Original Research Article

Assessing the baseline sensitivity of predominant soil-borne pathogens against carbendazim

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ABSTRACT

Fungicide resistance is reducing the effectiveness of fungicides in controlling plant diseases, leading to increased 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 EC50 of *F. oxysporum*and *M. phaseolina*is 9.970 and 16.294 µg/ml. The baseline sensitivity of *F.oxysporum* and *M. phaseolina* is determined based on the inhibition percentage at which the pathogen shows 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. 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. 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 (Russell, 2005). 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 (Smith, 1988). Soil-borne pathogens like *Macrophomina* spp. and *Fusarium* spp. causes substantial economic loss due to its effects on yield and quality in many agricultural and horticultural crops. Previously carbendazim resistance was studied by several scientific workers in *Fusarium*spp (Chen *et al*., 2007; Xu *et al*., 2019) and in *Macrophomina* spp. (Jagtap *etal*., 2017). 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. MATERIAL AND METHODS

2.1 Collection of test pathogens

*Fusarium oxysporum*and*Macrophominaphaseolina* were the pathogensobtained 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 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 0.2 µm syringe filter 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" (Schmitz, 1930) to determine the baseline sensitivity and fungicide resistance. A 9 mm-dia mycelial plug from the actively growing margins of the culture and placed at the centre of PDA amended with different concentrations of carbendazim for *F. oxysporum*(10, 50, 100, 500, 1000 µg/ml)and *M. phaseolina*(10, 50, 100, 150, 200 µg/ml). PDA without carbendazim and inoculated with fungal organisms alone served as a control. Each concentration was replicated thrice and the inoculated plates were incubated at a room temperature of 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 (1934).

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

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 soil-borne pathogens in PD broth, a 9mm diameter mycelial disc was inoculated in 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 EC50 value was determined using IBM SPSS Statistics Ver.22 software. The concentration beyond the EC50 value is 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 fungi

The sensitivity test of 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 of fungicide and expressed as a percent of inhibition. PDA plates inoculated with pathogen without fungicide served as a control.

3.1.1.*Fusarium oxysporum*

From the data shown in Table 2, at 50 µg/ml, the inhibition percent is 65.55%, and at 100 µg/ml, the mycelial growth was completely inhibited (Table 1) with the EC50 value of 9.970 µg/ml by the dose response curve (Fig. 1. b) which is similar to the EC50 values of carbendazim resistant collectionsaveraged 7.02 ± 11.86 µg/ml by (Chen *et* al.,2007) in *F. graminearum. F. oxysporum* shows a strong response to carbendazim at both 50 and 100 µg/ml, with higher inhibition at 50 µg/ml. The reduced response at 50 µg/ml compared to 10 µg/ml 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.

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

At 10 µg/ml, there is a significant growth inhibition of 57.77%. Even at the lowest tested concentration, there is 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.

50 µg/ml, which is beyond EC50 value was sub-cultured for four generations along with a control, growth inhibition was 65.55%, 65.22%, 64.88%, and 55.55% at 1st, 2nd, 3^{rd,} and 4th generation, respectively (Table 2). This decreasing 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)	Days after inoculation					Mycelial weight (mg)*		Inhibition over control* (%)
	2	4	6	8	10			
	Mycelial growth in (mm)*					Wet	Dry	
10	-	21	28	31.3	38	1150	105	57.77 (49.47)
50	-	20.3	28.1	28.5	31	1010	103	65.55 (54.07)
100	-	-	-	-	-	-	-	100 (89.32)
500	-	-	-	-	-	-	-	100 (89.32)
1000	-	-	-	-	-	-	-	100 (89.32)
Control	27.6	59	86.6	88	90	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.

