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EFFECT OF PASSAGE ON THE DEVELOPMENT OF CARBENDAZIM RESISTANCE IN MACROPHOMINA PHASEOLINA CAUSING CHARCOAL ROT OF MAIZE

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Abstract

Culturing of the sensitive *Macrophomina phaseolina* (Mp-3) isolate on carbendazim continuously for eight successive passages significantly increased resistance. Use of carbendazim alternately with Kocide, Mancozeb, Aliette and Ridomil for eight successive passages significantly reduced carbendazim resistance at 3rd passage only. Inoculation of *M. phaseolina* on maize plant continuously for eight successive passages increased the carbendazim resistance. Carbendazim alternately with Kocide, Mancozeb, Aliette and Ridomil completely prevented the infection of pathogen to maize plant at 3rd passage. Carbendazim in mixture with Kocide, Mancozeb, Aliette and Ridomil completely inhibited the growth of pathogen at 1st passage both *in vitro and in vivo*.

Keywords: charcoal rot, carbendazim, *Macrophomina phaseolina*, passage, resistance

1. Introduction

Maize (*Zea mays* L.) is a large grain plant domesticated by indigenous people in Mesoamerica in prehistoric time. It is best grown in warm, tropical regions as it requires warm soil to develop optimally. It is an annual grass belonging to Poaceae and is a staple food crop grown all over the world. It is also commonly grown as a feed for livestock.

Such an important crop suffers from many fungal diseases, such as common smut caused by *Ustilago maydis* (de Candole) Corda, head smut caused by *Sphacelotheca reiliana* (Kunhn) Clinton, brown rot caused by *Physoderma zeae maydis* F. J. Shaw, rust caused by *Puccinia sorghi* Schw., leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard *et* Suggs. Seedling blight and wilt caused *Fusarium moniliforme* var. *subglutinans* Wollenw and Reinking, seedling blight and top rot caused by *Giberella zeae* (Schwein) Petch, Charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid. Among these charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid. is serious.

Fungicide treatments are, and will remain, essential for maintaining healthy crops and reliable, high-quality yields. They form a key component of integrated crop management and their effectiveness must be sustained as long as possible. This disease is controlled by using various systemic and conventional fungicides by the farmers. Pathogen resistance to fungicides is widespread. The aim of the present study was to examine the effect of passage on the development of carbendazim resistance in charcoal rot of maize.

2. Material and Methods

Samples of maize exhibiting charcoal rot symptom were collected from different places in India. 16 isolates of the *Macrophomina phaseolina* were obtained from infected stem on the Czapek dox agar medium. Their sensitivity against Carbendazim was tested by Food poisoning technique (1). A series of carbendazim dilutions were prepared from a 1000µg/ml stock solution by dissolving in sterile distilled water. Each concentration was added to autoclaved Czapek dox agar medium. 30 ml treated agar was poured in 90 mm petri plates. 6 mm diameter agar disc, cut from the edges of actively growing fungal colonies, were placed at centre on each plate containing treated agar. The plates were then incubated at 30±2°C in the dark and linear growth was measured at different intervals. Plates without carbendazim served as control. For *in vivo* studies pot experiments were followed. Maize plants were treated with different concentrations of carbendazim solutions..

After determination of MIC of carbendazim the effects of passage on the development of carbendazim resistance in continuous, alternate and in mixture with other fungicides was studied *in vitro* and *in vivo*.

Continuous passage : To study the effect of continuous passage *in vitro*, wild sensitive isolate MP-3 in each passage was cultured on plates with 1µg/ml carbendazim in triplicate. Agar disc of 6 mm diameter from the previous passage of the same isolate was placed at the centre of each plate in triplicate. In each passage, linear mycelial growth was measured after 6 days. The development of resistance was studied up to 8 th passage.

Alternate passage: To study the effect of alternate passage *in vitro*, wild sensitive isolate MP-3 in each passage was cultured on plates with 1µg/ml carbendazim in triplicate. Agar disc of 6 mm diameter from the previous passage of the same isolate was placed at the centre of each plate in triplicate containing same concentration of different fungicides like Kocide, Mancozeb, Aliette and Ridomil. In each passage, linear mycelial growth was measured after 6 days. The development of resistance was studied up to 8 th passage.

Mixed passage: To study the effect of mixed passage *in vitro*, wild sensitive isolate MP-3 in each passage was cultured on plates with 1µg/ml mixer of carbendazim and respective fungicides in triplicate. Agar disc of 6 mm diameter from the previous passage of the same isolate was placed at the centre of each plate in triplicate. In each passage, linear mycelial growth was measured after 6 days. The development of resistance was studied up to 8th passage.

To study the effect of passage *in vivo*, earthen pots were used. In each pot five plantlets of maize were grown for 21 days and after 21 days each pot plants were treated with different fungicides having concentration
(0.5µg/ml). 24 hours of fungicide treatment the pot plants were inoculated with mycelial sclerotial suspension containing 52 sclerotia per ml and observation made after 6 days.

