Biological Forum – An International Journal **15(5): 372-376(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In vitro efficacy of fungicides on Uredospore Germination Inhibition of *Puccinia graminis tritici*

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(Received: 01 March 2023; Revised: 15 April 2023; Accepted: 19 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Wheat is one of the important cereals and is highest produced grain in the world after corn and rice. It is the second most important crop in India next to rice. Like several crops, wheat is also prone to diseases. Among various diseases, wheat stem rust is the main biotic constraint to wheat production and it is important to understand and analyse the disease in terms of pathological and biochemical responses. Systemic fungicides belonging to group of triazole, strobilurin and benzimidazoles exhibited effective mean uredospore germination inhibition in the range of 76.74 to 94.83 per cent. Significantly, the high mean inhibition of 95.33 per cent was recorded in propiconazole, followed by hexaconazole with 92.76 per cent germination inhibition. Least uredospore germination inhibition (76.74) was found in carbendazim. Effective inhibition of combined fungicides was ranged from 83.21 to 96.38 per cent. Nearly complete (99.22 %) inhibition of germination of uredospores was exhibited by azoxystrobin + tebuconazole at highest concentration of 500ppm. It was noticed that carbendazim + mancozeb at 100 ppm performed inferiorly compared to other treatments by recording the least uredospore germination inhibition of 79.85 per cent. Evaluation of various fungicides helps in the determination of suitable chemical for management and overcome fungicidal resistance. Emergence of new races also pose threat to current fungicide in use. Hence rigorous evaluation of combination fungicides help in understanding the efficacy against rust pathogen.

Keywords: Wheat, rust, inhibition, urediospores, fungicides.

INTRODUCTION

Wheat is one of the important cereals and is highest produced grain in the world after corn and rice. It is widely consumed as staple food in the world than any other cereal. It is the staple food for an estimated 35% of the world population. These countries contribute about 76% of the total world wheat production. Wheat is the second most important crop in India next to rice. It has been labelled as 'king of cereals'. Diseases are the major threat to wheat production and they are taking heavy toll of the crop in the country and elsewhere in the world. In the United States, stem rust disease is the most important wheat disease, and it is known to cause yield loss by affecting both quantity and quality of the plant (El-Gamal *et al.,* 2022). Among various diseases, wheat rusts are the most important biotic constraints to wheat production. The rusts are the worldwide distributed diseases of wheat. There are three rust diseases of wheat: stem (or black) rust, leaf (or brown) rust and stripe (or yellow) rust, caused by the pathogens *Puccinia graminis* f.sp. *tritici*, *P. triticina* and *P. striiformis* f. sp. *tritici*, respectively. Among all three rusts, black or stem rust caused by *P. graminis tritici* is potentially most dreadful when the variety is susceptible and conditions are favourable for their development. It is known as 'killer' disease of wheat because it kills wheat plant and this disease is responsible for economic loss in many countries including India. Application of fungicide has helped to control rust diseases loss to some extent.

MATERIAL AND METHODS

The stem rust infected leaves/stem of wheat were collected from the field. From these infected samples, the uredospores were collected and added in sterilized distilled water. The stock solutions of various fungicides with different level of concentration were prepared and then diluted the stock solution with distilled water to obtain desired concentration. Spore germination inhibition technique was employed to study the efficacy of different fungicides under *in vitro* condition (Table 1). Double strength than required concentration i.e., 50, 100 and 250 ppm for systemic and 100, 250 and 500 ppm for combination fungicides were obtained by dilution technique in sterilized distilled water. One drop of each fungicide suspension and of uredospore suspension was placed on a glass cavity slide, so concentration will be obtained as required for evaluation. The slides were placed in petri plates lined with moistened coarse filter paper to provide sufficient humidity for germination of uredospore (Plate I and II) for 6 hours at room temperature (25+1ºC). After 6 hours, observation of spore germination inhibition was recorded under light

microscope at 10X objective lens. Per cent inhibition of spore germination in each treatment was calculated by using following formula (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent spore germination inhibition
C = Number of spores germinated in control
T = Number of spores germinated in treatment

Table 1: List of fungicides evaluated for germination inhibition of uredospore *in vitro*.

