Int.J.Curr.Microbiol.App.Sci (2017) Special Issue-4: 53-60

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-4 (September-2017) pp. 53-60**
Journal homepage: http://www.ijcmas.com

Original Research Article

In Vitro Evaluation of Fungicides against Rice Blast Isolates to Assess Development of Fungicidal Resistance

Ramesh Babu Surapu[1*], Ch. Durga Rani[2], J. Aruna[1], S. Vijay[1], P. Narayan Reddy[1] and M. S. Prasad[1]

[1]Prof. Jayashankar Telangana State Agricultural University, Hyderabad (500030), Telangana, India
[2]Indian Institute of Rice Research, Hyderabad (500030), Telangana, India
**Corresponding author*

Keywords

Rice blast, Tricyclazole, Carbendazim, Resistance

A B S T R A C T

In vitro screening of two fungicides against all the 40 rice blast pathogen isolates revealed sensitivity at varied levels. For Carbendazim complete inhibition of mycelial growth (100% inhibition) was observed at 1000 and 1200 ppm concentrations, in case of Tricyclazole complete inhibitions was recorded at 800 ppm and maximum growth inhibition was at 600 ppm. These observations revealed that, no resistance was developed for both Tricyclazole and Carbendazim at optimal doses.

Introduction

Rice blast caused by *Magnaporthe grisea* (Hebert) Barr (Syn: *Pyricularia grisea* (Cooke) Sacc.) a filamentous ascomycetes fungus infecting more than 50 hosts. Rice blast was first recorded in China (1637) later from Japan (1704). In India, the disease gained importance when a devastating epidemic occurred in Thanjavur (Tanjore) delta of Tamilnadu India during 1919. In Andhra Pradesh, it was first identified in Chittoor district subsequently at Nijamabad in Telangana.

Fungicides are chemical compounds used to kill or inhibit and these are used for seed treatment or for foliar application to control the diseases during seedling stage, vegetative growth stage and reproductive stages. Fungicides that are commonly used for control of rice blast are Tricyclazole, Carbendazim, Kasugamycin, Isoprothiolane, Azoxystrobin, Propiconazole, Ediphenphos and Kitazin which are sprayed thrice at weekly interval starting from the initiation of the disease.

Materials and Methods

Collection of blast diseased specimens

A roving survey was conducted for collection of rice blast infected leaf tissue samples from different locations of Andhra pradesh and Telangana in 2013 and 2014. Total 40 isolates were collected from both Andhra Pradesh and Telangana.

Isolation of Pyricularia oryzae isolates

The fungus was isolated by tissue segmentation method (Bonman *et.al*. 1987). Blast infected leaf tissues stored in refrigerator were cut in to small bits. These bits were washed in sterilized distilled water twice, surface sterilized in 0.1% mercuric chloride for 30 seconds, rinsed three times in sterilized water and allowed for sporulation on sterilized glass slides by incubating in a moist chamber at 25 ^{0}C for 48 h. Well sporulated lesions were placed in double – distilled water in the test tubes and vortexed for 1 min. About 1 ml of spore suspension was added to sterilized plates and 2% agar was added. Single spores were located and picked up microscopically and transferred to fresh sterilized Petri plates containing OMA medium. The petri plates were incubated at 28 ^{0}C for 7 days and the fungus was identified following mycological description given by Ou (1985).

Evaluation of fungicides against P. oryzae In Vitro to assess fungicidal resistance

Two fungicides (Tricyclazole & Carbendazim) were evaluated against *M. grisea* isolates by poisoned food technique (Grover and Moore, 1962) at four different concentrations to assess fungicidal resistance. The recommended doses for Tricyclazole were at 200, 400, 600 and 800 ppm while for Carbendazim at 600, 800, 1000 and 1200 ppm. The required quantities of fungicides were weighed and mixed in the oat meal agar medium by thorough shaking for uniform mixing of the fungicide before pouring into Petri dishes so as to get the desired concentration of active ingredient of each fungicide separately. Twenty ml of amended medium was poured in 90 mm sterilized Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter from 10 day old culture was placed at the center of the Petri plate and then incubated at 28^{0}C for 15 days. Control was maintained without fungicide. Three replications were maintained for each treatment. Per cent inhibition of mycelial growth was calculated using the formula.

$$I = (C-T/C) \times 100$$

Where,

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (cm)

T = Colony diameter in treatment (cm)

Results and Discussions

The sensitivity of 40 *P. oryzae* isolates was tested against two commonly used fungicides Viz., Tricyclazole and Carbendazim by poisoned food technique at four different concentrations. All the fungicides evaluated showed significant decrease in the growth of *P. oryzae* under *in vitro* conditions compared to control.

