Plant Archives Vol. 26, No. 1, 2026 pp. 2119-2122 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.290

IN VITRO EVALUATION OF FUNGICIDES, BIOAGENTS, AND BOTANICALS AGAINST WHEAT LEAF RUST (*PUCCINIA TRITICINA* ERIKS)

V.A. Gardi[1]*, A.M. Navale[1], P.H. Gaikwad[1], B.M. Ilhe[2], M.A. Sushir[3], V.P. Chimote[4] and M.R. Patil[5]

[1]Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India – 413722.
[2] College of Agriculture, Dhule, Maharashtra, India
[3] Regional Wheat Rust Research Station, Mahabaleshwar, Satara, Maharashtra, India
[4]State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India
[5] Department of Statistics, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India
*Corresponding author E-mail: vinitagardi@gmail.com
(Date of Receiving : 30-01-2026; Date of Revision : 26-03-2026; Date of Acceptance : 07-04-2026)

ABSTRACT

Wheat leaf rust, caused by *Puccinia triticina* Eriks., is a major disease limiting wheat production worldwide. This study evaluated the *in vitro* efficacy of fungicides, bioagents, and botanicals against *P. triticina* Eriks to develop an integrated disease management strategy. Laboratory experiments were conducted using the cavity slide method under a completely randomized design with three replications. Among fungicides, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 18.2% + Difenoconazole 11.4% SC completely inhibited uredospore germination at all tested concentrations, while Hexaconazole 5% SC was effective only at the highest concentration (500 ppm). Among bioagents, *Trichoderma harzianum* and *Bacillus subtilis* showed moderate inhibition, and among botanicals, Neem oil and Pongamia oil exhibited partial efficacy. Maximum uredospore germination (63.03%) was recorded in the untreated control. The results indicate that a combination of chemical, biological, and botanical treatments could contribute to an effective and sustainable management of wheat leaf rust.

Keyword **:** *Puccinia triticina,* Uredospore, Wheat.

Introduction

Wheat (*Triticum aestivum*) serves as a staple food for nearly one billion people worldwide, supplying approximately 20% of the total caloric intake for the human population. It is primarily consumed in the form of flour, which is used to prepare chapatis and pasta. Additionally, wheat is utilized in the production of bread, biscuits, cookies, snacks, noodles, dalia, maida, vermicelli, and various other food products. The crop contains gluten, an essential component for baking (Anonymous, 2009). Beyond its role in human nutrition, wheat straw is employed as animal fodder and as a material for packaging. The chemical composition of wheat consists of roughly 70% carbohydrates, 12% crude protein, 1.7% fat, 2.7% minerals, 2% fiber, and 12% moisture (Gupta *et al*., 2002).

Its production, however, is severely affected by various biotic stresses, among which rust diseases are particularly destructive. Leaf rust, caused by *Puccinia triticina* Eriks., is one of the most prevalent and economically significant wheat diseases worldwide. The pathogen can infect wheat in almost all growing regions, leading to substantial yield losses ranging from 10% to 50%, especially under favourable environmental conditions (Singh *et al*., 2016; Bhardwaj *et al*., 2019).

Management of wheat leaf rust traditionally relies on resistant cultivars and chemical fungicides. Triazole and strobilurin-based fungicides, such as Tebuconazole and Azoxystrobin, are widely used due to their effectiveness in suppressing the pathogen. However, the continuous evolution of *P. triticina* Eriks., and the indiscriminate use of fungicides raise concerns about resistance development and environmental safety. Consequently, alternative strategies, including the use of biological control agents such as *Trichoderma harzianum* and *Bacillus subtilis*, and botanical extracts

like Neem and Pongamia oils, are being explored as sustainable solutions.

Integrated disease management, combining chemical, biological, and botanical approaches, offers a promising and eco-friendly strategy to control wheat leaf rust effectively. The present study was undertaken to evaluate the *in vitro* efficacy of selected fungicides, bioagents, and botanicals against *P. triticina*, Eriks., with the aim of identifying effective and environmentally sustainable measures for leaf rust management.

Material and Method

All laboratory experiments for the present investigation were conducted in the Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. To evaluate the *in vitro* efficacy of fungicides, bioagents and botanicals against the causal fungus, fungicides belonging to different chemical groups were procured from the Department of Plant Pathology and Microbiology. These fungicides were stored at 25°C in the dark to preserve their biocidal activity. The fungicides tested in this study

were: *Trichoderma harzianum*, *Bacillus substilis*, Neem oil, Pongamia oil, Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG, Azoxystrobin 18.2%+ Difenconazole 11.4% w/w SC, Azoxystrobin 18.2%+ Difenconazole 11.4% w/w SC.

The required concentrations of fungicides were prepared by dissolving known quantity of fungicides in known quantity of sterile distilled water separately under aseptic conditions. Twenty-five microliters of each fungicide were pipetted out on cavity slides and uredospores were added by scraping single uredia of infected wheat leaf with a sterilized scalpel. Three replications were maintained for each treatment. Effect of fungicides and their concentrations on the germination of uredospores were observed after 24 hrs of incubation in a moist chamber (Chaudhari *et al*. 2013). The control was maintained with distilled water. Per cent inhibition over the control was calculated by using the formula given by Vincent (1947). Later data was analyzed using completely randomized design.