Sr. No.	Name of fungicide	Concentration (ppm)		
Systemic fungicides				
1.	Propiconazole 25 EC	50	100	250
2.	Tebuconazole 50 WG	50	100	250
3.	Hexaconazole 5 EC	50	100	250
4.	Azoxystrobin 20 SC	50	100	250
5.	Carbendazim 50 WP	50	100	250
Combination fungicides				
6.	Azoxystrobin 11% + Tebuconazole 18.3% SC	100	250	500
7.	Propiconazole 10.7% + Tricyclazole 34.2% SC	100	250	500
8.	Mancozeb 63% + Carbendazim 12% WP	100	250	500
9.	Tebuconazole 50% + Trifloxystrobin 25% WG	100	250	500
10.	Propiconazole 13.9% + Difenconazole 13.9% EC	100	250	500

RESULT AND DISCUSSION

Stem rust of wheat have resulted due to systemic infection caused by *Puccinia graminis tritici*, hence systemic fungicides belonging to groups like triazoles (hexaconazole, propiconazole and difenconazole), benzimidazoles (carbendazim) and strobilurin (azoxystrobin) were investigated for its *in vitro* efficacy in reducing the germination of uredospores.

A. Effect of systemic fungicides

Irrespective of different concentrations, systemic fungicide exerted significant effect ($P < 0.05$) on the germination inhibition of uredospores. Average inhibition in uredospore germination exerted by various systemic fungicides was ranged 76.74 to 94.83 per cent. Significantly the highest mean inhibition of 94.83 per cent was recorded in propiconazole, followed by hexaconazole with 92.76 per cent germination inhibition. Azoxystrobin was the next effective fungicide with 88.89 per cent inhibition in uredospore germination. Tebuconazole recorded the 86.05 percent uredospore germination inhibition and proved comparatively the least performing fungicide among its other azole group fungicides. Least per cent of uredospore germination inhibition was found in carbendazim which recorded 76.74 per cent uredospore germination inhibition (Fig. 1).

(i) Effect of concentration. Irrespective of systemic fungicides, concentration indicated significant effect ($P < 0.05$) in the germination inhibition of uredospores. Successive reduction in germination of uredospores was observed with subsequent increase in the concentration from 50 to 250 ppm. Highest inhibition of 90.70 per cent was recorded with 250 ppm concentration followed by 88.22 per cent in 100 ppm. Lowest inhibition of 84.65 per cent was recorded in 50 ppm concentration of the fungicides.

(ii) Interaction effect of systemic fungicide × concentration. Interaction of systemic fungicide × concentration exerted a significant effect ($P < 0.05$) in the reduction of germination of uredospores. Germination inhibition ranged from 71.32 to 97.67 per cent in various fungicides at its different concentrations. Highest inhibition in uredospore germination of 97.67 per cent was recorded at 250 ppm propiconazole, followed by same fungicide recording 94.57 per cent inhibition at 100 ppm concentration and both were at par with each other. This was closely followed by hexaconazole that recorded 94.57 per cent reduction in the germination at 250 ppm. Propiconazole at 50 ppm again proved effective in reducing uredospore germination by 92.25 per cent. Three fungicides *viz.*, azoxystrobin at 250 ppm and hexaconazole at 100 and 50 ppm exhibited similar inhibition effect by reducing uredospore germination to 92.25, 92.25 and 91.47 per cent, respectively. Azoxystrobin exhibited moderate inhibition of 89.15 per cent at 100 ppm, which was close to tebuconazole at both 250 and 100 ppm showing 88.37 and 86.82 per cent uredospore germination inhibition. Further reduction in uredospore germination was recorded by azoxystrobin and tebuconazole at 50 ppm by 85.27 and 82.95 per cent. Least uredospore germination inhibition of 71.32, 78.29 and 80.62 was recorded by carbendazim at 50, 100 and 250 ppm respectively and thus proved less efficaceous in managing rust pathogen.

Azole and strobilurin group of fungicides exerted an effective inhibition on uredospore germination of uredospores. Higher inhibition potential of azoles (propiconazole, hexaconazole and tebuconazole) and strobilurin (azoxystrobin) fungicides was due to its peculiar mode of action *viz.*, disruption of membrane fluidity and cell integrity by the suppression of ergosterol synthesis, respiration inhibition, respectively. Amaresh and Nargund (2003) reported that propiconazole was the most inhibitory fungicide (78.7%), followed by hexaconazole (78.4%) in managing sunflower rust caused by *Puccinia helianthi.* Kalappanavar *et al.* (2008) also reported propiconazole (95.73%) to be the best systemic fungicide in managing leaf rust. Propiconazole was found the best for inhibition of 86.03 per cent uredospores germination followed by hexaconazole and penconazole with 77.40 and 72.29 per cent, respectively (Chaudhary *et al.*, 2015). Hence, the results obtained in the present investigation are in accordance to the findings of previous researchers.

