



## Effect of Passage on the Development of Carbendazim Resistance

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**Abstract:** Effect of passage in the development of carbendazim resistance in the sensitive isolate indicated that culturing of the isolate continuously for 4 successive passages increased resistance. But culturing of the isolate alternately with dithane Z-78 and mancozeb the resistance was decreased. Moreover, use of carbendazim in mixture with these fungicides reduced the resistance. The spray programme of carbendazim may influence on the development of resistance in pathogens. Therefore, the effect of passage on agar medium containing carbendazim alone, alternately or in mixture with other fungicides like benomyl, dithane Z-78 and mancozeb was evaluated.

### 1. Introduction

Post harvest diseases of *Zizyphus* fruits were studied. The infected fruits were collected from different places. It was seen that altogether five diseases caused by *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp., *Fusarium oxysporum* and *Rhizoctonia solani* were recorded. However, fruit rot by *Aspergillus niger* was recorded at maximum places and on more varieties. Carbendazim is recommended for the management of *Zizyphus* diseases; hence this fungicide was undertaken for detailed study. Reports on fungicide resistance in plant pathogens of various crops are very few although the use of fungicides in disease management is increasing day by day. The aim of the present study was therefore to examine the possibility of development of resistance in *Aspergillus niger* against carbendazim.

### 2. Material and Methods

After determining the MIC carbendazim against 10 isolates, the effect of continuous and alternate treatments of fungicides having different modes of action and a mixture of both on the development of resistance in the wild sensitive isolates of *Aspergillus niger* was studied on agar plates and on the fruits as well.

In order to study the effect of passage *in vitro* wild sensitization isolate AN-9 in each passage was cultured on agar plates containing a sublethal dose of carbendazim (2.5µg/ml). The plates without fungicide served as control. Inoculum from the colony of previous passage of the same isolate was placed at the center of each plate. In each passage linear growth was measured after 8 days. A percentage increase of growth of the isolate from passage to passage was considered an increase in carbendazim resistance. The development of resistance was studied up to 4<sup>th</sup> passage. Alternate passage of carbendazim with Benomyl, Dithane Z-78, Mancozeb and mixed passage with the same fungicides were also carried out.

*In vivo* studies were carried out on the fruits. A fruit wrapping technique using tissue papers was followed for this purpose. Fruits were dipped in the solution of carbendazim alone and in alternate or in mixture with other fungicides. They were inoculated with the wild sensitive isolate. An infected portion of the fruits of first passage was used for re-isolation and used as the source of inoculum for second passage. This was continuously carried out up to the last passage.

### 3. Experimental Result

For *in vitro* studies a carbendazim sensitive isolate AN-9 was selected. It was cultured in Czapek Dox agar medium containing sublethal dose (2.5µg/ml) of

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carbendazim individually and alternately with other fungicides for four successive passages. Results are shown in Table 1 and Fig. 1a & 1b. It was observed that culturing the isolate continuously for four successive passages on carbendazim increased the growth from passage to passage. Carbendazim was altered with benomyl, dithane Z-78 and mancozeb. When the isolate was cultured alternately with benomyl there was a significant increase in its growth when compared with the first passage. But when the isolate was cultured alternately with dithane Z-78 or mancozeb the growth was reduced. Alteration of carbendazim with dithane Z-78 inhibited the complete growth at 4<sup>th</sup> passage.

When the carbendazim was mixed with other fungicides somewhat different results were obtained. Use of carbendazim continuously increased the growth of isolate AN-1 from passage to passage and at 4<sup>th</sup> passage, there was significant growth when compared with first passage. But mixing of carbendazim with benomyl increased the growth of isolating at 4<sup>th</sup> passage. However, mixing of carbendazim with dithane Z-78 or mancozeb was found to be inhibitory for the isolate. Mixing of carbendazim with dithane Z-78 completely inhibited the growth at 3<sup>rd</sup> passage only while mixing with mancozeb inhibited the growth at 4<sup>th</sup> passage (Table 2).

For *in vivo* studies the fruits of *Zizyphus* were dipped into the fungicide solutions and then inoculated with the carbendazim sensitive isolate AN-9. Percentage infection zones were measured after 7 days. Carbendazim was altered with benomyl, dithane and mancozeb. Results are in Table 3 and Fig. 2.

