International Journal of Plant & Soil Science

Volume 35, Issue 20, Page 333-339, 2023; Article no.IJPSS.106556
ISSN: 2320-7035

Assessing the Baseline Sensitivity of Predominant Soil-Borne Pathogens against Carbendazim

Chandana Y. Kundargi [a], I. Johnson [a*], M. Karthikeyan [b], R. Anandham [c] and M. Jayakanthan [d]

[a] Department of Plant Pathology, TNAU, Coimbatore - 641003, Tamil Nadu, India.
[b] Department of Crop Protection, AC&RI, Vazhavachanur - 606601, Tamil Nadu, India.
[c] Department of Agricultural Microbiology, TNAU, Coimbatore - 641003, Tamil Nadu, India.
[d] Department of Plant Molecular Biology and Bioinformatics, TNAU, Coimbatore - 641003, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i203813

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/106556

Original Research Article

Received: 11/07/2023
Accepted: 14/09/2023
Published: 20/09/2023

ABSTRACT

Fungicide resistance is reducing the effectiveness of fungicides in controlling plant diseases, leading to increase crop losses and the need for alternative control strategies. Addressing this issue requires careful fungicide use and the development of new management approaches. The main objective of present study was to determine the baseline sensitivity and development of fungicide resistance in predominant soil-borne pathogens against carbendazim at various concentrations. It was observed that the EC_{50} of *Fusarium oxysporum* and *Macrophomina phaseolina* was 9.970 and 16.294 µg ml^{-1}, respectively. The baseline sensitivity of both fungi was determined based on the inhibition percentage at which the pathogen showed sensitivity. Repeated transfer of these cultures at same concentrations for some generations showed the progressive decline in growth inhibition across generations resulting in the development of fungicide resistance.

**Corresponding author: E-mail: johnson.i@tnau.ac.in;*

These studies have demonstrated that overreliance on certain fungicides can lead to the development of resistant fungal strains, compromising the efficacy of disease management. This emphasizes alternative use of fungicides with different mode of action or following integrated disease management strategies to mitigate fungicide resistance and sustainably protect crops.

Keywords: Carbendazim; baseline sensitivity; fusarium; macrophomina.

1. INTRODUCTION

Plant pathogenic fungi cause severe crop diseases with global repercussions, and their management involves a careful balance of chemical and non-chemical approaches. Chemical management of plant diseases plays a significant role in modern agriculture and horticulture by effectively controlling and preventing the spread of plant diseases. Fungicides have been employed by farmers for more than 200 years to safeguard their crops against fungi, despite the toxic residues they leave behind and have a negative impact on other organisms like beneficial and non-target organisms, birds, fish etc [1,2]. Over time there's a failure in control of plant diseases in field even after lumpsum application of recommended fungicides. This is the first sign of fungicide resistance development in the field.

The baseline serves as the reference point for the acknowledged fungal responsiveness to a particular fungicide. To observe any potential shift towards resistance in the reference, it is imperative to be aware of the sensitivity baseline for the fungus-fungicide combination. This knowledge enables the monitoring of fungicide effects on the fungus, allowing for the detection of any changes in its susceptibility over time [3]. Fungicide resistance poses significant challenges to effective disease control and ecosystem health, emphasizing the need for sustainable and responsible agricultural practices. Addressing fungicide resistance requires a comprehensive approach that considers economic implications, optimizes fungicide use, and preserves beneficial fungi. Educating the farmers about fungicide resistance, proper fungicide use, and the necessity of fungicide rotation strategies is essential. The ultimate goal is not just to manage resistance but to prevent or delay its development by understanding baseline sensitivity and tracking fungicide's impact on fungi.

Around 1970, benzimidazoles began to exhibit resistance to many pathogens after only two years of commercial use [4]. Soil-borne pathogens like *Macrophomina* spp. and *Fusarium* spp. cause substantial economic loss due to their effects on yield and quality in many agricultural and horticultural crops. Previously carbendazim resistance was studied by several scientific workers in *Fusarium* spp. [5,6] and in *Macrophomina* spp. [7]. Considering the risk of resistance development against recommended fungicide, it is obvious to document the shift in sensitivity of pathogens and hence present study is done.