Table 2: Germination inhibition of *P. graminis tritici* uredospores by systemic fungicides at different concentrations *in vitro.*

Sr. No.	Systemic fungicides	Germination inhibition (%) Concentration (ppm)			Mean
		50	100	250	
1.	Propiconazole 25 EC	73.87^{c} (92.25)*	76.59^{b} (94.57)	81.22^{a} (97.67)	77.23^{a} (94.83)
2.	Tebuconazole 29.9 EC	65.62^{gh} (82.95)	68.77^{ef} (86.82)	70.12^{e} (88.37)	68.17^{d} (86.05)
3.	Hexaconazole 5 EC	73.06^{c} (91.47)	73.87^{c} (92.25)	76.59^{b} (94.57)	74.51^{b} (92.76)
4.	Azoxystrobin 25 EC	67.45^{fg} (85.27)	70.79^{de} (89.15)	73.87^{c} (92.25)	70.70^{c} (88.89)
5.	Carbendazim 50 WP	57.62^{j} (71.32)	62.24^{i} (78.29)	63.89^{hi} (80.62)	61.25^{e} (76.74)
Mean		67.52^{c} (84.65)	70.45^{b} (88.22)	73.14^{a} (90.70)	
C.V.%		2.09			

*Figures in parentheses are retransformed values of arc-sine transformation
Treatment means with common letter(s) are not significant by Duncan's New Multiple Range Test at 5% level of significance.

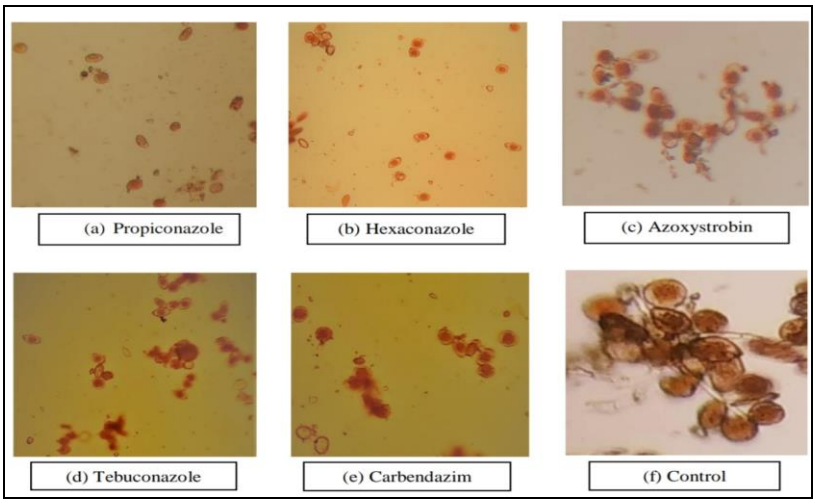

Fig. 1. *In vitro* germination of *P. graminis tritici* uredospores by systemic fungicides.

B. Efficacy of combination fungicides

Combined fungicides with more than one active ingredient offers better inhibition potential with more than one target in the pathogen cell and also has less chance for the development of resistance in pathogens. Therefore, combination of strobilurin and azole (azoxystrobin + tebuconazole, tebuconazole + trifloxystrobin), azole and azole (propiconazole + tricyclazole, propiconazole + difenconazole) and benzimidazole and dihtiocarbamate (carbendazim + mancozeb) groups of fungicides were tested against uredospore germination inhibition of *P. graminis tritici.* Their uredospore germination inhibition potential is discussed below:

(i) Effect of combination fungicides. Irrespective of different concentrations, combined fungicides investigated exhibited a significant effect ($P < 0.05$) on the uredospore inhibition of germination *P. graminis tritici*. Average inhibition in uredospore germination exerted by various combined fungicides ranged from 96.38 to 83.21 per cent. Propiconazole + tricyclazole recorded 87.60 percent uredospore germination inhibition and was the least performing fungicide among its azole +azole group of fungicides. Least per cent of uredospore germination inhibition was found in carbendazim + mancozeb which recorded 83.21 per cent uredospore germination inhibition (Fig. 2).

(ii) Effect of concentration. Irrespective of combined fungicides, concentration exerted a significant effect on the inhibition of the uredospore germination. Inhibition per cent increased significantly by increase in the level of concentration from 100 ppm to 500 ppm. Highest germination inhibition per cent of 93.33 was recorded in 500 ppm, succeeded by 90.85 per cent in 250 ppm. However, comparatively less inhibition per cent of 88.22 was recorded in 100 ppm concentration.