Indicated that treatment of carbendazim for four successive passages increased infection significantly. Treatment of carbendazim alternately with benomyl also increased the percentage infection. However, the alteration of carbendazim with dithane Z-78 or mancozeb decreased the infection significantly when compared with first passage. Carbendazim was mixed with benomyl, dithane Z-78 and mancozeb and *Zizyphus* fruits were treated for four successive

passages. It was observed that continuous treatment of only carbendazim increased infection from passage to passage. Treatment of fruits with a mixture of carbendazim and benomyl gave similar results. But treatment of carbendazim with dithane or mancozeb reduced the infection significantly at 3<sup>rd</sup> passage only (Table 4 and Fig. 3).

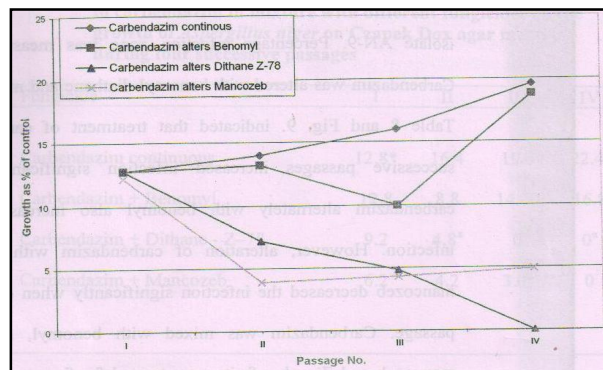


Fig. 1a. *In Vitro* continuous and alternate use of carbendazim with other fungicides on the growth of *Aspergillus niger* isolate AN-9.

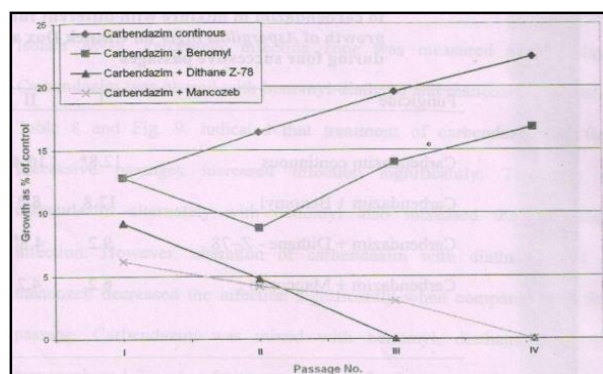


Fig. 1b. *In Vitro* continuous and application of carbendazim in mixture with other fungicides on the growth of *Aspergillus niger* isolate AN-9.

Table 1. Effect of continuous exposure to carbendazim alone alternately with other fungicides on the growth of *Aspergillus niger* on Czapek Dox agar medium during four successive passages.

| Fungicide                        | I     | II   | III   | IV    |
|----------------------------------|-------|------|-------|-------|
| Carbendazim continuous           | 12.8* | 14.0 | 16.0  | 19.6a |
| Carbendazim alters Benomyl       | 12.8  | 13.2 | 10.00 | 18.8a |
| Carbendazim alters Dithane Z-78  | 12.8  | 7.2  | 4.8   | 0.0   |
| Carbendazim alters with Mancozeb | 12.2  | 3.9  | 4.3   | 4.9a  |

\*-Growth as % control; a - Significant increase or decrease over the first passage by a Wilcoxon sum rank test at  $p=0.05$ .

Table 2. Effect of continuous exposure to Carbendazim individually and to Carbendazim in mixture with different fungicides on the growth of *Aspergillus niger* on Czapek Dox agar medium during four successive passages.

| Fungicide                         | I     | II   | III   | IV    |
|-----------------------------------|-------|------|-------|-------|
| Carbendazim continuous            | 12.8* | 16.4 | 19.6  | 22.4a |
| Carbendazim + Benomyl             | 12.8  | 8.8  | 14.00 | 16.8  |
| Carbendazim alters + Dithane Z-78 | 9.2   | 4.8a | 0     | 0a    |
| Carbendazim + Mancozeb            | 6.2   | 4.2  | 3.0a  | 0     |

\*-Growth as % of control; a - Significant increase or decrease over the first passage of a Wilcoxon sum rank test at  $p=0.05$ .

