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RESEARCH ARTICLE

EFFECT OF CONTINUOUS AND ALTERNATE PASSAGES ON THE DEVELOPMENT OF BENOMYL RESISTANCE IN *FUSARIUM SOLANI* CAUSING DRY ROT OF ELEPHANT FOOT YAM

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Abstract

Effect of continuous and alternate passages on the development of benomyl resistance in sensitive isolate (FS-9) of *Fusarium solani* causing dry rot of elephant foot yam, obtained through MIC, indicated that there is increase in the resistance of the pathogen to benomyl when cultured continuously for eight successive passages in vitro and in vivo. While, use of benomyl alternately with kocide, ridomil, roko and dhanuka reduced benomyl resistance significantly.

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INTRODUCTION

Elephant foot yam [*Amorphophallus paeoniifolius* (Dennst.) Nicolson] is a tropical tuber crop belonging to family Araceae. It is a crop of Southeast Asian origin. It grows in wild form in the Philippines, Malaysia, Indonesia and Southeast Asian countries. In India, it is cultivated in Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh, and Jharkhand. The corms and corms of this plant are usually boiled and eaten. In some countries (Philippines), corms are boiled and also fed to pigs. It is also used as common source of mannose. It is generally used for making vegetables, pickles and indigenous ayurvedic preparations for various ailments. The tuberous roots of the plant possess blood purifying properties and have been used traditionally for the treatment of piles, abdominal disorders, tumors, enlargement of spleen, asthma and rheumatism [1]. Such important plant is attacked by many diseases among them; dry rot of yam caused by *Fusarium solani* is very serious. Diseases caused by Deuteromycetous pathogens were controlled by benomyl due to its systemic properties and its great efficacy in controlling plant diseases [2]. The present study was, therefore, undertaken to examine the possibility of development of benomyl resistance due to continuous and alternate passages of benomyl.

MATERIAL AND METHODS**In vitro studies****Continuous passage**

The effect of continuous passage on the development of benomyl resistance in the pathogen, wild sensitive isolate was cultured on Czapek Dox agar plates containing benomyl at the concentration of 25 µg ml⁻¹. It was kept constant for all the passages. Agar disc (8 mm) was taken from the culture of previous passage of same isolate and placed upside down on agar surface.

Alternate passage

Wild sensitive isolate was cultured on Czapek Dox agar plates containing 25 µg ml⁻¹ benomyl. After eight days, 8

mm disc was taken from the previous passage and transferred to the plates containing another fungicide at the same concentration. The process of such alternation of benomyl to another fungicide was continued up to 8th passage.

In vivo studies

Continuous passage For this purpose, mycelial suspension using one culture petriplate of wild sensitive isolate FS-9 was prepared. 10 ml mycelial suspension was inoculated on the healthy yam corms, treated with $25 \mu\text{g ml}^{-1}$ benomyl 24 hrs before as described earlier to determine MIC in vivo. After 8 days, mycelial suspension from infected corms was prepared and applied to healthy corms, treated with same concentration of benomyl. Same procedure was followed up to 8th passage.

Alternate passage

To study the effect of passage, 10 ml mycelial suspension of wild sensitive isolate was inoculated on the healthy yam corms as described earlier, treated with $15 \mu\text{g ml}^{-1}$ benomyl 24 hrs. before it. After 8 days, mycelial suspension from such infected corms was prepared and inoculated on healthy corms treated with another fungicide at same concentration. Same procedure was followed up to 8th passage.

RESULTS AND DISCUSSION

In vitro studies:-

Continuous and alternate treatment with Benomyl:-

It was seen that growing of *Fusarium solani* on the medium containing Benomyl for eight successive passages continuously significantly increase the resistance. When Benomyl was altered with kocide, ridomil, roko and dhanuka. It was observed that *F. solani* cultured alternately with other fungicides, there was decrease in the development of benomyl resistance. Use of benomyl alternately with these fungicides completely inhibited the growth of pathogen at 5th and 6th passages (Table: 1).

Table: 1 Effect of exposure of *Fusarium solani* to Benomyl continuous and alternating with other fungicides on the development of resistance during eight successive passages (In Vitro).

Fungicides (25 $\mu\text{g/ml}$)	Passage number							
	1	2	3	4	5	6	7	8
Benomyl continuous	10.67	14.66	18.33	20.00	21.67	22.33	24.66	25.33
Benomyl alters Roko	11.00	23.33	15.33	12.33	10.00	00.00	00.00	00.00
Benomyl alters Ridomil	11.67	53.33	20.33	17.66	15.33	00.00	00.00	00.00
Benomyl alters Kocide	11.00	24.00	12.33	10.66	00.00	00.00	00.00	00.00
Benomyl alters Dhanuka	11.33	44.00	17.00	12.33	10.33	09.00	00.00	00.00

In vivo studies

It was observed that continuous treatment of Benomyl in alone for eight successive passages increased the resistance of pathogen. The wild sensitive isolate was inoculated on benomyl treated yam corms alternately with kocide, ridomil, roko and dhanuka. It was seen that use of benomyl with kocide, ridomil, roko completely prevented the infection of pathogen at 3rd passage only. When benomyl altered with dhanuka, there was complete inhibition of growth at 4th passage only (Table: 2).

These results are agreeing with other workers. Use of ediphenphos alternate with Benomyl reduced Benomyl resistance in *Septoria nodorum* and *Cercospora herpotrichoides* [3]. A mathematical model to test different fungicides for their alternate use given was by [4]. Multisided action of Benomyl with mancozeb, benomyl, captafol and thiram might be responsible for the complete inhibition or the development of resistance in the *Macrophomina phaseolina* causing charcoal rot of potato [5]. Alternate treatment with copper oxychloride, ziram and mancozeb significantly reduced aluminium phosphite resistance in *Pythium aphanidermatum* from passage to passage [6].

Table: 2 Effect of exposure of *Fusarium solani* to Benomyl continuous and alternating with other fungicides on the development of resistance during eight successive passages (In Vivo)

Fungicides (25 $\mu\text{g/ml}$)	Passage number							
	1	2	3	4	5	6	7	8
Benomyl continuous	10.67	14.66	18.33	20.00	21.67	22.33	24.66	25.33
Benomyl alters Roko	11.00	23.33	15.33	12.33	10.00	00.00	00.00	00.00

Benomyl alters Ridomil	11.67	53.33	20.33	17.66	15.33	00.00	00.00	00.00
Benomyl alters Kocide	11.00	24.00	12.33	10.66	00.00	00.00	00.00	00.00
Benomyl alters Dhanuka	11.33	44.00	17.00	12.33	10.33	09.00	00.00	00.00

CONCLUSION

Culturing of sensitive isolate of *Fusarium solani* on benomyl continuously for eight successive passages significantly increased the fungicide resistance. Use of benomyl alternately with kocide completely inhibited the growth of pathogen at 5th passage. When benomyl used alternately with roko, ridomil and dhanuka, there was reduction in resistance after 6th and 7th passage respectively.

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