Results:

From Table. 1 it was observed that during the continuous passage there was significant increase in the resistance of the pathogen while there was decrease in the resistance of the pathogen during alternate passage. In *in vivo* studies it was observed that use of Kocide, Mancozeb, Aliette and Ridomil showed complete inhibition of pathogen at III rd passage only. In *in vitro* studies, in mixture passage it was interesting to note that when carbendazim was used along with Kocide, Mancozeb, Aliette and Ridomil completely inhibited the growth of M. phaseolina in first passage only (Table. 3 and 4).

Table 1 : Effect of exposure of *Macrophomina phaseolina* (*In vitro*) to carbendazim continuous and alternating with other fungicides on the development of resistance during eight successive passages

Fungicides	Passage Number							
	1	2	3	4	5	6	7	8
Carbendazim Continuous	8.33	10.33	13.00	14.33	16.33	18.66	18.66	18.66
Carbendazim Alters kocide	8.33	32.33	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Mancozeb	8.33	23.66	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Aliette	8.66	28.66	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Ridomil	8.33	25.66	00.00	00.00	00.00	00.00	00.00	00.00

Table 2 : Effect of exposure of *Macrophomina phaseolina* (*In vivo*) to carbendazim continuous and alternating with other fungicides on the development of resistance during eight successive passages

Fungicides		Passage Number							
		1	2	3	4	5	6	7	8
Carbendazim Continuous	Percentage of infection	6.66	13.20	13.20	20.00	20.00	26.60	26.60	33.66
Carbendazim Alters kocide	Percentage of infection	6.66	26.60	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Mancozeb	Percentage of infection	6.66	20.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Aliette	Percentage of infection	6.66	33.66	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Ridomil	Percentage of infection	6.66	33.66	00.00	00.00	00.00	00.00	00.00	00.00

Table 3 : Effect of exposure of *Macrophomina phaseolina* (*In vitro*) in the mixture of carbendazim other fungicides on the development of resistance (growth in mm) during eight successive passages

Fungicides	Passage Number							
	1	2	3	4	5	6	7	8
Carbendazim Continuous	8.33	10.33	13.00	14.33	16.33	18.66	18.66	18.66
Carbendazim + kocide	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Mancozeb	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Aliette	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim +Ridomil	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00

Table 4 : Effect of exposure of *Macrophomina phaseolina* (*In vivo*) to carbendazim mixed with other fungicides on the development of resistance during eight successive passages

Fungicides		Passage Number							
		1	2	3	4	5	6	7	8
Carbendazim + kocide	Percentage of infection	0.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Mancozeb	Percentage of infection	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Aliette	Percentage of infection	0.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Ridomil	Percentage of infection	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00

3. Discussion

Resistance to fungicides has in fungal pathogen become a serious issue in the management of crop diseases and has threatened the potential of some highly effective commercial products. Incidence of resistance to fungicides has remained restricted mainly to systemic fungicides that operate against single bio-chemical targets also known as single site inhibitors (2,3,4). Alternate use of ediphenphos with carbendazim reduced carbendazim resistance in *Septoria nodorum* and *Cercosporella herpotrichoides* (5). There is a mathematical model to test different chemicals for their alternate use (6). Late blight of potato can be controlled by alternate use of metalaxyl with mancozeb (7). Effect of successive passage on metalaxyl resistant and metalaxyl sensitive isolates of *P. infestans* collected from Nilgiris to metalaxyl used individually, alternately with contact fungicides or in mixture revealed that treatment of metalaxyl in mixture with carbendazim and mancozeb reduced development of metalaxyl resistance in the pathogen (8). Development of cross resistance to related products is another area that has attracted attention of the workers. The build up of fungicide resistance among the pathogen would be less if a combination of fungicides used is both systemic in nature and a multisite inhibitor (2).Use of carbendazim alternately with captafol, Mancozeb, thiram and benomyl reduced the carbendazim resistance in *M. phaseolina* causing charcoal rot of pigeon pea (9). Use of carbendazim alternately with thiram and mancozeb reduced the carbendazim resistance in *Sclerotium rolfsii* causing fruit rot of cucumber (10). Carbendazim when used in mixture with captan, zineb and mancozeb there was reduction in carbendazim resistance. Mutisite action of carbendazim with captan, zineb and Mancozeb in combination might be responsible for inhibition of *M. phaseolina* causing charcoal rot of sweet potato. Carbendazim when used in mixture with mancozeb and zineb completely inhibited the growth of *M. phaseolina* at first passage only, while in mixture with Metalaxyl and difolatum inhibited the pathogen at 2 nd and 6 th passage (11). Wadikar (9) found that carbendazim with thiram in mixture inhibited growth of *M. phaseolina* causing charcoal rot of pigeonpea completely at 7 th passage. Carbendazim when used in mixture with benomyl completely inhibited growth of *Sclerotium rolfsii* causing fruit rot of cucumber (11).

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