



Residue and bioefficacy of propiconazole against stripe rust on wheat (*Triticum aestivum*) in Haryana region

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ABSTRACT

The experiment was conducted during 2019 to 2021 at Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana to evaluate the effectiveness of six different fungicides against stripe rust on wheat (*Triticum aestivum* L.) crop. Propiconazole is recommended for spray against stripe rust, therefore, its residues were analyzed at six different growth stages (Tillering; Stem elongation; Booting; Awn emergence, i.e. 50% ear head emergence; Flowering (complete anthesis) and Milk development stage in wheat and straw using GC-MSMS after its application. The experiment was laid out in a randomized block design (RBD) in triplicate plot size of 2.5 m × 2 m. Studies showed that, propiconazole 25% EC (Tilt) was found to be economically best for controlling stripe rust as well as enhancing the grain yield among tested chemicals. The residuals were less than the quantitation limit (0.01 mg/kg) at tillering, stem elongation and booting stage. However, residues were above maximum residual limit (0.05 mg/kg) as well as quantitation limit in grains at awn, milk development and dough stage. Thus, from the current study it can be concluded that use of propiconazole is safe only when good agricultural practice is adopted and sprayed before ear head emergence stage.

Keywords: Dissipation, Efficacy, Fungicides, GC-MSMS, QuEChERS, Yellow rust

Wheat (*Triticum aestivum* L.) is grown across the world and considered as a major staple cereal crop, it provides near about 71% carbohydrates and daily needed vital nutrients to approximately 85% world's total populace (Zargar *et al.* 2017, Shewry and Hey 2015), with a yield of roughly 763.2 million tons, around 219 million hectares of land are used to cultivate wheat worldwide. India holds second position after China in the production of wheat (Bhardwaj *et al.* 2019). Contribution of nation's total land together account for 60% by the three states that produce the most wheat are Uttar Pradesh, Punjab, and Haryana. There are number of components which reduces the production of wheat, viz. change in climate, infection caused by rusting (Singh *et al.* 2016). Stripe or yellow rust is the most commonly occurring disease in wheat due to the bio tropic fungus *Puccinia striiformis* (Pst) (Chen 2014). Urediniospores of stripe rust are yellowish to slightly orange in color grouped in a linear pattern along the veins, generating pustules on susceptible leaves, leaf sheaths, glumes and awns when a long winter, moist and cold spring season is available. If the

weather is congenial for stripe rust, it can infect plants from seedling to maturity. Many countries have experienced yield losses of millions of tonnes in a single crop season (Chen *et al.* 2014). A 5% yield loss owing to rusts might result in a loss of ₹39,200 million in 2007, while in north India, yellow rust might cause a 25% yield loss, which could cost ₹10,000 million. (Bhardwaj *et al.* 2016). When the genetic resistance is ineffective, an emergent strategy for managing wheat rusts through fungicides becomes useful. But due to rigorous use of these fungicides, residues remain there at the time of harvest in grains. Presence these residues is a big reason of rejections or bans on our food products in international markets. Therefore, it's necessary to monitor the presence of these residues from consumers health as well as from export point of view. Thus, the current research was conducted to find the best fungicide as an alternative for curing the stripe rust, as well as to measure residual toxicity in wheat grain and straw at various phenological growth stages.

MATERIALS AND METHODS

The experiment was conducted during 2019 to 2021 at Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana to evaluate the effectiveness of six different fungicides against stripe rust on wheat (*Triticum aestivum* L.) crop.