2. MATERIALS AND METHODS

2.1 Collection of Test Pathogens

The two test pathogens i.e., *Fusarium oxysporum* and *Macrophomina phaseolina* were obtained from the Department of Plant Pathology, TNAU, Coimbatore – 641 003.

2.2 Fungicide and Stock Solution Preparation

The commercial formulation of carbendazim was purchased from a pesticides shop in Coimbatore. Preparation of 1000 µg ml^{-1} fungicide stock solution was prepared by dissolving carbendazim in methanol and further in distilled water. Before usage, the stock solution was sterilized by filtering through a 0.22 µm syringe filter to remove any potential microbial contaminants or any particulate matter or undissolved solids from solution that might interfere with the experiment and stored in a cool, dry place away from direct sunlight or heat source for further studies.

2.3 Sensitivity of Test Pathogens to Carbendazim

In vitro screening of fungi against Carbendazim was carried out by "Poisoned food technique" [8] to determine the baseline sensitivity and fungicide resistance. Mycelial plugs (9 mm in diameter) taken from the actively growing margins of the culture and placed at the centre of PDA amended with different concentrations of carbendazim for *F. oxysporum,* being 10, 50,

100, 500, 1000 µg ml^{-1} and *M. phaseolina,* being 10, 50, 100, 150, 200 µg ml^{-1}, respectively. PDA without carbendazim and inoculated with fungal organisms alone served as a control. Each concentration was replicated thrice and the inoculated plates were incubated in an incubator at 27±2°C. The mycelial growth of the fungal colonies were measured in mm at every 24 hours until the control plates were completely covered. The percent inhibition over control was calculated by the following formula as given by Bliss [9].

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

Where;

PI = Percent inhibition in mycelial growth
C = Mycelial growth in control plates
T = Mycelial growth in treated plates

2.4 Effect of Carbendazim on Mycelial Biomass of Test Pathogens

To test the effect of carbendazim on mycelial biomass of the two test soil-borne pathogens in PD broth, a 9mm in diameter mycelial disc was used to inoculate each 100 ml PD broth amended with the above-mentioned fungicide concentration. To estimate the weight of the mycelial mat, the fungal mycelium was harvested after 10 days of incubation, by separating it from the PD broth by filtering through Whatman No. 1 filter paper, and the wet weight was calculated. Further, the dry weight of the fungus was determined after drying the mycelial mat at 45°C overnight.

2.5 Determination of Baseline Sensitivity

The baseline sensitivity concentration was determined at which the response is closest to the baseline (no fungicide effect). The dose-response curve was plotted and the EC_{50} value was determined using IBM SPSS Statistics Ver.22 software. The concentration beyond the EC_{50} value was selected at which there was minimal mycelial growth and sub-cultured on the PDA plates containing the same concentration for several generations. For each generation, the control plate was, also, maintained without any fungicide amendment. Mycelial growth was recorded at 24 hrs interval until the control plate reached complete growth. The dose-response curve and the statistical analysis were performed using the software IBM SPSS Statistics Ver.22.

3. RESULTS AND DISCUSSION

3.1 Effect of Carbendazim on the Mycelial Growth of the Two Test Fungi

The sensitivity test of the two pathogens was carried out against carbendazim by the Poisoned food technique. Fungicide sensitivity was determined by measuring radial growth on PDA plates containing different concentrations from 0-1000µg ml^{-1} of fungicide and expressed as a percent of inhibition. PDA plates inoculated with any of the two pathogens without fungicide served as a control.