(iii) Interaction effect of combination fungicide × concentration. Interaction of combined fungicide × concentration indicated significant effect on the germination of the uredospores. Effective inhibition exceeding 85 per cent was recorded in the higher concentration (500 ppm) and more than 82 percent in 250 ppm concentration of all the five compound fungicides. Nearly Complete (99.22%) inhibition of germination of uredospores was exhibited by azoxystrobin + tebuconazole and tebuconazole + trifloxystrobinat highest concentration of 500 ppm. The inhibition in uredospore germination of 96.12, 96.90 and 95.35 per cent at 250, 500 and 250 ppm of azoxystrobin + tebuconazole and tebuconazole +

trifloxystrobin indicated its superior efficacy to prevent germination of uredospores. This was followed by azoxystrobin + tebuconazole, propiconazole + difenconazole and tebuconazole + trifloxystrobin which emerged as the next effective fungicide by an effective germination inhibition of 93.80 per cent at 100 ppm, 93.80 per cent at 500 ppm and 93.02 per cent at 100 ppm, respectively. Propiconazole + difenconazole exhibited significant germination inhibition of uredospores of 89.15 to 92.25 per cent at 100 and 250 ppm concentrations. Germination inhibition of 89.92 per cent was recorded in propiconazole + tricyclazole at 500 ppm. Carbendazim + mancozeb recorded relatively lower germination inhibition of 86.82 and 82.95 per cent at 500 and 250 ppm concentrations compared to its counterparts. This was followed by propiconazole + tricyclazole which recorded least efficacy at lower concentrations with 87.60, and 85.27 per cent germination inhibition at 250 and 100 ppm concentrations, respectively. It was noticed that carbendazim + mancozeb at 100 ppm performed inferiorly compared to other treatments by recording the least uredospore germination inhibition of 79.85 per cent.

Effective reduction in the germination of uredospores by combination fungicides was due to combined action of antisporulation and respiration inhibition by strobilurins, ergosterol suppression by azoles, cell division inhibition by benzimidazoles, and multisite inhibition of contact fungicides like inactivation of sulphahydral enzymes (respiratory enzymes) by dithiocarbamates.

Sunil (2013) reported that azoxystrobin + difenconazole, tebuconazole, picoxystrobin + propiconazole, trifloxystrobin + tebuconazole, tebuconazole completely inhibited uredospore germination even at 5 µl whereas other fungicides *viz*., azoxystrobin + cyproconazole and propiconazole caused complete inhibition of uredospore germination at 10 µl. The maximum mean reduction of uredospore germination (84.48%) in the treatment of pyraclostrobin 13.3% + epoxyconazole 5% WP followed by tebuconazole 50% + trifloxystrobin 25% WG with 76.34 per cent inhibition (Khunt *et al.,* 2019). Therefore, the results in the present investigation relates to the findings of previous researchers.

Table 3: Germination inhibition of *P. graminis tritici* uredospores by combination fungicides at different concentrations *in vitro.*

Sr. No.	Combination fungicides	Germination inhibition (%) Concentration (ppm)			Mean
		100	250	500	
1.	Azoxystrobin 11% + Tebuconazole 18.3% SC	75.64^{cd} (93.80)*	78.76^{bc} (96.12)	87.07^{a} (99.22)	80.49^{a} (96.38)
2.	Propiconazole 10.7% + Tricyclazole 34.2% SE	67.45^{ij} (85.27)	69.40^{hi} (87.60)	71.52^{fgh} (89.92)	69.46^{d} (87.60)
3.	Carbendazim 12% + Mancozeb 63% WP	63.33^{k} (79.85)	65.62^{jk} (82.95)	68.73^{hij} (86.82)	65.89^{e} (83.21)
4.	Tebuconazole 50% + Trifloxystrobin 25% WG	74.68^{d} (93.02)	77.55^{bcd} (95.35)	80.00^{b} (96.90)	77.41^{b} (95.09)
5.	Propiconazole 13.9% + Difenconazole 13.9% EC	70.79^{gh} (89.15)	73.87^{efg} (92.25)	75.64^{cd} (93.80)	73.43^{c} (91.73)
Mean		70.38^{c} (88.22)	73.04^{b} (90.85)	76.59^{a} (93.33)	
C.V.%		2.86			

*Figures in parentheses are retransformed values of arc-sine transformation

Treatment means with common letter(s) are not significant by Duncan's New Multiple Range Test at 5% level of significance.

