Effect of passage on the development of Benomyl resistance *In Vitro* against *Fusarium udum*

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ABSTRACT

In vitro effect of passage on the development of benomyl resistance in sensitive isolate FU-1 indicated that there is increase in the resistance of the pathogen when the cultured continuously for 6 successive passage. When the pathogen is cultured alternately with carbendazim, difolatan, thiophanate methyl, aureofungin and thirum there was reduction in the resistance. Mixing of benomyl with difolatan gave more favorable result.

Key words: Benomyl, Passage, *In Vitro*, Resistance.

INTRODUCTION

Fungicide application programme may influence the development of resistance in the pathogen. Hence the effect of passage on the agar medium containing benomyl individually, alternately or in mixture with other fungicides (carbendazim, thirum, thiophanate methyl, difolatan and aureofungin) having different mode of action was studied. Transfer of isolates through five successive passages also increased resistance in *Aspergillus flavus* (Gangawane and Reddy, 1985). Adoption of the *Furarium udum* isolate to benomyl during the passage might be the major factor involved in the development of resistance. This view can be supported by the work of Horsten (1979), who found that *Septoria nodorum* causing glum blotch of wheat was able to develop maximum resistance to casbendazim. There are few more evidences for the increase of resistance due to continuous exposure of pathogen to fungicides (Reddy, 1986; Gangawane and Shaikh, 1988 ; Kamble, 1991).

MATERIALS AND METHODS

After determining the MIC of benomyl against the isolates, the effect of continuous and alternate treatments of fungicides with two different mode of action and a mixture of both on the development of resistance in wild sensitive isolates of *Fusarium udam* was studied on Czapek Dox agar medium.

In order to study the effect of passage on agar plate's wild sensitive isolate FU-1 in each passage was cultured on plates with benomyl (1.5 mg/ml). The plates without fungicides served as control. A 4 mm diameter disc of freshly grown culture taken from the culture of previous passage of the same isolate was placed at the centre of each plate in triplicate. In each passage linear growth was measured after a week. Percentage increase of the growth of the isolate from passage to passage was considered as increase in the benomyl resistance. The development of resistance was studied up to 6^{th} passage.

RESULTS AND DISSCUSSION

It is evident that culturing of the isolate (FU-1) continuously for six successive passage increased the growth of the pathogen with the increase of passage number. At sixth passage there was significant increase in the growth indicating the increase in the benomyl resistance. However, when the isolate was culture alternately with carbendazim, thiophanate methyl, thirum, difolatan and aureofungin the results were variable. When benomyl was altered with carbendazim there was slight increase in the growth of the pathogen at 2^{nd} passage but there was significant reduction from 3^{rd} to 6^{th} passage. In case of thiophante methyl, thirum, difolatan and aureofungin the growth was highly increased when compared with benomyl alone at 2^{nd} passage.

But at third passage there was again decrease in the growth on benomyl. Again at the 4th passage the growth was increased and decreased at 5th passage. At the 6th passage it was noted that there was significant decrease over benomyl passage alone in the growth of the pathogen. In general use of benomyl alternately with other fungicides there was decrease in the resistance (Table 1, Fig.1)

Table 1: Effect of continuous exposure to benomyl and to benomyl alternately with other fungicides on the growth of *Fusarium udum* on agar medium during 6 successive passage.

Sr. No.	Fungicide (µg/ml)	Passage Number					
		I	Ii	III	IV	V	VI
1	Benomyl continuous (1.5 µg/ml)	15*	31	41	46	55	67[b]
2	Benomyl (1.5 µg/ml) altars Carbendazim (1.5 µg/ml)	15	29	17	22	8	14
3	Benomyl (1.5 µg/ml) altars thiophanate methyl (1.5 µg/ml)	15	75	36	49	29	22[a]
4	Benomyl (1.5 µg/ml) altars thirum (1.5 µg/ml)	15	79	53	75	70	36[a]
5	Benomyl (1.5 µg/ml) altars difolatan (1.5 µg/ml)	15	78	41	34	27	21[a]
6	Benomyl (1.5 µg/ml) alters aureofungin (1.5 µg/ml)	15	70	48	88	71	32[a]

a and b significant increase over first passage by wilcoxon's sum sank test at P = 0.05 and P = 0.01 respectively.
* Radial growth as % of control.

Table 2 Effect of continuous exposure to benomyl individual and to benomyl in mixture with various Fungicides on the growth of *Fusarium udum* on agar medium during 6 successive passage.

Sr. No.	Fungicide (µg/ml)	Passage Number					
		I	Ii	III	IV	V	VI
1	Benomyl individual (1.5 µg/ml)	15*	31	41	46	55	67[b]
2	Benomyl + arbendazim (1.5 µg/ml)	00	00	00	00	00	00
3	Benomyl +thiophanate methyl (1.5 µg/ml)	09	30	37	38	41	38[b]
4	Benomyl + thirum (1.5 µg/ml)	05	17	24	31	32	32[b]
5	Benomyl + difolatan (1.5 µg/ml)	12	20	20	24	27	29[a]
6	Benomyl + aureofungin (1.5 µg/ml)	08	16	17	32	32	36[b]

a and b significant increase over first passage by wilcoxon's sum sank test at P = 0.05 and P = 0.01 respectively.
* Radial growth as % of control.