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In vitro study of different fungicides against Puccinia striiformis f. sp. tritici (Pst): Six fungicides namely propiconazole 25% EC (Tilt), mancozeb 75% WP (Dithane M-45), zineb 75% WP (Dithane Z-78), tebuconazole 430% SC (Folicur), trifloxystrobin 25% WG + tebuconazole 50% WG (Nativo) and azoxystrobin 11% + tebuconazole 18.3% SC (Custodia) were appraised against stripe rust *in vitro* conditions by applying poison food technique on double strength water agar medium using different concentrations (5, 10, 20, 50, 100, 250, 500 and 1000 ppm), prepared from required quantity (formulation basis) of fungicide. Double strength agar medium was prepared in water and sterilized in autoclave for 20 min at a pressure of 15 psi. To get the required concentration of each fungicide, stock solution of respective fungicide was mixed with sterilized agar medium thereafter the medium was poured into petri-plates under aseptic conditions for solidification. Then we have sprinkled the fresh urediniospores of Pst with a painting brush on solidified medium and put in the BOD (biochemical oxygen demand) incubator at a temperature of $12 \pm 1^\circ\text{C}$. For higher precision each individual treatment was repeated thrice. For appropriate check, fresh urediniospores were scattered on agar medium in petri-plates with no fungicide. Data of different parameters were recorded after 12 h of incubation, and per microscopic field, the germinated urediniospores were counted under compound microscope, and Vincent (1947) formula was used to calculate the per cent germination inhibition of urediniospores.

Cavity slide technique: The suspension of urediniospores was prepared 20 per microscopic field harvested from research area during December 2019 to March 2021 at above said concentrations of fungicides. Equal amount of urediniospores suspension and different concentrations of different fungicides were added in equal proportion. The germination of urediniospores was recorded in different treatments of fungicides. A log probit scale was used to plot the percent spore inhibition of Pst against the fungicide concentration in order to identify the fungicidal concentrations (EC₅₀ and EC₉₀) that cause 50% and 90% growth inhibition, respectively.

Bio-efficacy of fungicides against stripe rust under field conditions: The experiment was conducted on susceptible variety WH 147 under field conditions during 2019–2021 for three years continuously. The wheat cv. WH 147 was sown on 15 November 2019; 25 November 2020 and 12 November 2021 in plots size of 4 m × 2.5 m by adopting recommended cultural practices. Each fungicidal treatment was replicated thrice. The suspensions of Pst urediniospores were inoculated artificially on seedlings planted retained in respective plot in December. Fungicides, viz. propiconazole 25% EC, mancozeb 75% WP, zineb 75% WP, tebuconazole 430% SC, trifloxystrobin 25% WG + tebuconazole 50% and azoxystrobin 11% + tebuconazole 18.3% SC were evaluated. First spray of these fungicides was done on disease appearance (10S) at a stage of stem enlargement and second spray at the interval of fifteen days. Terminal disease severity was recorded in the month of March by using modified Cobb

scale (Peterson *et al.* 1948). Final yield/plot computed based on kg/ha. The data were analyzed statistically by using randomized block design (RBD).

Sampling and processing: To estimate the residues of propiconazole in wheat grain and straw at six different growth stages (Zadoks 1974), viz. (i) Tillering; (ii) Stem elongation; (iii) Booting, (iv) Awn emergence i.e. 50% ear head emergence; (v) flowering (complete anthesis); and (vi) milk development stage. The experiment was laid out in a randomized block design (RBD) in triplicate plot size of 2.5 m × 2 m. Propiconazole 25% EC was sprayed @0.1% in the field of wheat at just appearance of stripe rust and repeated at the interval of 15 days of each growth stage. Samples of wheat grain (2 kg) and straw (1 kg) were collected at each growth stage, processed in pesticide residue laboratory using QuEChERS method (Anastassiades 2003) and subjected for residue analysis using GC-MSMS.

Extraction and cleanup of samples

Wheat grain: Samples of wheat grains (2 kg) were crushed in Robot coupe homogenizer to form fine granular powder from the three replicates. Then 10 g of the sample was weighed and taken in microcentrifuge tube of volume 50 ml. To it, 20 ml chilled water and 20 ml acetonitrile (ACN) was added and allowed to stand for 10 min. Homogenized the samples on homogenizer at $14\text{--}15 \times 1000$ rpm until it becomes chalky. Added 2 g of sodium chloride and shook the samples briskly and centrifuged the samples on 3000 rpm for 2 min. Then the upper layer (10 ml) was collected and added 5 g anhydrous sodium sulphate in it. 6 ml aliquot from this is transferred in other centrifuge tube (15 ml) having 0.2 g primary secondary amine (PSA) and 0.6 g anhydrous magnesium sulphate and centrifuged it again for 5 min @3500 rpm. Take out 4 ml aliquot from it and reconstitute in *n*-hexane after being dried on rotovap.