3.1.1 *Fusarium oxysporum*

From the data presented in Table (2), at 50 µg ml^{-1}, the inhibition percent was 65.55%, and at 100 µg ml^{-1}, the mycelial growth was completely inhibited (Table 1) with the EC_{50} value of 9.970 µg ml^{-1} by the dose response curve (Fig. 1. b) which is similar to the EC_{50} values of carbendazim resistant collections averaged 7.02 ± 11.86 µg ml^{-1} by Chen et al. [5] in *F. graminearum. F. oxysporum* shows a strong response to carbendazim at both 50 and 100 µg ml^{-1}, with higher inhibition at 50 µg ml^{-1}. The reduced response at 50 µg ml^{-1} compared to 10 µg ml^{-1} suggests that *F.oxysporum* is showing some level of reduced sensitivity to carbendazim. This could indicate the possibility of early signs of carbendazim resistance where 100% growth inhibition is a strong indicator of resistance. In the study conducted by Datta and Tarafder [10], *F. solani* SF0301 isolate could grow in 100 µg ml^{-1} of carbendazim with an ED_{50} value of 0.98 µg ml^{-1} while in our study 100% inhibition is observed at the same concentration suggesting a significant development in carbendazim resistance.

At higher concentrations (100, 500 and 1000 µg ml^{-1}), the fungal biomass showed complete inhibition, indicating sensitivity or effective control. The two fungi were affected by the carbendazim at lower doses (10 and 50 µg ml^{-1}), which potentially indicated the development of carbendazim resistance (Fig. 1. a).

At 10 µg ml^{-1}, there was a significant growth inhibition of 57.77%. Even at the lowest tested concentration, there was an observable effect on inhibiting *F. oxysporum* growth. This concentration represents the lowest effective concentration that inhibits *F. oxysporum* growth and serves as a baseline sensitivity for

comparing responses and potential resistance across different concentrations.

At 50 µg ml^{-1}, which is beyond EC_{50} value was sub-cultured for four generations along with a control, in which growth inhibition was 65.55, 65.22, 64.88, and 55.55% at 1st, 2nd, 3rd, and 4th generation, respectively (Table 2). This decreasing in inhibition indicates that *F. oxysporum* response to carbendazim was diminishing over generations and it was adapting and becoming resistant to the carbendazim.

Table 1. Effect of carbendazim on mycelial growth and fungal biomass of *Fusarium oxysporum*

Concentration (µg ml^{-1})	Days after inoculation					Mycelial weight (mg)*		Inhibition over control* (%)
	2	4	6	8	10			
	Mycelial growth in (mm)*					Wet	Dry	
10	-	21.0	28.0	31.3	38.0	1150	105	57.77 (49.47)
50	-	20.3	28.1	28.5	31.0	1010	103	65.55 (54.07)
100	-	-	-	-	-	-	-	100 (89.32)
500	-	-	-	-	-	-	-	100 (89.32)
1000	-	-	-	-	-	-	-	100 (89.32)
Control	27.6	59.0	86.6	88.0	90.0	6020	292	0 (0.67)
SEd								0.63
CD(0.01)								1.92

**Mean of three replications. The figure within the parenthesis are arcsine transformed values*

Table 2. Effect of carbendazim on mycelial growth of *Fusarium oxysporum* at different generations

Generations	Days after inoculation										Inhibition over control (%)*
	Control					Treated					
	Mycelial growth (mm)*										
	2	4	6	8	10	2	4	6	8	10	
I	27.6	59.0	86.6	88.0	**90.0**	-	20.3	28.1	28.5	**31.0**	65.55
II	37.6	62.0	79.0	85.0	**90.0**	-	28.0	29.6	29.0	**31.3**	65.22
III	41.0	65.0	83.0	**90.0**	**90.0**	-	28.5	30.0	30.0	**31.6**	64.88
IV	48.3	67.0	88.0	**90.0**	**90.0**	-	29.6	32.0	36.0	**40.0**	55.55

