان وآثارهما على مرض اللفحه , المحصول ومكوناته, جوده الحبوب والمكونات الكيميائيه للحبوب في الارز

رباب ممدوح أحمد العماوي[1]، فاطمه عبد المطلب مصطفي[2] و ربيع عبد الفتاح سعد الشافعي[3]

[1] قسم بحوث أمراض الأرز - معهد بحوث أمراض النباتات - مركز البحوث الزراعية –سخا- كفر الشيخ- مصر

[2] بحوث المكافحة المتكاملة - معهد بحوث أمراض النباتات - مركز البحوث الزراعية – جيزه - مصر

[3] مركز البحوث و التدريب في الأرز- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية- سخا- كفر الشيخ- مصر

تستخدم المبيدات الفطرية الجهازية (tricyclazole (Beam 75% و isoprothiolane (Fuji-one 40% EC)علي مطاق واسع في مكافحه مرض لفحه الارز في مصر. اجريت تجربه حقليه بالمزرعة البحثية لمركز البحوث و التدريب بالارز- سخا- كفر الشيخ خلال موسمي 2015 و 2016 – علي الصنف الحساس سخا 101. تم تقييم تأثير كلا من tricyclazole (0.5جم/لتر) و isoprothiolane (2 مل/لتر) على الاصابه بمرض اللفحه، المحصول ومكوناته, جوده الحبوب والمكونات الكيميائية للحبوب. بالاضافه الي تحليل المتبقيات في قش الارز, السرسه , والارز الابيض باستخدام طريقه QuEChERS مع التحليل الكروموتوجرافي HPLC/MS/MS بعد 30 و 60 يوم من الحصاد. أشارت النتائج إلى أن tricyclazole وisoprothiolane حققت فاعليه مرتفعة في مكافحه مرض لفحه أوراق في الأرز بناء على استخدم القياسات المرضية حيث خفضت شدة الإصابة ،والمساحة تحت منحني تطور المرض (AUDPC) ومعدل تطور المرض (r-value) في موسمي 2015 و 2016. اوضحت دراسة الاثر المتبقي ان متبقيات مبيد tricyclazole في قش الأرز (1.06 مجم/كجم) كانت أعلى عنها في حبوب الأرز, حيث كان المتبقيات منه في السرسه و حبة الأرز (0.19 و 0.14 مجم/كجم علي التوالي) أقل بكثير من الحد المسموح به (<LOQ) عند 0.001 مجم/ كجم بعد30 من الحصاد. تم الكشف عن متبقيات isoprothiolane في حبوب الأرز ووجدت انها أعلى من LOQ عند 0.58 مجم/كجم بعد 60 يومًا بعد الحصاد. باستخدام كلا المبيدين زادت معظم صفات المحصول ومكوناته بشكل معنوي مقارنه بغير المعامل. اظهرت المعامله بكلا المبيدين تحسين معنوي في صفات جودة الحبوب مثل نسبة التقشير والتبيض وتصافي الحبوب نسبة الي غير المعامل. حسنت المعاملة بمبيد tricyclazole وisoprothiolane المحتوي الكيميائي للحبوب مثل الكربوهيدرات والبروتين والدهون والرماد والألياف .وأخيرا ، تشير هذه الدراسة الي أن tricyclazole وisoprothiolane تعتبر مبيدات فطريه فعاله في مكافحه مرض لفحه الأرز و تعتبر آمنه طبقا للمواصفات الأوربية لسلامه الاغذيه. وبناء علي نتائج تحليل المتبقيات و الاستهلاك السنوي لحبوب الارز للفرد ,نقترح تحديد الحد الأدنى المسموح به الخاص بالظروف المصرية. حيث ان المجموع الكلي للاستهلاك وصل الي 4 مليون طن/سنه ويمثل هذا 4 اضعاف الاستهلاك الاوروبي (واحد مليون طن /سنه).