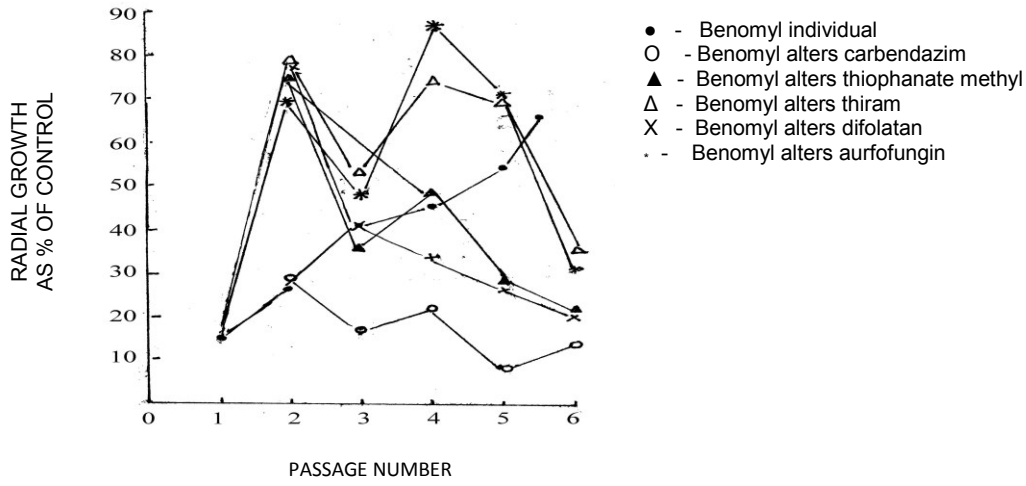

Fig. 1: Effect of continuous exposure to benomyl and to benomyl alternately with other fungicides on the growth of *Fusarium udum* on agar medium during 6 successive passage.

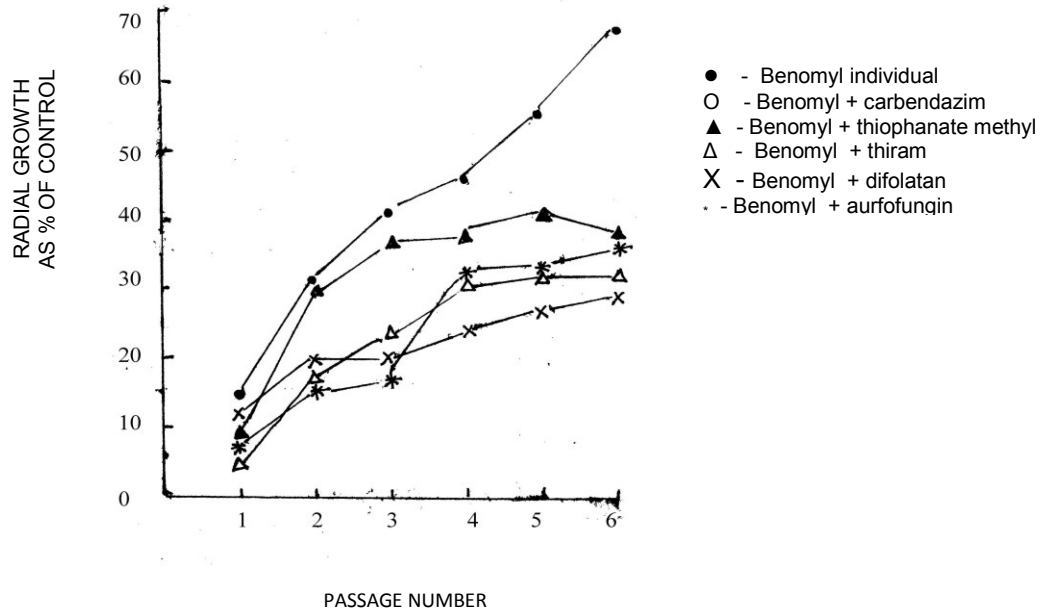

Fig. 2: Effect of continuous exposure to benomyl individual and to benomyl in mixture with various Fungicides on the growth of *Fusarium udum* on agar medium during 6 successive passage

It was seen that when the benomyl was mixed with carbendazim, thiophante methyl, thirum, difolatan and aureofungin, there was reduction in growth of the isolate when compaired with the passage on benomyl alone. However, with the increase of the passage there was increase in the growth of the pathogen. But his increase was always less than that of benomyl passage alone and significant decrease was seen at the 6th passage. At this last passage reduction in the growth was more prominent due to mixing of benomyl with difolatan followed by thirum, aureofungin and thiophanate methyl in decreasing manner. No growth of pathogen was observed when benomyl was mixed with carbendazim. This indicated that mixing of benomyl with other fungicides reduce the resistance with increase of passage (Table 2, fig. 2)

It is possible that fungicide application programmes may influence the development of resistance in pathogen. Hence, effects of successive passages of *Fusarium udum* (wild isolate sensitive to benomyl) on benomyl individually, alternately or in mixture with other fungicides with different mode of action was studied *In Vitro*.

In the present investigation it was noted that there is increase in the resistance of the sensitive isolate when cultured continusoly on benomyl for 6 successive passage. But when the pathogen is cultured alternately with carbendazim difolatan, thiophanate methyl, aureofungin and thirum, there was reduction in the resistance. Alternation of benomyl with carbendazim found to be more beneficial than with other fungicides. Culturing of the pathogen on plates containing benomyl in combination with carbendazim, thiophanate methyl, thirum, difolatan and aureofungin also reduced resistance. Benomyl and difolatan mixrue gave more favorable results. According to Graffin (1981) the alternately used fungicide must have different mode of action and in the present invesitation there might have less chance to mutate or to adopt resistance in this pathogen due to use of different specific site inhibiting fungicide. Similarly, benomyl when used in mixture with other fungicides also reduced benomyl resistance. These results also agree with earlier work of Horsten (1979), Kareppa (1990) and Waghmare (1991) in case of *Septoria nodorum, Puccinia arachidis and Phytopthora drechsleri* f.sp. *canjani* respectively.

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