

Bio-efficacy of new fungicide, Tebuconazole- 430SC against coffee leaf rust disease, *Hemileia vastatrix* Berk and Broome



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Summary

Coffee is an important plantation crop cultivated worldwide and used as stimulating drink. Coffee leaf rust (CLR) disease is one of the devastating disease affecting foliage of Coffee, limiting Coffee production worldwide. It is caused by an obligate parasite *Hemileia vastatrix*, which reduce photosynthetic area in turn results in heavy defoliation and die-back of branches. In India CLR management is accomplished with one contact fungicide spray (Bordeaux mixture @ 0.5%) and effective systemic fungicide spray during pre and post monsoon periods respectively. However, to find an alternative to the recommended fungicide the studies were conducted to know the efficacy of new fungicide Tebuconazole 430 SC against CLR. The results revealed that, in the laboratory conditions, complete inhibition of the uredospore germination was observed in Tebuconazole 430 SC @ 0.5 ml/L and 0.75 ml/L and Hexaconazole 5 EC @ 2 ml/L. However, in lower concentration Tebuconazole 430 SC @0.25 ml/L there was 98.97 per cent inhibition and in Copper Oxychloride 50 WP @ 5.5 g/L 87.49 per cent inhibition was observed over control. Field trials indicated that, at all the locations, Tebuconazole 430SC @ 0.75ml/L was found very effective which recorded maximum disease reduction followed by Tebuconazole 430SC @ 0.5ml/L which was found on-par with the currently recommended fungicide Hexaconazole 5 EC @ 2 ml/L and minimum disease reduction was observed in Copper oxychloride 50 WP @ 5.5g/L.

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Keywords: *Coffea Arabica*; disease; fungicide; *Hemileia vastatrix*; leaf rust; management

INTRODUCTION

Coffee is a perennial plantation crop, cultivated in tropics and sub-tropics of the world. It produces one of the most important horticultural product, which is internationally traded and ranked next to petroleum. It is one of the flavoured beverages that is non-alcoholic and is consumed in many parts of the world. Coffee leaf rust is one of the most devastating disease historically and economically important affecting coffee crop. Among the two commercially cultivated species of coffee, *Coffea arabica* is more susceptible to CLR disease than *Coffea canephora*. The disease is caused by fungus *Hemileia vastatrix*. It was first reported in 1861 by a British explorer on uncultivated coffee in Lake Victoria of Kenya in East Africa. In cultivated coffee, it was reported from Sri Lanka in 1869, and completely devastated coffee production in

that country within 10 years. Small chlorotic spots measuring 2-4 mm in diameter on lower surface are the initial macroscopic symptoms on leaves which later turn to orange yellow powdery mass of urediniospores. The spots enlarge and become more irregular as they coalesce with the adjacent spots thus resulting in the reduction of photosynthetic activity of infected leaves. With ageing, the central portion of the spots becomes brownish and later necrotic, whereas the peripheral zone of the lesions continues to sporulate. Severe infection can cause heavy premature defoliation and die-back of branches.^{10, 15, 1} This will reduce yields by 50 per cent and have a cumulative weakening effect on the trees for succeeding years.^{5, 14} Hence, managing the rust fungus is crucial to enhance production of coffee. In India, currently, integrated disease management includes use of tolerant varieties, optimum shade, bush management and chemical sprays which are accomplished with one contact spray with Bordeaux mixture and a systemic fungicide during pre and post monsoon respectively.⁶

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However, chemical management has been critical in preventing the losses due to disease. Although many fungicides have been evaluated and found effective by many researchers against the disease, to add one more substitute to the effective fungicides. The present study was undertaken to know efficacy of the new fungicide Tebuconazole 430 SC for the management of coffee leaf rust disease.

Materials and Methods

In vitro evaluation of the fungicide tebuconazole 430 SC

In vitro evaluation of fungicide Tebuconazole 430 SC was carried out in the Plant Pathology Division of Central Coffee Research Institute (CCRI), Chikkamagaluru, Karnataka, India by following spore germination studies. The matured uredospores of leaf rust pathogen, *Hemileia vastatrix* were collected from infected leaves of susceptible *Coffea arabica* variety S.795 in gelatin capsules at CCRI farm. Inhibition of the spore germination was studied by following spore germination technique.² The fungicide Tebuconazole 430SC 0.25 ml/L, 0.50 ml/L and 0.75 ml/L was tested and Bordeaux mixture @ 0.5%, Copper Oxychloride @ 5.5g/L, Hexaconazole 5 EC @ 2ml/L were compared with new fungicide and untreated control was also maintained for comparison. The treatments details for in vitro and in vivo studies are presented in table 1. After incubation period microscopic observations of the germination of uredospores were carried out. Germination inhibition

percentage of uredospores was calculated by using the formula below,

$$\text{Germination inhibition (\%)} = \left(C - \frac{T}{C} \right) \times 100$$