Wheat straw: 5g straw was weighed to which 75 ml ACN was added and then shaken for 1 h 30 min. The sample was filtered with cotton plug and concentrated up to 5 ml. For cleanup, the glass column (length 60 cm and internal diameter 22 mm) was packed with 2 g florisil in between 1g of anhydrous sodium sulphate. The column was first prewetted (10 ml) and then eluted (10 ml) with same hexane:acetone solution having ratio of 9:1 v/v. The eluate was concentrated on rotavap and then reconstituted with *n*-hexane. Residue analysis was performed on gas chromatography MSMS.

Instrumentation: Gas chromatography MSMS was used to quantify the residues of propiconazole. Capillary column of SH-Rxi-5 Sil MS of length 30 m, internal diameter of the column as 0.25 μm and film thickness 0.25 mm. For sensitive analysis, the multiple reactions monitoring (MRM) mode was used. During analysis, programming was done by ramping, in which, the oven temperature of 80°C was kept merely for two minutes with escalating temperature @20°C each minute up to 180°C just for few seconds and further expanded at the rate of 5°C every min up to a temperature of 300°C for two minutes. The temperature of sample injection port was 250°C and detector temperature

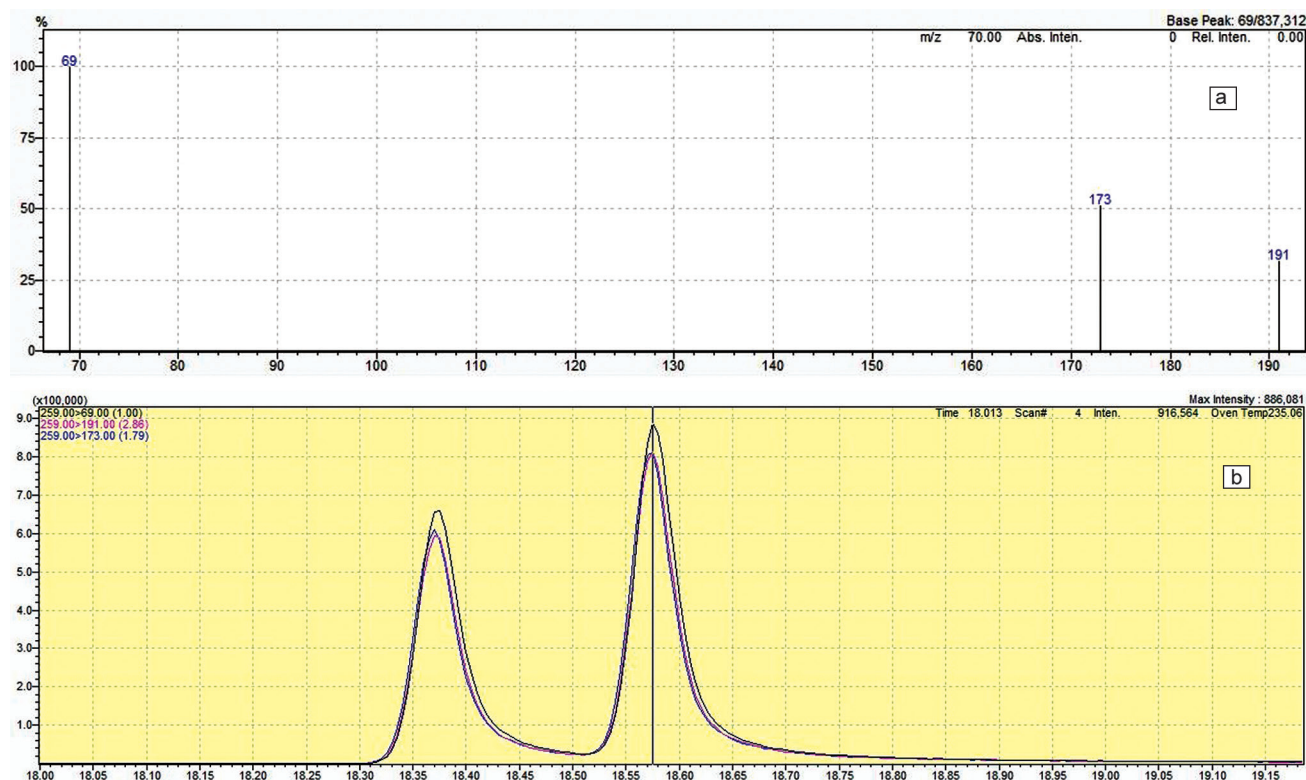


Fig. 1 (a) Mass spectra showing mass to charge (m/z) fragmentation during MRM mode (b) Chromatogram of propiconazole with retention time

was 300°C. Under these conditions, propiconazole produce parent ion of mass 259 which further dissociated into three smaller fragments (m/z value $259 > 191$, 173, 69) and peak was obtained at retention time (R_t) of 18.365 and 18.568 min (Fig. 1a, 1b).

Method validation, calibration, limit of quantification and limit of detection of propiconazole: The analytical method was validated prior to test the analyte according to guidelines of SANTE (2017). Parameters like linearity, repeatability, precision, specificity, reproducibility, ruggedness, limit of detection (LOD) and limit of quantification (LOQ) and recovery of the product were evaluated by validation method as discussed in materials and methods. Results obtained were satisfactory. To check the linearity seven-point calibration curve (0.01–1.00 mg/kg) was prepared by matrix match standards. Efficiency of the method was tested based on recovery rates for the test samples i.e., wheat grains and straw. Test samples were fortified at 0.01, 0.05, 0.1 and 0.25 mg/kg five replicates of each. LOD was considered equivalent to thrice of noise level ($S/N = 3$) and LOQ was tenfold the noise ($S/N = 10$).