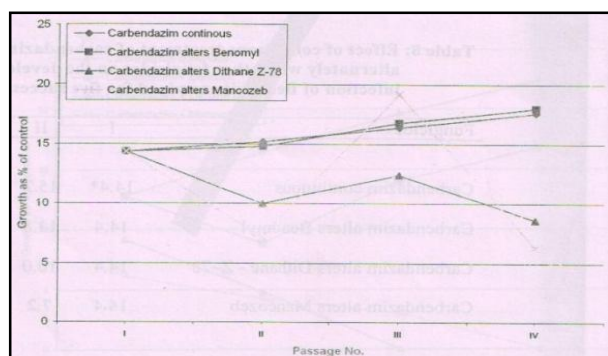


Fig. 2. *In Vivo* continuous and alternate use of carbendazim with other fungicides on the growth of *Aspergillus niger* during four successive passages.

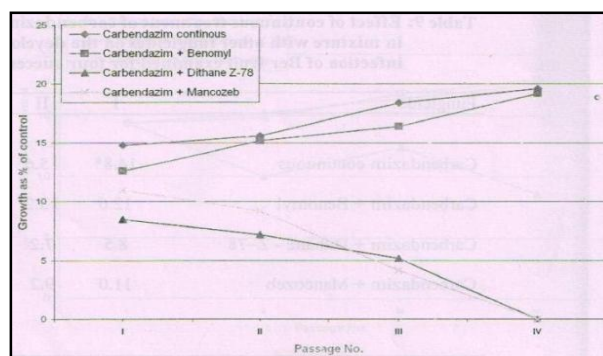


Fig. 3. *In Vivo* continuous and in treatment of carbendazim in mixture with other fungicides on the growth of *Aspergillus niger* isolate AN-9.

Table 3. Effect of continuous of Carbendazim individual and alternately with other fungicides on the development of infection of Ber fruit examined five successive passages.

| Fungicide                        | I     | II   | III  | IV    |
|----------------------------------|-------|------|------|-------|
| Carbendazim continuous           | 14.4* | 15.2 | 16.9 | 17.6a |
| Carbendazim alters Benomyl       | 14.4  | 14.8 | 16.8 | 18.0  |
| Carbendazim alters Dithane Z-78  | 14.4  | 10.0 | 12.4 | 8.6a  |
| Carbendazim alters with Mancozeb | 14.4  | 7.2  | 19.2 | 6.3a  |

\*-Infection zone as % of control; a - Significant increase or decrease over the first passage by a Wilcoxon sum rank test at  $p=0.05$ .

Table 4. Effect of continuous treatment of Carbendazim individual and in mixture with other fungicides on the development of infection of fruit examine for four successive passages.

| Fungicide                  | I     | II   | III  | IV    |
|----------------------------|-------|------|------|-------|
| Carbendazim continuous     | 14.8* | 15.6 | 18.4 | 19.6a |
| Carbendazim + Benomyl      | 12.6  | 15.2 | 16.4 | 19.2a |
| Carbendazim + Dithane Z-78 | 8.5   | 7.2  | 5.2a | 0     |
| Carbendazim + Mancozeb     | 11.0  | 9.2  | 4.2a | 0     |

\*- Infection zone; a - Significant increase over first passage by

#### 4. Discussion

In order to see whether the development of carbendazim resistance in *Aspergillus niger* could be prevented or delayed by continuous, alternate or in mixture with other fungicides experiments were carried out on agar plates. It was noted that culturing of the sensitive isolates continuously for four successive passages on carbendazim increased the resistance. But culturing the isolate alternately with dithane Z-78 and mancozeb reduced the carbendazim resistance. Moreover, use of these fungicides in the mixture also reduced the resistance. According to Griffin (1981) alternately used fungicides must have different modes of action and in the present study, it is proved that there is less chance of mutation in *Aspergillus niger* due to use of the different specific site inhibiting fungicides like dithane Z-78 and mancozeb. Alternate use of Edifenphos in reducing carbendazim resistance in *Septoria nodorum* and *Cercospora herpotrichoides* have been shown by Horsten (1979). A mathematical model to test alternate use of fungicides has been also suggested by Kable and Jeffery (1980). The work of Anitha *et al.*, (1989) also supports the present findings in *Macrophomina phaseolina* for acquiring and cross

fungicide tolerance to thiophanate methyl. The Multisite action of carbendazim with dithane Z-78 and mancozeb in combination might be responsible for inhibition of resistance development.

#### References

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