The radial growth of all the 40 pathogenic isolates was completely inhibited at 1000 and 1200 ppm concentrations of Carbendazim. While in 800 ppm, complete inhibition was observed in Nalgonda, Palem, Nizamabad, Nandyal and Aler. At 800 ppm, the inhibition of *P. oryzae* isolates ranged from 86.29 (Vijayawada) to 94.81% (Nellore). The differences were observed in IIRR, Bapatla, Veeravasaram, Karempudi, Madira, Podur and Palakollu were non-significant. Carbendazim 600 ppm concentration showed reduction in growth of all the *P. oryzae* isolates which varied from 78.14 (Vijayawada) to 92.22% (Tuni). The differences observed on inhibition of growth among the 40 isolates of *P. oryzae* were non-significant at 600 ppm (Table-1).

Fig.1 *In vitro* evaluation of commonly used fungicides against *P. oryzae* isolates

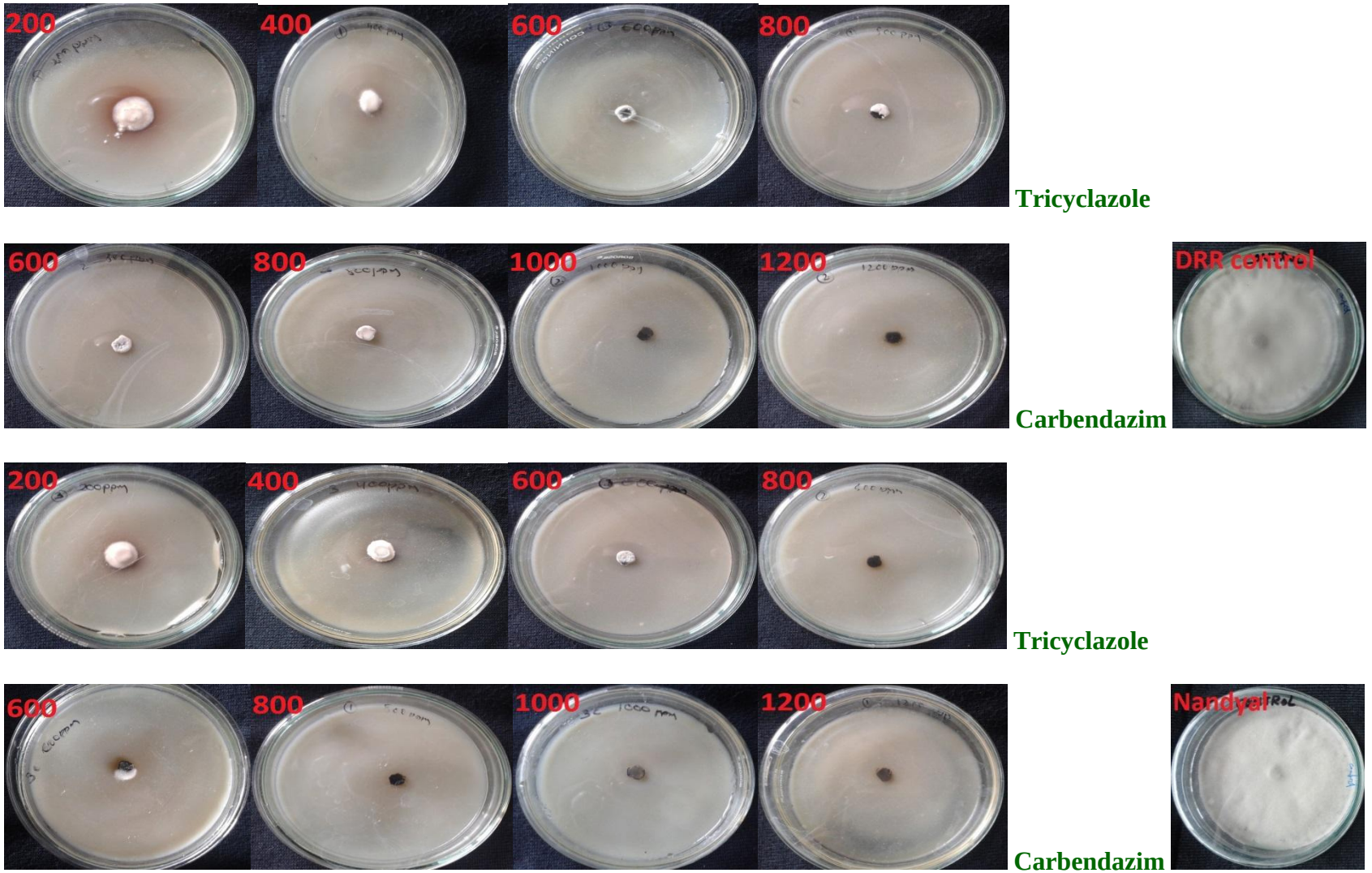

Fig.2 *In vitro* evaluation of fungicides (Tricyclazole)

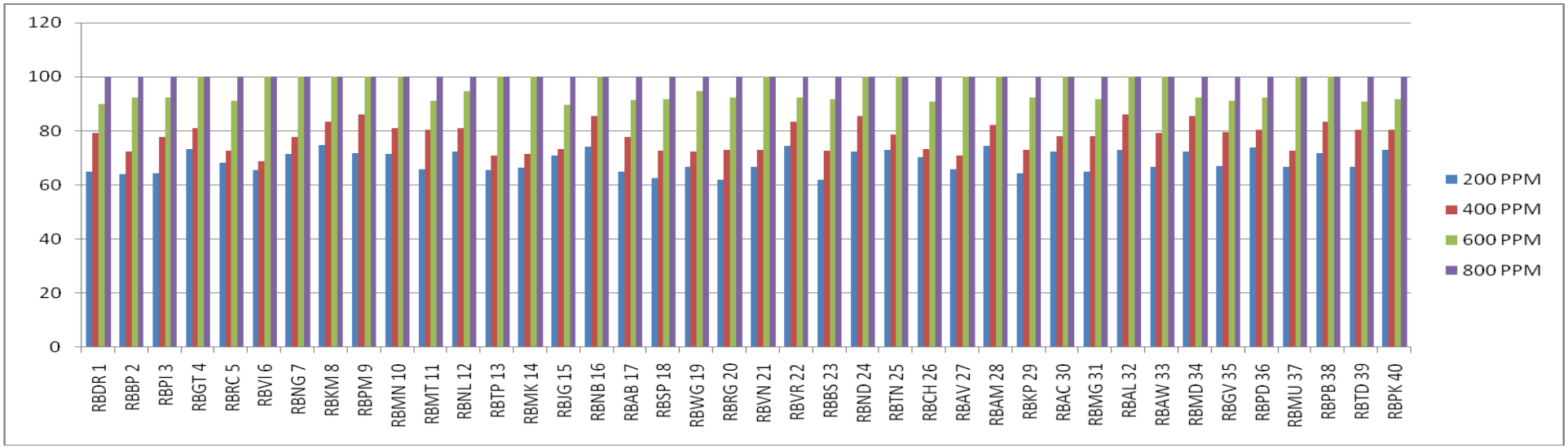

Fig.3 *In vitro* evaluation of fungicides (Carbendazim)