**Mean of three replications*

Fig. 1. (a) Effect of carbendazim on the mycelial growth of *F. oxysporum*

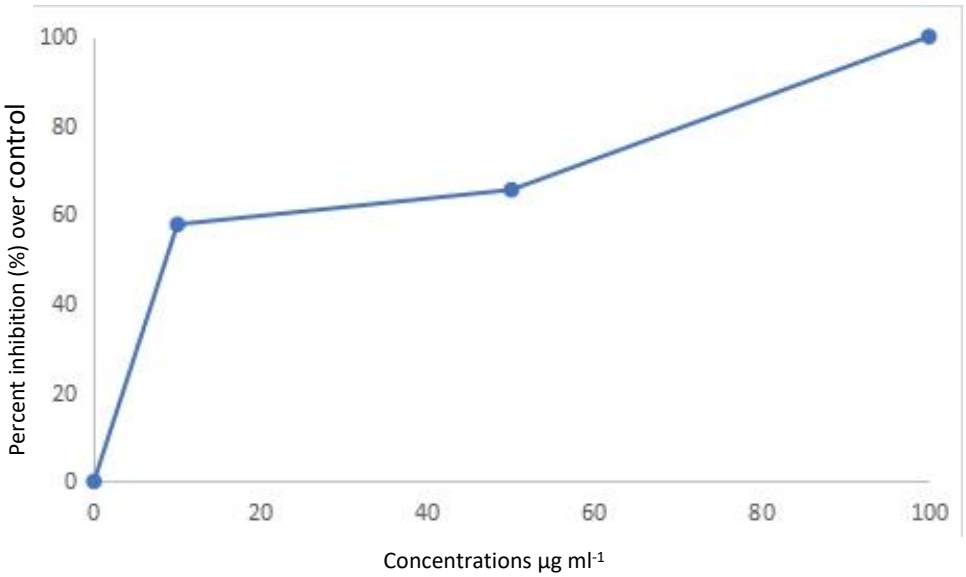

Fig. 1. (b) Dose response curve of *F. oxysporum* to carbendazim on agar plate.

3.1.2. *Macrophomina phaseolina*

Mycelial growth (Fig. 2. a) was, completely inhibited at 150 µg ml^{-1} of carbendazim while, at decreased concentrations of 100, 50, and 10 µg ml^{-1} there was a decreased growth inhibition of 78.14, 65.55, and 44.81% respectively (Table 3). The decreasing effectiveness of carbendazim at lower concentrations suggested the possibility of carbendazim resistance with the EC_{50} value of 16.294 µg ml^{-1} by the dose response curve (Fig. 2. b).

The carbendazim was effective at concentrations of 10 to 100 µg ml^{-1}, as evidenced by the reduction in dry weight of the fungus. However, at 150 and 200 µg ml^{-1}, the mycelial growth was completely inhibited, indicating possible resistance to the carbendazim at higher concentrations.

Considering the concentration beyond EC50 value with minimal mycelial growth *i.e*., 100 µg ml^{-1} was sub-cultured for generations along with a control, in which growth inhibition started decreasing on generations *i.e*., 78.14, 45.22, 21.55, and no growth inhibition (0%) at 1st, 2nd, 3rd and 4th generation, respectively (Table 4). The progressive decline in growth inhibition across generations revealed the development of carbendazim resistance in *M. phaseolina* as like in the sensitive *M. phaseolina* (Mp-3) isolate's resistance was markedly boosted by continuous carbendazim culture for eight successive passages in the experiment studied by Japtap et al. [7] which caused rot of Maize. The complete loss of growth inhibition in the fourth generation indicates that the pathogen has likely acquired a level of resistance that renders it unaffected by carbendazim.