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

I = Per cent inhibition

C = Germination of uredospores in control

T = Germination of uredospores in treatment

Result and Discussion

Three fungicides, two bioagent and two botanicals were evaluated against uredospore germination and inhibition of *P. triticina* Eriks using cavity slide method. Also, observations were interpreted on per cent uredospore germination inhibition and inhibition of germ tube elongation after 24 hours of incubation at 20^0C.

Effect of different fungicides, bioagent and botanicals on germination inhibition of uredospores of *P. triticina* Eriks.

Data presented in (Table 1, Fig. 1, and Plate 1) showed that, among three fungicides tested, Tebuconazole 50% + Trifloxystrobin 25% w/w WG (T_5) and Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC (T_6) were found cent per cent effective against, *P. triticina* which completely inhibited uredospore germination at all three concentrations, viz. 50 ppm, 100 ppm, 500 ppm. However, Hexaconazole 5% SC (T_7) were found cent per cent effective against test pathogen only at 500 ppm. Among bioagent, *Trichoderma harzianum* (T_1) and *Bacillus substilis* (T_2) showed effective treatments to some extent with 48.62, 66.60, 79.17 and 54.16, 75.31, 82.91 per cent germination inhibition at 50 ppm, 100 ppm and 500 ppm respectively. Among botanicals, Neem oil (T_3) and Pongamia oil (T_4) showed effective treatments to some extent with 42.60, 60.06, 64.09 and 12.98, 33.08, 42.16 per cent germination at 50 ppm, 100 ppm and 500 ppm respectively. Pongamia oil (T_4) was not effective at 50 ppm concentrations. Maximum uredospore germination (63.03 %) was recorded in control.

Similar results were cited by Kalappanavar *et al*., (2008) who reported the in botanicals the neem leaf extract was superior against uredospores germination of *P. recondita* f. sp. *tritici* followed by parthenium leaf extract and duranta leaf extract.

In the present investigation, fungicides showed the highest inhibition of uredospore germination compared to bioagents and botanicals. The effectiveness of fungicides may be attributed to their ability to interfere with essential physiological processes of the pathogen. Triazole fungicides are known to inhibit ergosterol biosynthesis, which is essential for fungal cell membrane formation and germ tube development. Earlier studies have reported that systemic fungicides significantly inhibit spore germination and germ tube growth in rust fungi (Roelfs *et al*., 1992).

Similarly, Kolmer (2005) reported that fungicides targeting sterol biosynthesis effectively suppress wheat

rust pathogens by preventing uredospore germination and subsequent infection.

The inhibition of uredospore germination observed in this study is consistent with previous findings that fungicides are highly effective in controlling rust diseases by affecting early stages of pathogen development. According to Agrios (2005), fungicides may prevent fungal spore germination by disrupting enzymatic activity and membrane integrity, thereby limiting pathogen growth and infection.

Among the biological control agents tested, species of *Trichoderma* and *Pseudomonas* exhibited moderate inhibitory effects on uredospore germination. The antagonistic activity of these microorganisms may be due to the production of antibiotics, volatile metabolites, and hydrolytic enzymes that inhibit fungal growth. The ability of *Trichoderma* species to suppress plant pathogenic fungi through antibiosis, competition, and mycoparasitism has been widely documented (Harman *et al*., 2004). These mechanisms may contribute to the reduction of uredospore viability and germination.

Table 1 : Effect of different fungicides, bioagent and botanicals on germination inhibition of uredospores of *P. triticina* Eriks.

Tr. No.	Treatment	Germination of uredospores after 24 hrs					
		50 ppm		100 ppm		500 ppm	
		% Germination	% Inhibition	% Germination	% Inhibition	% Germination	% Inhibition
T_1	*Trichoderma harzianum*	32.39 (34.69)	48.62 (44.21)	21.06 (27.31)	66.60 (54.70)	13.06 (21.18)	79.17 (62.86)
T_2	*Bacillus substilis*	28.89 (32.51)	54.16 (47.39)	15.56 (23.23)	75.31 (60.21)	10.71 (19.10)	82.91 (65.60)
T_3	Neem oil	36.18 (36.98)	42.60 (40.74)	25.18 (30.12)	60.06 (50.80)	22.51 (28.33)	64.09 (53.18)
T_4	Pongamia oil	54.85 (47.78)	12.98 (21.11)	42.18 (40.50)	33.08 (35.11)	36.26 (37.02)	42.16 (40.49)
T_5	Tebuconazole 50% + Trifloxystrobin 25% w/w 75 WG	0.00 (0.00)	100 (90.00)	0.00 (0.00)	100 (90.00)	0.00 (0.00)	100 (90.00)
T_6	Azoxystrobin 18.2%+ Difenconazole 11.4% w/w SC	0.00 (0.00)	100 (90.00)	0.00 (0.00)	100 (90.00)	0.00 (0.00)	100 (90.00)
T_7	Hexaconazole 5 % SC @ 1ml/l	25.33 (30.22)	59.81 (50.66)	8.67 (17.12)	86.24 (68.23)	0.00 (0.00)	100 (90.00)
T_8	Control (Water spray)	63.03 (52.55)	-	63.03 (52.55)	-	63.03 (52.55)	-
	SEm ±	0.65	-	0.60	-	0.50	-
	CD at 1%	1.89	-	1.77	-	1.47	-