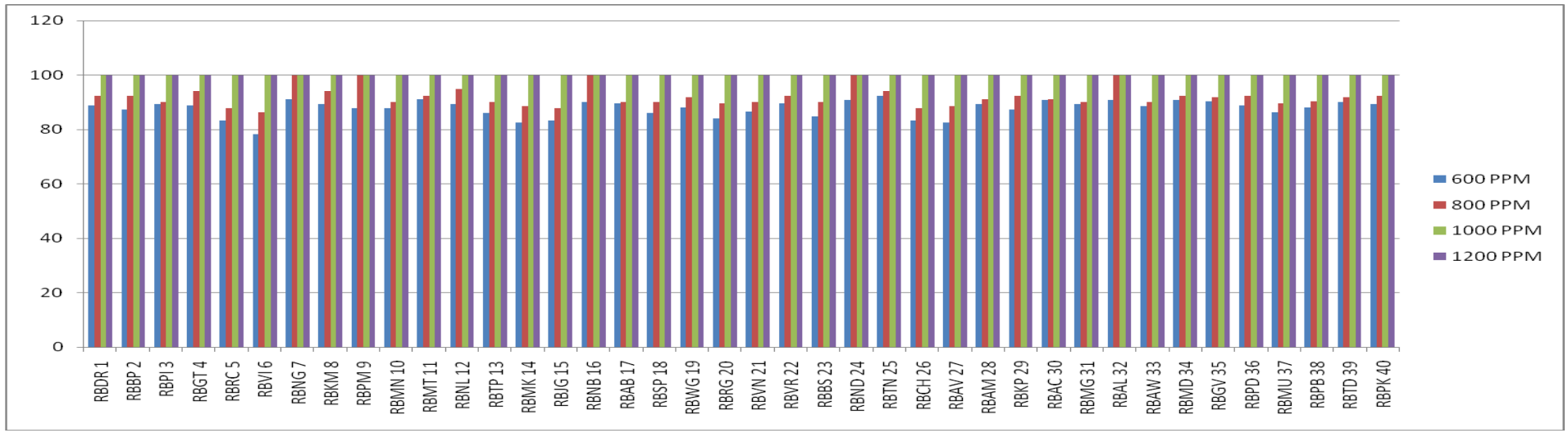

Table.1 Sensitivity of *P. oryzae* rice blast isolates collected from different rice growing areas of Telangana and Andhra Pradesh against the commonly used fungicides under *in vitro* conditions

S.no	Isolates	Tricyclazole concentrations (ppm)				Carbendazim concentrations (ppm)			
		200	400	600	800	600	800	1000	1200
1	IIRR	64.810 (53.59)	79.253 (62.88)	90.000 (71.53)	100.00 (90.00)	88.880 (70.49)	92.22 (73.77)	100.00 (90.00)	100.00 (90.00)
2	Bapatla	64.07 (53.15)	72.22 (58.16)	92.22 (73.77)	100.00 (90.00)	87.40 (69.19)	92.22 (73.80)	100.00 (90.00)	100.00 (90.00)
3	Piduguralla	64.44 (53.37)	77.77 (61.84)	92.22 (73.77)	100.00 (90.00)	89.25 (70.84)	90 (71.53)	100.00 (90.00)	100.00 (90.00)
4	Guntur	73.33 (58.89)	81.11 (64.23)	100.00 (90.00)	100.00 (90.00)	88.88 (70.49)	94.07 (78.41)	100.00 (90.00)	100.00 (90.00)
5	Rentachintala	68.14 (55.61)	72.59 (58.41)	91.11 (72.68)	100.00 (90.00)	83.33 (65.87)	87.77 (69.50)	100.00 (90.00)	100.00 (90.00)
6	Vijayawada	65.55 (54.03)	68.89 (56.08)	100.00 (90.00)	100.00 (90.00)	78.14 (62.10)	86.29 (68.25)	100.00 (90.00)	100.00 (90.00)
7	Nalgonda	71.480 (57.70)	77.770 (61.84)	100.00 (90.00)	100.00 (90.00)	91.106 (72.68)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
8	Khammam	74.81 (59.85)	83.33 (65.87)	100.00 (90.00)	100.00 (90.00)	89.25 (70.84)	94.07 (78.41)	100.00 (90.00)	100.00 (90.00)
9	Palem	71.85 (57.93)	85.92 (67.93)	100.00 (90.00)	100.00 (90.00)	87.77 (69.50)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
10	Mahanandi	71.48 (57.69)	81.11 (64.28)	100.00 (90.00)	100.00 (90.00)	87.77 (69.53)	90.00 (71.53)	100.00 (90.00)	100.00 (90.00)
11	Marteru	65.92 (54.26)	80.37 (63.68)	91.11 (72.62)	100.00 (90.00)	91.11 (72.69)	92.22 (73.77)	100.00 (90.00)	100.00 (90.00)
12	Nellore	72.22 (58.16)	81.11 (64.23)	94.81 (79.18)	100.00 (90.00)	89.25 (70.84)	94.81 (79.18)	100.00 (90.00)	100.00 (90.00)
13	Tirupathi	65.55 (54.03)	70.74 (57.24)	100.00 (90.00)	100.00 (90.00)	85.92 (67.93)	89.99 (71.58)	100.00 (90.00)	100.00 (90.00)
14	Patancheru	66.29 (54.48)	71.48 (57.70)	100.00 (90.00)	100.00 (90.00)	82.59 (65.32)	88.51 (70.16)	100.00 (90.00)	100.00 (90.00)