RESULTS AND DISCUSSION

In vitro bio-efficacy of fungicides against stripe rust: Fungicides become an important means to manage when severe epidemics of rust occur, however, their usage results in increased production cost for farmers. The present study was conducted to evaluate bioefficacy of fungicides against Pst *in vitro* for its management. The studies revealed that all the fungicides significantly inhibited urediniospores

germination at different concentrations on the basis of check (distilled water). The mean of both the urediniospores germination methods (poison food and cavity slide) has been presented in Table 1. *In vitro* evaluation of eight different fungicides at 5, 10, 20, 50, 100, 250, 500 and 1000 $\mu\text{g/L}$ concentration was carried out against Pst. The data of present study showed that all the fungicides at different doses reduced the germination of urediniospores significantly as compared to control. The data of inhibition of urediniospores germination (Table 1) demonstrated that tebuconazole 430% SC, trifloxystrobin 25% WG +tebuconazole 50% WG, resulted in complete inhibition of urediniospores germination at 5 $\mu\text{g/L}$, whereas, propiconazole 25% EC and azoxystrobin 11% + tebuconazole 18.3% SC inhibited the germination of urediniospores completely at a concentration of 10 $\mu\text{g/L}$. Mancozeb 75% WP and zineb 75% WP were able to inhibit the germination of urediniospores only up to 50 and 80% at 50 and 100 $\mu\text{g/L}$ concentrations, respectively. All the fungicides completely inhibited the germination of Pst at 250 $\mu\text{g/L}$. As per Shabana *et al.* (2017) report fungicide Sumi-8 100% inhibit the germination of urediniospores. Kalappanavar *et al.* (2008) evaluated three fungicides at three doses (0.025%, 0.05% and 0.1%). Propiconazole (95.73%) was most effective against uredospores germination of *Puccinia recondita* f.sp. tritici followed by triadimefon and hexaconazole.