Where,

C- Uredospore Germination in Control

T- Uredospore Germination in Treatment

Field evaluation of the fungicide tebuconazole 430 SC

Based on in vitro studies, field evaluation of the fungicide was carried out at five locations owned by CCRI which is located in Karnataka state, India. The locations are CCRI farm; Technology Evaluation Centre (TEC) Mudigere; TEC Chickmagalur; and two locations at Coffee Research Sub-Station (CRSS), Chettalli, Madikeri. The experiment was laid out in randomized block design with seven treatments and three replications at all the locations. The experiment was conducted for two consecutive years i.e., 2017-18 and 2018-19 seasons. The test material *Coffea arabica* variety S.795, most susceptible variety to coffee leaf rust was used for the study. Treatments were imposed as foliar spray in two applications by using high volume sprayer during pre and post monsoon season. The observations on the incidence of coffee leaf rust (CLR) were recorded 30, 60, 90 days after each spray in all locations. The per cent disease incidence and per cent disease reduction was calculated by using the formula below:^{13,9}

$$\text{Disease incidence (\%)} = \frac{\text{Total number of diseased leaves}}{\text{Total number of leaves examined}} \times 100$$

$$\text{Percent Disease Reduction (PDR)} = \frac{PDIC - PDIT \times 100}{PDIC}$$

Where,

PDR = Per cent disease reduction

PDIC = Per cent disease incidence in control

PDIT = Per cent disease incidence in treatment

Table.1 Treatment details of in vitro and in vivo studies

Tr. No.	Fungicide	Dosage
1	Tebuconazole 430 SC	0.25 ml/L
2	Tebuconazole 430 SC	0.5 ml/L
3	Tebuconazole 430 SC	0.75 ml/L
4	Copper Oxy Chloride	5.5 g/L
5	Bordeaux Mixture	0.5%
6	Hexaconazole 430 SC	2 ml/L
7	Control	Water Sprayed

Evaluation of phytotoxicity of new fungicide tebuconazole 430 SC on sprayed plants

To study phytotoxicity of the fungicide molecule Tebuconazole 430 SC, identified plants were sprayed with different dosage of test fungicide viz., 0.75ml/L and

1.5 ml/L of the fungicide. The plants were observed for leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty on the sprayed arabica coffee plants after 3, 7 and 15 days of application of the fungicide.

Persistence studies of new fungicide tebuconazole 430 SC

In-planta persistence of the fungicide Tebuconazole 430 SC was studied against coffee leaf rust by following resporulation technique. Infected plants were selected and sprayed with different concentrations i.e. 0.25ml/L, 0.50ml/L and 0.75ml/L. The plants were observed regularly for the healing of infected pustules and for resporulation of cured pustules.¹³

Results and Discussions

Usage of fungicides is very essential for effective management of CLR disease. Continuous usage of single fungicide molecule in coffee plantation may result in development of resistance in the pathogen. New classes of fungicides with novel modes of action are being developed and are likely to have a significant impact on disease management in 21st century. Tebuconazole is active ingredient from the triazole group of fungicides, it is taken up by the plants acropetally and is known as demethylation inhibiting fungicide, it is a systemic fungicide and has broad-spectrum activity with excellent plant compatibility. It delivers both curative and preventive

action for the management of plant diseases.⁸ It disturbs the cell wall of fungi by suppressing the spore germination and fungal growth by interfering with the production of ergosterol. This unique mode of action of Tebuconazole is considered to be fungistatic or growth-inhibiting fungicide. It works systemically, absorbing into the target plant to protect it against diseases, prevent further spread or can eliminate the disease entirely depending on the severity level. Field trials remain to be the dominating methods for characterizing new compounds in terms of efficacy. Screening of the new fungicide molecule Tebuconazole 430 SC was carried out in five locations and results are presented here under;

Efficacy of fungicide tebuconazole 430 SC under *in vitro* conditions

Statistical analysis of the data indicated that, treatments were found significant. Observations on spore germination indicated that, the fungicide Tebuconazole @ 0.5 ml-L, and 0.75 ml-L and the recommended fungicide Hexaconazole @ 2ml-L were highly effective and recorded 100 per cent inhibition of uredospore germination over control. Whereas, maximum inhibition 98.97 per cent was recorded in Tebuconazole 430SC@ 0.25 ml/L and in copper-based fungicides the inhibition percent was 96.4 per cent and 87.49 per cent in Bordeaux mixture@ 0.5% and Copper oxychloride @ 0.55g/L respectively (Table 2).