15	Jagityal	70.74 (57.23)	73.33 (58.88)	89.62 (71.20)	100.00 (90.00)	83.33 (65.87)	87.77 (69.50)	100.00 (90.00)	100.00 (90.00)
16	Nizamabad	74.07 (59.36)	85.55 (67.63)	100.00 (90.00)	100.00 (90.00)	90 (71.53)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
17	Adilabad	64.81 (53.59)	77.77 (61.84)	91.48 (73.00)	100.00 (90.00)	89.63 (71.18)	90 (71.53)	100.00 (90.00)	100.00 (90.00)
18	S.M Puram	62.59 (52.27)	72.59 (58.40)	91.85 (73.39)	100.00 (90.00)	85.92 (68.12)	90.00 (71.53)	100.00 (90.00)	100.00 (90.00)
19	Warangal	66.66 (54.71)	72.22 (58.16)	94.81 (79.18)	100.00 (90.00)	88.14 (69.84)	91.85 (73.39)	100.00 (90.00)	100.00 (90.00)
20	Ragolu	61.85 (51.83)	72.96 (58.64)	92.22 (73.77)	100.00 (90.00)	84.07 (66.46)	89.63 (71.18)	100.00 (90.00)	100.00 (90.00)
21	Vizianagaram	66.66 (54.71)	72.96 (58.64)	100.00 (90.00)	100.00 (90.00)	86.66 (68.55)	90 (71.53)	100.00 (90.00)	100.00 (90.00)
22	Veeranasaram	74.44 (59.60)	83.33 (65.87)	92.22 (73.77)	100.00 (90.00)	89.63 (71.18)	92.22 (73.77)	100.00 (90.00)	100.00 (90.00)
23	Basara	61.85 (51.83)	72.59 (58.40)	91.85 (73.39)	100.00 (90.00)	84.81 (67.04)	90 (71.53)	100.00 (90.00)	100.00 (90.00)
24	Nadyal	72.22 (58.16)	85.55 (67.63)	100.00 (90.00)	100.00 (90.00)	90.74 (72.28)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
25	Tuni	72.96 (58.64)	78.51 (62.35)	100.00 (90.00)	100.00 (90.00)	92.22 (73.77)	94.07 (78.41)	100.00 (90.00)	100.00 (90.00)
26	Chirala	70.37 (56.99)	73.33 (58.88)	90.74 (72.29)	100.00 (90.00)	83.33 (65.87)	87.77 (69.50)	100.00 (90.00)	100.00 (90.00)
27	Adivi	65.92 (54.26)	70.74 (57.23)	100.00 (90.00)	100.00 (90.00)	82.59 (65.32)	88.51 (70.16)	100.00 (90.00)	100.00 (90.00)
28	Amaravati	74.44 (59.61)	82.22 (65.05)	100.00 (90.00)	100.00 (90.00)	89.25 (70.84)	91.11 (72.62)	100.00 (90.00)	100.00 (90.00)
29	Karempudi	64.44 (53.37)	72.96 (58.64)	92.22 (73.77)	100.00 (90.00)	87.4 (69.19)	92.22 (73.80)	100.00 (90.00)	100.00 (90.00)
30	Atchempet	72.22 (58.17)	78.14 (62.10)	100.00 (90.00)	100.00 (90.00)	90.74 (72.26)	91.11 (72.68)	100.00 (90.00)	100.00 (90.00)
31	Mangalagiri	64.81	78.14	91.85	100.00	89.25	90	100.00	100.00