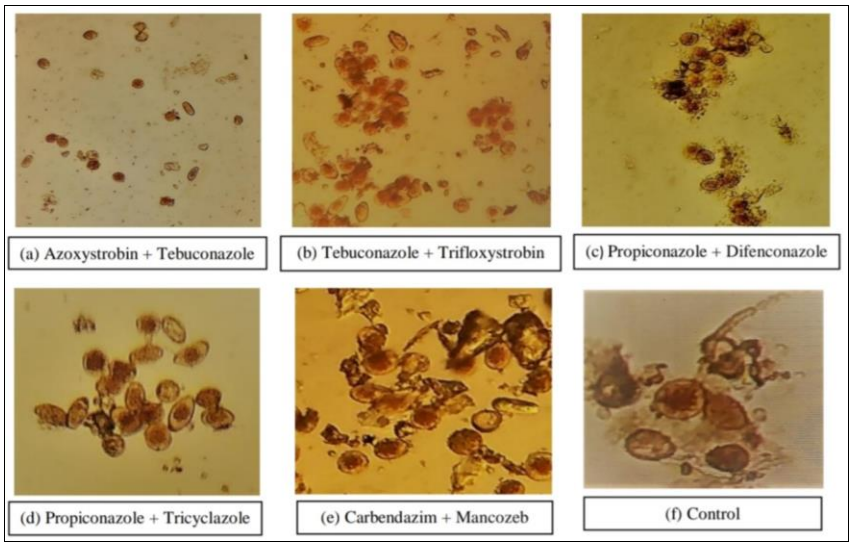

Fig. 2. *In vitro* germination of *P. graminis tritici* uredospores by combination fungicides.

CONCLUSIONS

Propiconazole and hexaconazole were found to be the best performing fungicides *in vitro* by recording mean germination inhibition of 94.83 and 92.76 per cent. The uredospore germination inhibition was comparatively low (76.74%) in carbendazim. Among the combined fungicides, Azoxystrobin + tebucanazole and tebuconazole + trifloxistrobin proved to be the best fungicides *in vitro* with mean uredospore germination inhibition of 96.38 and 95.09 per cent. Carbendazim + mancozeb resulted in 83.21 per cent mean uredospore germination inhibition and thereby proved comparatively inferior.

A rapid method of quantifying the fungicide effectiveness with detached leaves was developed to study wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*. The results revealed that 0.5% water agar containing 75 µg/ml of 6-benzylaminopurine and filter paper worked the best for maintaining wheat leaves. The disease incidences of different concentrations of spore suspension were compared. When the spore concentrations were 5 and 10 mg/ml, the disease incidences had no significant differences at 12 and 15 days after inoculation ($P < 0.05$). Fungicide treatment tests revealed that there were no significant differences in the efficacies of triadimefon on rust suppression between detached leaves in the culture dishes and direct spray on seedlings (Peng *et al.*, 2020).

The spore germination inhibition of *Puccinia triticina* with different combination of fungicides in laboratory conditions was investigated by Khunt *et al.* (2019). The relative efficacies of eight different fungicides combination were tested at different concentrations *viz.*, 200, 500 and 1000 ppm. The data indicated that the maximum mean reduction of uredospore germination (84.48%) was found in the treatment of pyraclostrobin 13.3% + epoxyconazole 5% WP followed by tebuconazole 50% + trifloxystrobin 25% WG with 76.34 per cent inhibition. The other fungicide *viz.*, cymoxanil 8% + mancozeb 64% WP (71.90%) was found moderately effective.

FUTURE SCOPE

The fungicides remains a major phytosanitary challenge to wheat cultivation and a threat to wheat production worldwide; its global economic impact remains elusive. Our analysis suggests that fungicides will continue to play a key role in stem rust management, even when wheat cultivars with high level of resistant to the greatest possible number of Pgt races become available.

Acknowledgements. The author acknowledges the facilities provided by Department of Plant Pathology, Chimanbhai Patel College of Agriculture SDAU, Sardarkrushinagar, Gujarat, India, for the smooth conduction of this research work.

Conflicts of Interest. None.

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How to cite this article: Shipra Sharma, Rashmi D. and S. I. Patel (2023). *In vitro* efficacy of fungicides on Uredospore Germination Inhibition of *Puccinia graminis tritici*. *Biological Forum – An International Journal*, *15*(5): 372-376.