Table 3. Effect of carbendazim on mycelial growth and fungal biomass of *Macrophomina phaseolina*

Concentration (µg ml^{-1})	Days after inoculation					Mycelial weight (mg)*		Inhibition over control* (%)
	2	4	6	8	10			
	Mycelial growth in (mm)*					Wet	Dry	
10	-	21.0	34.0	40.0	49.6	5630	664	44.81 (42.01)
50	-	-	24.0	28.0	31.0	4828	332	65.55 (54.35)
100	-	-	-	18.0	19.66	3150	221	78.14 (62.12)
150	-	-	-	-	-	-	-	100 (89.32)
200	-	-	-	-	-	-	-	100 (89.32)
Control	36.0	90.0	90.0	90.0	90.0	11104	1107	0 (0.67)
SEd								2.91
CD(0.01)								6.34

**Mean of three replications. The figure within the parenthesis are arcsine transformed values*

Table 4. Effect of carbendazim on mycelial growth of *Macrophomina phaseolina* at different generations

Generations	Days after inoculation										Inhibition over control (%)*
	Control					Treated					
	Mycelial growth (mm)*										
	2	4	6	8	10	2	4	6	8	10	
I	36.0	**90.0**	**90.0**	**90.0**	**90.0**	-	-	-	18.0	**19.6**	78.14
II	36.0	71.0	**90.0**	**90.0**	**90.0**	19.0	21.3	29.0	41.3	**49.3**	45.22
III	48.0	78.6	**90.0**	**90.0**	**90.0**	23.1	35.3	50.3	55.6	**70.6**	21.55
IV	50.0	80.3	**90.0**	**90.0**	**90.0**	27.0	48.0	66.0	69.3	**90.0**	0