Table 2: Efficacy of Tebuconazole 430 SC on inhibition of uredospore germination under *in vitro* condition

Treatments	Uredospore germination over control (%)
T ₁ - Tebuconazole 430 SC @0.25ml/liter	98.9
T ₂ - Tebuconazole 430 SC @0.50ml/liter	100.0
T ₃ - Tebuconazole 430 SC @ 0.75ml/liter	100.0
T ₄ - Copper oxychloride 50 WP @ 5.5 g/liter	87.4
T ₅ - Bordeaux mixture 0.5%	96.4
T ₆ - Hexaconazole 5 EC @ 2ml/liter	100.0
S.Em±	0.85
CD 1%)	3.68

Efficacy of fungicide tebuconazole 430 SC under field conditions

During the experimental period all the tested fungicides were effective when compared to control. The statistical analysis of the data revealed that, treatments were found to significant at all locations tested. Observations revealed that fungicide Tebuconazole 430 SC was effective against coffee leaf rust when compared to control at all the concentrations tested. Data of mean disease incidence ranged from 1.68 per cent to 24.51 per

cent. During the year 2017-18, maximum disease suppression of 85.65%, 88.59%, 85.2%, 82.87% & 86.81% was recorded in Tebuconazole 430 SC @ 0.75 ml/L at all the locations tested, whereas the minimum disease suppression of 37.52%, 38.0%, 42.5%, 52.38% and 29.41% was recorded in Bordeaux mixture @ 0.5% at CCRI, TEC-Mudigere, TEC- Chikkamagaluru, CRSS- Chettalli- 1 and CRSS- Chettalli- 2 respectively. However, the recommended fungicide Hexaconazole 5 EC recorded the disease suppression of 75.16%, 82.9%, 83.5%, 77.95% and

83.33% at CCRI, TEC- Mudigere, TEC- Chikkamagaluru, CRSS - Chettalli- 1 and CRSS- Chettalli- 2 respectively, and was found on par with Tebuconazole 430 SC @ 0.5ml/L at all the locations. Tebuconazole 430 SC @ 0.75ml/L

was superior among all the treatments. Whereas, Copper oxy chloride @5.5g/L and recommended contact fungicide Bordeaux mixture@ 0.5% were on par with each other (Table. 3).

Table 3: Bio-efficacy of the fungicide Tebuconazole 430 SC at different locations during 2017-18

Treatments	Disease reduction over control in all locations (%)				
	CCRI	TEC, Mudigere	TEC, Ckm	CRSS, Chettalli-1	CRSS, Chettalli-2
T ₁ - Tebuconazole 430 SC @0.25ml/liter	62.65	66.0	70.1	73.79	73.58
T ₂ - Tebuconazole 430 SC @0.50ml/liter	71.44	70.6	73.4	79.17	77.29
T ₃ - Tebuconazole 430 SC @ 0.75ml/liter	85.65	88.2	85.2	82.87	86.81
T ₄ - Copper oxycloiride 50 WP @ 5.5 g/liter	42.16	41.5	56.8	62.82	46.87
T ₅ - Bordeaux mixture 0.5%	37.52	38.0	42.5	52.38	29.41
T ₆ - Hexaconazole 5 EC @ 2ml/liter	75.16	82.9	83.5	77.95	83.33