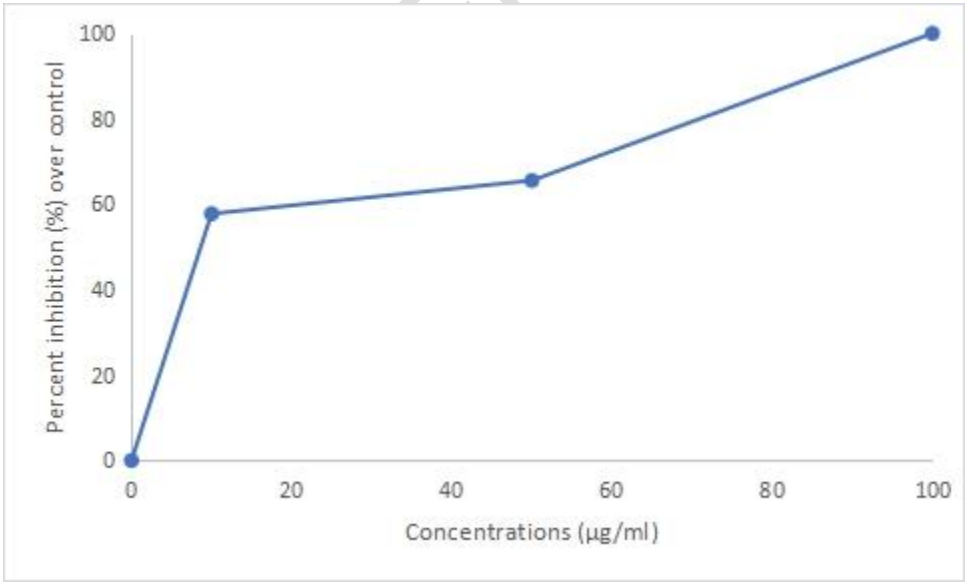
Generations	Days after inoculation										Inhibition over control (%)*
	Control					Treated					
	Mycelial growth (mm)*										
	2	4	6	8	10	2	4	6	8	10	
I	27.6	59	86.6	88	**90**	-	20.3	28.1	28.5	**31**	65.55

II	37.6	62	79	85	**90**	-	28	29.6	29	**31.3**	65.22
III	41	65	83	**90**	**90**	-	28.5	30	30	**31.6**	64.88
IV	48.3	67	88	**90**	**90**	-	29.6	32	36	**40**	55.55

*Mean of three replications

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

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



3.1.2. *Macrophominaphaseolina*

Mycelial growth (Fig. 2. a) was, completely inhibited at 150 µg/ml of carbendazim while, at decreased concentrations of 100, 50, and 10 µg/ml 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 EC50 value of 16.294 µg/ml by the dose response curve (Fig. 2. b).

The carbendazim was effective at concentrations of 10 to 100 µg/ml, as evidenced by the reduction in dry weight of the fungus. However, at 150 and 200 µg/ml, 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 was sub-cultured for generations along with a control, 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*aslike 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.,2017) 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 *Macrophominaphaseolina*

Concentration (µg/ml)	Days after inoculation					Mycelial weight (mg)*		Inhibition over control* (%)
	2	4	6	8	10			
	Mycelial growth in (mm)*					Wet	Dry	
10	-	21	34	40	49.6	5630	664	44.81 (42.01)
50	-	-	24	28	31	4828	332	65.55 (54.35)
100	-	-	-	18	19.66	3150	221	78.14 (62.12)
150	-	-	-	-	-	-	-	100 (89.32)
200	-	-	-	-	-	-	-	100 (89.32)
Control	36	90	90	90	90	11104	1107	0 (0.67)
							SEd	2.91
							CD(0.01)	6.34