**Mean of three replications*

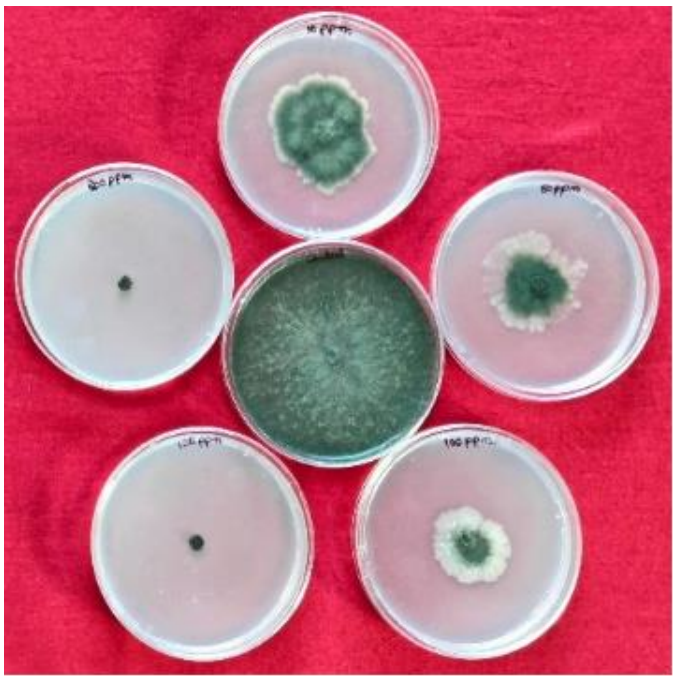

Fig. 2. (a) Effect of carbendazim on the mycelial growth of *M. phaseolina*

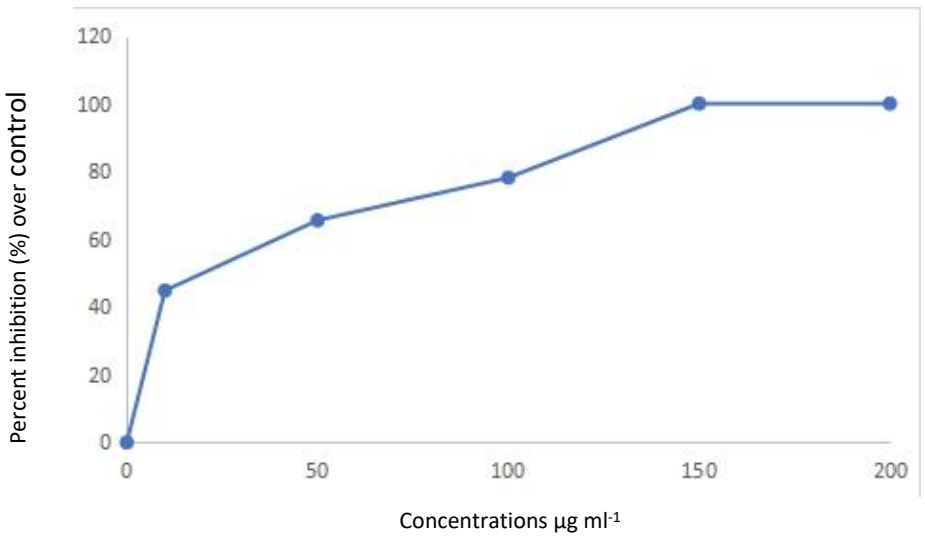

Fig. 2. (b) Dose response curve of *M. phaseolina* to carbendazim on agar plate

4. CONCLUSION

The present study investigated the baseline sensitivity and potential resistance of predominant soil-borne pathogens i.e., *F. oxysporum* and *M. phaseolina* to the carbendazim. The results highlighted the diverse responses of two fungi to carbendazim exposure, reflecting the complex nature of fungicide-fungus interactions. The findings demonstrated that *F. oxysporum* showed a strong sensitivity to carbendazim at lower concentrations, indicating effective control. However, signs of reduced sensitivity were observed at even lower concentrations in the subsequent generations, suggesting the early stages of resistance. *M. phaseolina,* also, displayed decreasing sensitivity to carbendazim at lower concentrations, implying the possibility of resistance development.

In conclusion, addressing fungicide resistance requires a multifaceted approach that considers the specific characteristics of each fungus-fungicide interaction. However, the preliminary study of the Poison food technique did not provide a comprehensive understanding of the underlying mechanisms causing resistance. Hence, molecular basis analysis is necessary to identify specific genetic changes or mutations that might be responsible for the resistance. However, this information is crucial for developing effective strategies to manage and combat fungicide resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dignam BE, Marshall SD, Wall AJ, Mtandavari YF, Gerard EM, Hicks E, Cameron C, Aalders LT, Shi S, Bell NL. Impacts of soil-borne disease on plant yield and farm profit in dairying soils. Journal of Sustainable Agriculture and Environment. 2022 Mar;1(1):16-29.
2. Thurston AM, Waller L, Condron L, Black A. Sensitivity of the soil-borne pathogen Phytophthora agathidicida, the causal agent of kauri dieback, to the anti-oomycete fungicides ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin. New Zealand Plant Protection. 2022 May 3;75:14-8.
3. Russell PE. A century of fungicide evolution. The Journal of Agricultural Science. 2005 Feb;143(1):11-25.
4. Smith CM. History of benzimidazole use and resistance.
5. Chen C, Wang J, Luo Q, Yuan S, Zhou M. Characterization and fitness of carbendazim-resistant strains of *Fusarium graminearum* (wheat scab). Pest Management Science: Formerly Pesticide Science. 2007 Dec;63(12):1201-7.
6. Xu S, Wang J, Wang H, Bao Y, Li Y, Govindaraju M, Yao W, Chen B, Zhang M. Molecular characterization of carbendazim resistance of Fusarium species complex that causes sugarcane pokkah boeng disease. Bmc Genomics. 2019 Dec;20(1): 1-4.
7. Jagtap SG, Kamble SS, Kavale TR. Effect of passage on the development of carbendazim resistance in *Macrophomina phaseolina* causing charcoal rot of maize. International Journal of Researches in Biosciences, Agriculture and Technology. 2017;5(3):313-6.
8. Schmitz, Henry. A suggested toximetric method for wood preservatives. Industrial & Engineering Chemistry Analytical Edition. 1930;2(4):361-363.
9. Bliss CI. The method of probits. Science. 1934 Jan 12;79(2037):38-9.
10. Tarafder M, Datta B. Deciphering β-tubulin gene of carbendazim resistant *Fusarium solani* isolate and its comparison with other *Fusarium species*. Current Genetics. 2022 Aug;68(3-4):429-47.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/106556