After plotting per cent inhibition of Pst urediniospores against different concentrations of fungicides values of ED_{50} and ED_{90} obtained (Table 1). Values of ED_{50} ranged between 2.5–7.8 ppm a.i. and ED_{90} values ranged between

Table 1 *In vitro* bio-efficacy of fungicides against *P. striiformis* f. sp. *Tritici* with ED₅₀ and ED₉₀ values of various fungicides

Fungicides	Concentration range (ppm a.i.) 5–1000		Concentration (µg/L)							
	ED ₅₀ ppm a.i.	ED ₉₀ ppm a.i.	Urediniospores germination inhibition (%)							
			5	10	20	50	100	250	500	1000
Propiconazole (Tilt 25% EC)	7.8	15.0	80	100	100	100	100	100	100	100
Mancozeb (Dithane 75% WP)	2.6	5.1	10	20	30	50	80	100	100	100
Zineb (Dithane 75% WP)	2.5	4.9	10	25	35	50	80	100	100	100
Trifloxystrobin 25 % WG + tebuconazole 50 % WG (Nativo75 WG)	2.6	5.1	100	100	100	100	100	100	100	100
Tebuconazole 430 % SC (Folicur 430 SC)	2.5	4.9	100	100	100	100	100	100	100	100
Azoxystrobin 11 % SC + tebuconazole 18.3 % SC (Custodia 29.3 SC)	2.5	4.9	95	100	100	100	100	100	100	100
CD (<i>P</i> =0.05)	-	-	12.5	10.8	NS	NS	NS	NS	NS	NS
CV	-	-	15.0	14.09	-	-	-	-	-	-

4.9–15 ppm a.i. Best inhibitory results were shown by tebuconazole 430% SC, azoxystrobin 11% + tebuconazole 18.3% SC and zineb 75% WP with ED₅₀ and ED₉₀ values 2.5 and 4.9 ppm a.i. followed by trifloxystrobin 25% WG + tebuconazole 50% WG and mancozeb 75% WP 2.6 and 5.1 ppm a.i., respectively.

Field evaluation of fungicides against stripe rust: After artificial inoculation of Pst under field conditions, it was evaluated that trifloxystrobin 25% WG + tebuconazole 50% WG showed minimum disease severity of 3.33 % and highest grain production of 75.93% followed by propiconazole 25% EC. The minimum grain yield (10.74%) was recorded on application of mancozeb 75% WP because of the maximum disease severity (56.66%) (Table 2). There was non-significant effect on thousand grains weight, hence not included in the Table 2. The maximum benefit to cost ratio

was shown by Tilt 25% EC (60.73:1) followed by folicur 430% SC (42.97:1). Basandari *et al.* (2020) reported that out of eleven different fungicides tebuconazole 25 EC @ 0.1% was found best in controlling the severity of disease (1.84%) and mean disease control (99.64%) after that Nativo 75 WG @0.05%, Amistar 250 SC, Propiconazole and Amistar Top 325 SC @0.1% were found effective. Bawari *et al.* (2020) concluded that Folicur 250 EW was best in controlling the disease (1.67%) with maximum grain production of 2050 kg/ha followed by Nativo 75 WG and propiconazole 25% EC. Bajoriya *et al.* (2023) applied four different fungicides on two different wheat varieties and concluded that tebuconazole 50% EC was best in reducing the disease severity (1.67%), increased the biological and grain yield ranging from 169–185% followed by propiconazole 25 EC. The findings corroborated those of Devlash *et al.* (2015),

Table 2 Field evaluation of fungicides against stripe rust of wheat during 2019–2021 (pooled data)

Fungicides	Dose (ml/g/ha)	Initial disease severity (%) at time of application of fungicide	Mean terminal disease severity (%)	Mean yield (kg/ha)	Yield grain (%) over check	B:C ratio
Propiconazole 25% EC (Tilt)	500 ml	10	5.00	4790	62.37	60.73
Azoxystrobin 11% + tebuconazole 18.3% SC (Custodia)	500 ml	10	5.00	4180	41.69	15.22
Tebuconazole 430% SC (Folicur)	500 ml	10	5.00	4810	63.05	42.97
Trifloxystrobin 25% WG + tebuconazole 50% (Nativo)	300 g	10	3.33	5190	75.93	32.34
Mancozeb 75% WP (Dithane M-45)	2000 g	10	56.66	3267	10.74	7.32
Zineb 75% WP (Dithane Z-78)	2000 g	10	53.33	3335	13.05	8.34
Check (water)	500 ml	-	100	2950	-	-
CD (<i>P</i> =0.05)	-		8.05	506.84	-	-
CV	-		13.7	6.549	-	-
SEM	-		2.54	162.31	-	-