In the following year, comparable results were the obtained in the following year of the experimental period but the percentage of disease suppression was maximum in the tested fungicide when compared to the previous year. Maximum disease suppression was observed in Tebuconazole 430 SC @ 0.75 ml/L and was 95.25%, 92.92%, 83.03%, 91.87% and 81.2% at CCRI, TEC- Mudigere, TEC- Chikkamagaluru, CRSS- Chettalli- 1 and CRSS- Chettalli- 2 respectively tested followed by Tebuconazole 430 SC @ 0.5ml/L and was found to be on par with the recommended fungicide Hexaconazole 5 EC , whereas the minimum disease suppression of 32.70%, 27.83%, 43.91%, 47.43% and 23.9% was recorded in Bordeaux mixture @ 0.5% at CCRI, TEC- Mudigere, TEC- Chikkamagaluru, CRSS- Chettalli- 1 and CRSS- Chettalli- 2 respectively. Tebuconazole 430 SC @ 0.75ml/L was superior among all the treatments. Whereas, Copper oxy chloride @ 5.5g/L and recommended contact fungicide Bordeaux mixture@ 0.5% recorded less disease suppression and was found on par with each other (Table 4). The self-same molecule Hexaconazole 5% EC but at

the lower active ingredient and different formulation was found effective against coffee leaf rust at 25 and 75ppm as per the studies of Daivasikamani & Govindarajan 1989.³ Strobilurins are the compounds which are gaining importance recently in the management of the plant diseases, the fungicide pyraclostrobin was efficient than epoxyconazole in reducing the coffee leaf disease by Honorato et al. 2015.⁴ In addition, combination of different group of fungicides also manage the coffee leaf rust effectively. Santoshreddy et al. 2019¹³ found that the fungicide Fluxapyroxad 167 g/l + Pyraclostrobin 333 g/l @ 0.5 ml/l was effective against coffee leaf rust and recorded minimum disease incidence. Shivaprasad et al. 2021¹² revealed that the fungicide Pyraclostrobin 133g/l + Epoxiconazole 50g/l SE @ 1.50 ml/L showed the least coffee leaf rust disease severity. Silva et al. (2003)¹⁷ found that the fungicides Triadimenol 6GR and Triadimenol + Disulfuton could manage the coffee leaf rust effectively when compared to test fungicide Copper oxychloride 50%.

Table 4: Bio-efficacy of the fungicide Tebuconazole 430 SC at different locations during 2018-19

Treatments	Disease reduction over control in all locations (%)				
	CCRI	TEC, Mudigere	TEC, Ckm	CRSS, Chettalli-1	CRSS, Chettalli-2
T ₁ - Tebuconazole 430 SC @0.25ml/liter	89.71	85.95	59.41	86.60	70.7
T ₂ - Tebuconazole 430 SC @0.50ml/liter	92.93	88.36	68.35	88.86	74.0
T ₃ - Tebuconazole 430 SC @ 0.75ml/liter	95.25	92.92	83.03	91.87	81.2
T ₄ - Copper oxycloiride 50 WP @ 5.5 g/liter	49.77	48.91	61.31	57.16	38.1
T ₅ - Bordeaux mixture 0.5%	32.70	27.83	43.91	47.43	23.9
T ₆ - Hexaconazole 5 EC @ 2ml/liter	92.56	85.93	2.52	86.10	77.3

Evaluation of phytotoxicity of new fungicide tebuconazole 430 SC on sprayed plants

Phytotoxic studies revealed that, there was no phytotoxic symptoms like leaf injury, wilting, vein clearing, necrosis,

epinasty and hyponasty on the sprayed arabica coffee plants after 3, 7 and 15 days of application of the fungicide.

Persistence studies of new fungicide tebuconazole 430 SC

In persistence studies, it was observed that in all concentrations of the fungicide, healing of infected spots was observed from three days after spray. After 20 days of spray, it was observed that there was complete death of the uredospores. In the plants sprayed with 0.25 ml/litre and 0.50 ml/litre, re-sporulation of the infected spot was observed after 65 days of spray. However, in the higher concentration 0.75ml/L re-sporulation was extended upto 70 days indicating the complete dissipation of the fungicide on the sprayed plants. But Nithi et al. 2004⁷ revealed that the persistence of the another triazole group of fungicide Fluzilazole @ 0.015% and 0.020% was upto 91 days indicating its efficacy in a superior way than the fungicide used in the present study. Tebuconazole 430 SC was effective against the coffee leaf rust when compared to untreated plants. The disease suppression of the fungicide was relatively maximum in the higher concentration when compared to presently recommended fungicide Hexaconazole 5 EC. Hence, the fungicide Tebuconazole 430 SC@ 0.75ml/L can be used for the management of the coffee leaf rust effectively.

Declaration of interests

The authors have no conflict of interest to declare.

Data sharing

All relevant data are within the manuscript.

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