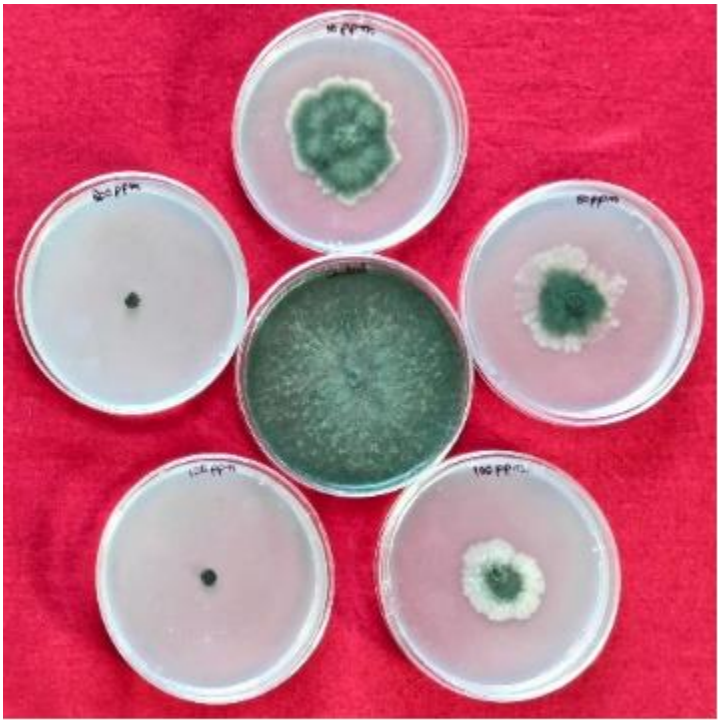
*Mean of three replications. The figure within the parenthesis are arcsine transformed values.

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

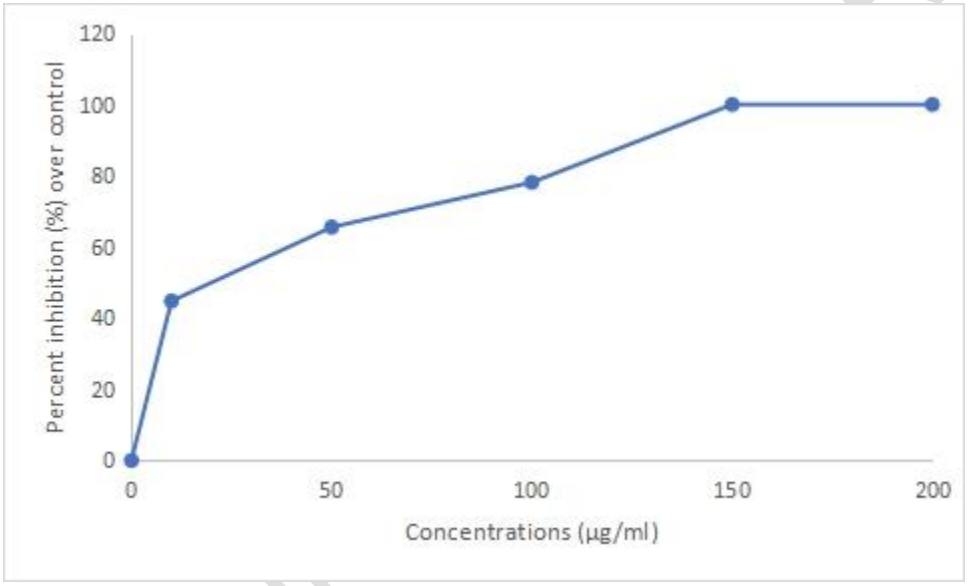
Generations	Days after inoculation										Inhibition over control (%)*
	Control					Treated					
	Mycelial growth (mm)*										
	2	4	6	8	10	2	4	6	8	10	
I	36	**90**	**90**	**90**	**90**	-	-	-	18	**19.6**	78.14
II	36	71	**90**	**90**	**90**	19	21.3	29	41.3	**49.3**	45.22
III	48	78.6	**90**	**90**	**90**	23.1	35.3	50.3	55.6	**70.6**	21.55
IV	50	80.3	**90**	**90**	**90**	27	48	66	69.3	**90**	0

*Mean of three replications

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



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



4. CONCLUSION

The study investigated the baseline sensitivity and potential resistance of predominant soil-borne pathogens to the carbendazim. The results highlighted the diverse responses of two fungus 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’ntprovide 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 DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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