Note: *Figures in parenthesis are square root transformation (√n+1) values, **Figures in parenthesis are arc sin value

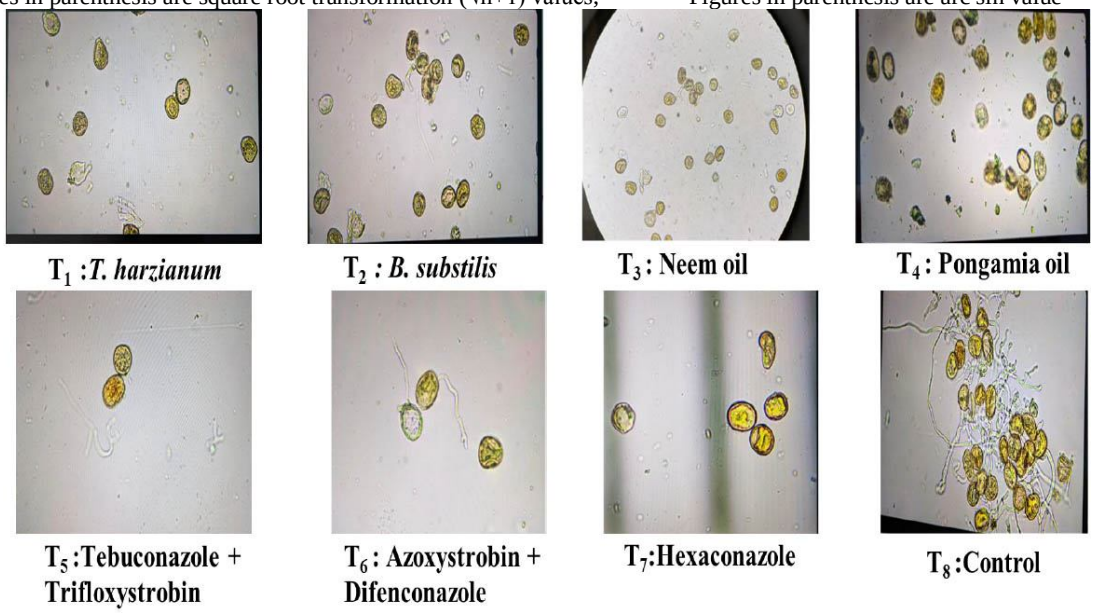

Plate 1 : Germination inhibition of uredospores after 24 hrs in 500 ppm conc. of bioagents, oils and fungicides

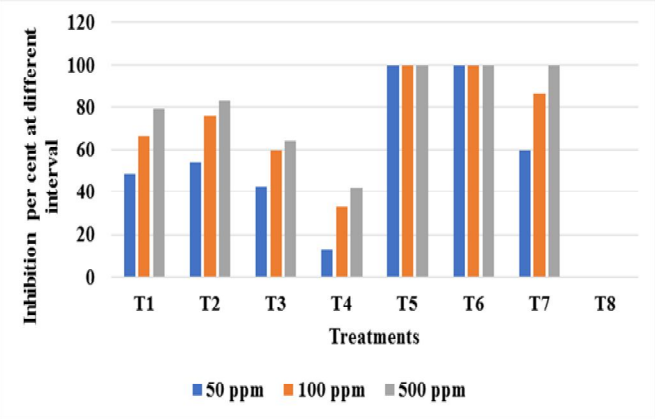

Fig. 1 : Graphical presentation of uredospore germination (*P. triticina* Eriks.) inhibition at different concentrations of fungicides, botanicals and bioagents.

Conclusion

The current study's finding showed that under *in vitro* studies, the fungicides (Tebuconazole 50 % + Trifloxystrobin 25 % w/w WG) and treatment (Azoxystrobin 18.2 % + Difenoconazole 11.4 % w/w SC) were found effective against, leaf rust which completely inhibited uredospore germination at all three concentrations, viz. 50 ppm, 100 ppm, 500 ppm. Similarly, the bioagent, *Trichoderma harzianum* and *Bacillus substilis* showed moderate inhibition and in case of botanicals neem oil and pongamia oil showed least effective in inhibition of uredospore germination.

Acknowledgement

The authors sincerely acknowledge the Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India, for providing the necessary facilities to conduct this research. Deep gratitude is extended to the research guide and colleagues for their invaluable encouragement, guidance, and constructive suggestions throughout the course of this work.

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