		(53.59)	(62.10)	(73.39)	(90.00)	(70.84)	(71.53)	(90.00)	(90.00)
32	Aleru	72.96 (58.64)	85.92 (67.93)	100.00 (90.00)	100.00 (90.00)	90.74 (72.28)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
33	Aswaraopet	66.66 (54.71)	79.26 (62.88)	100.00 (90.00)	100.00 (90.00)	88.51 (70.16)	90 (71.53)	100.00 (90.00)	100.00 (90.00)
34	Madhira	72.22 (58.16)	85.55 (67.63)	92.22 (73.77)	100.00 (90.00)	90.74 (72.26)	92.22 (73.77)	100.00 (90.00)	100.00 (90.00)
35	Gopavaram	67.03 (54.93)	79.62 (63.14)	91.11 (72.62)	100.00 (90.00)	90.37 (71.89)	91.85 (73.39)	100.00 (90.00)	100.00 (90.00)
36	Poduru	73.7 (59.12)	80.37 (63.68)	92.22 (73.77)	100.00 (90.00)	88.88 (70.49)	92.22 (73.77)	100.00 (90.00)	100.00 (90.00)
37	Muttukuru	66.66 (54.71)	72.59 (58.40)	100.00 (90.00)	100.00 (90.00)	86.29 (68.24)	89.63 (71.18)	100.00 (90.00)	100.00 (90.00)
38	Penubarti	71.85 (57.93)	83.33 (65.87)	100.00 (90.00)	100.00 (90.00)	88.14 (69.84)	90.37 (71.89)	100.00 (90.00)	100.00 (90.00)
39	Tandur	66.66 (54.71)	80.37 (63.68)	90.74 (72.26)	100.00 (90.00)	90 (71.53)	91.85 (73.39)	100.00 (90.00)	100.00 (90.00)
40	Palakollu	72.96 (58.64)	80.37 (63.68)	91.85 (73.39)	100.00 (90.00)	89.25 (70.84)	92.22 (73.77)	100.00 (90.00)	100.00 (90.00)

	Standard error (d)	**Standard error (m)**	**CD**
A	0.06	0.04	0.12
B	0.09	0.06	0.17
C	0.28	0.20	0.56
A*B	0.12	0.09	0.25
A*C	0.40	0.28	0.79
B*C	0.57	0.40	1.12
A*B*C	0.81	0.57	1.59

The radial growths of all the 40 pathogenic isolates were completely inhibited at 800 ppm of Tricyclazoe. While in 600 ppm, complete inhibition was observed in Guntur, Vijayawada, Nalgonda, Khammam, Palem, Mahanadi, Tirupati, Medak, Nijamabad, Vizianagaram, Nandyal, Tuni, Adivi, Amaravati, Achutapuram, Aler, Aswaraopet, Muttukur and Penubrati. The other remaining isolates showed maximum reduction (94.81%) in growth. The percent inhibition of *P. oryzae* isolates ranged from 89.62 (Jagityal) to 94.81% (Nellore) at 600 ppm. There was no significant difference among the isolates with respect to per cent inhibition at 600 ppm concentration. Tricyclazole at 400 ppm concentration showed maximum reduction (85.92%) in Palem and Aler and lowest growth reduction (68.89) in Vijayawada.

The differences among Piduguralla, Nalgonda, Adilabad, Guntur, Mahanandi, Nellore, Tandur, Palakollu, Podur, Tirupati, Medak and Maruteru isolates with respect to per cent inhibition at 400 ppm concentration were non-significant. Tricyclazole at 200 ppm concentration showed reduction in growth of all the *P. oryzae* isolates which varied from 61.85 (Ragolu and Basara) to 74.81% (Karimnagar). The differences observed on inhibition of growth among the 40 isolates of *P. oryzae* were significant at 200 ppm. However, the isolates of Karimnagar, Nizamabad, Veeravasaram and Karempudi did not differ significantly with respect to inhibitions at this concentration (Figure. 1, 2, 3).

These results indicated that majority of isolates did not develop any resistance against optimal doses of fungicides however isolates showed higher sensitivity at lower concentration. Intensive use of a particular fungicide with specific action leads to the development of resistance in the pathogen population.

Acknowledgments

I thank P. Narayan Reddy and M. Srinivas Prasad for giving opportunity to work in Lab.

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