*mean of three replications

Table 3 Residual toxicity of propiconazole 25% EC (tilt) sprayed @0.1% at different phenological growth stage of wheat against stripe rust during 2019–2021

Crop growth stage	Grain residue (mg/kg)	Straw residue (mg/kg)
Tillering	<LOQ	<LOQ
Stem elongation	<LOQ	<LOQ
Booting	<LOQ	0.122
Awn (50% earhead emergence)	0.126	0.169
Milk development	0.153	0.196
Dough	0.211	0.268
Maximum residual limit		
FSSAI*	0.05	0.2
Codex	0.09	0.2

In grain and straw LOQ = 0.01 mg/kg, *FSSAI = Food Security and Safety Authority of India; Codex, European Union.

who observed that spraying propiconazole 25% EC (Tilt) @0.1% at intervals of 15 days produced maximum disease control of 91.5% and increased grain output (36.7 q/ha). Results are in agreement with Gad *et al.* (2020), Tilt 25 EC shows minimum disease severity (1.67%), disease control (98.07%) and increased grain yield by 29.92%.

Recovery studies: The mean recoveries of propiconazole in fortified wheat grains and straw at different concentrations were found to be consistent and >85%. No recovery factor was used as recovery obtained in both the matrices is good. LOD and LOQ for propiconazole were found to be 0.003 and 0.01 mg/kg using matrix match. The detection limit is all the time kept lower than the level of quantification because co-extractives were associated with matrix, which intruded with the elution of analyte.

Residues of propiconazole 25% EC (Tilt) at different phenological growth stages of wheat: At tillering and stem elongation stage residues of propiconazole were found to be below maximum residual limit (MRL) value (0.05 mg/kg) in samples of grains and straw (Table 3). The MRL value, as per Food Security and Safety Authority of India (FSSAI), in wheat grains and straw was 0.05 and 0.2 mg/kg, respectively. However, at booting stage grain samples were found to be free from residual toxicity but in straw residues (0.122 mg/kg) were detected.

In grains, residues of propiconazole were found above MRL as well as LOQ value at awn (50% earhead emergence), milk development and dough stage with the value of 0.126, 0.153 and 0.211 mg/kg, respectively. In straw, the residues of propiconazole at awn, milk development and dough stage were 0.169, 0.196 and 0.268 mg/kg, respectively.

Gad *et al.* (2020) applied eight different fungicides on wheat crop twice at disease emergence stage and checked residues in grain and straw at harvest time. Concentration was higher in grains as compared to straw and above MRL value. Kumar *et al.* (2018) studied harvest time residues of azoxystrobin, difenconazole, and penicuron in unpolished rice, husk, and soil and reported that residues

were lesser than the LOQ (0.01 mg/kg). Kaur *et al.* (2012) applied mix formulation of trifloxystrobin and tebuconazole (Nativo 75 WG) on wheat crop @ X and 2X g/ha and found that the residues were below the quantification limit i.e. 0.01 mg/kg in wheat grains collected at the time of harvesting. Kundu *et al.* (2011) did a trial to check tebuconazole residues at harvest time in paddy and groundnut. The application rate was 750 and 1500 ml/ha. In each case, the residues were found below the LOQ (0.01 mg/kg).

Trifloxystrobin + tebuconazole and propiconazole was found to be most effective combination in controlling the disease and enhancing the grain yield. During tillering and stem elongation stage in wheat grain and straw, residuals were less than LOQ. However, residues of propiconazole at awn, milk development and dough stage were above LOQ and MRL values. Hence, it can be concluded that the present study will aid to enforce grain production advisory which will help the farm producer and policy/decision makers, in case of foreign exchange, for the export of wheat grains and to avoid rejection of their produce because of chemical residues. Chemicals or fungicides application becomes imperative when disease appear in epidemic form due to the breakdown of resistance in deployed cultivars and emergence of new race or pathotype(s), but care needs to be taken during and after their application.

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