Scholarly Research Journal for Interdisciplinary Studies,
Online ISSN 2278-8808, SJIF 2018 = 6.371, www.srjis.com
PEER REVIEWED & REFERRED JOURNAL, MAY-JUNE, 2019, VOL- 6/51

THE SURVIVAL ABILITY OF CARBENDAZIM RESISTANT MUTANT OF ASPERGILLUS NIGER IN MIXED POPULATION ON ZYZUPUS FRUIT

Purnima Sable, Ph. D.

Associate Professor, S.M.B.S.T. College Sangamner, purnimasable@gmail.com

Abstract

In natural population of plant pathogenic fungi, there is potential danger due to development of fungicide resistance in the pathogen. Hence it is important to know the ability of such mutant for its survival in the sensitive population. Fitness of resistant Aspergillus niger mutant in the absence and presence of carbendazim was evaluated and its significance in the management of resistance is discussed.

INTRODUCTION

The Ber (*Zizyphus mauritiana* Lamk.) is an important fruit crop of arid and semi-arid tropics in our country. It is xerophytic in nature and survives well under water stress conditions. The fruits are very nutritious and are available at low cost. Hence it is really a poor man's fruit. Nowadays it is also grown in the parts of central Asia, China and Taiwan. In India it is grown on marginal land in the hot and arid region of Rajasthan, Punjab, Haryana, Uttar Pradesh and Gujarat. In Maharashtra the *Zizyphus* plant is found in every field wherever irrigation facilities are less. The genus *Zizyphus* belongs to the family Rhaminaceae and consist of 40 species in tropical and subtropical regions. Of these *Zizyphus jujube* and *Zizyphus mauritiana* are more common in Maharashtra. There are more than 125 cultivars grown in India developed by selection in different regions. However the ber suffers from various diseases like powdery mildew, sooty mold, *Alternaria* leaf spot and *Phoma* leaf spot. The fruits are attacked by many pathogens at pre and post harvest condition and spoil the taste and market quality. Under WTO the fruits are supposed to be free from the diseases in order to export them aboard. Therefore it was considered to study the post harvest diseases of *Zizyphus* fruits in market and storage conditions in Maharashtra and workout the management strategies.

The samples of *Zizyphus* fruits infected with different pathogens were collected from the market at different places in Maharashtra. The pathogens like *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp., *Fusarium oxysporum* and *Rhizoctonia bataticola* were recorded. However, *Aspergillus niger* was found to be dominant on maximum fruits. Hence isolates of *Aspergillus niger* were isolated for the study. The sensitivity of isolates was tested against carbendazim and ED_{50} and MIC was confirmed. There appeared to be quite a large variation in the sensitivity of the isolates.

Some mutants obtained through different mutagenic agents were selected to study the resistant factor and their pathogenicity. Mutants were inoculated onto the Czapek Dox agar medium containing multiple MIC of sensitive strain up to 10x and categorised as resistant, moderately resistant and highly resistant strains. It was noted that 3 strains were resistant, 8 were moderately resistant and 4 were highly resistant (Table 12). Pathogenicity varied according to the resistance. Resistant strains showed pathogenicity ranging from 49.0 to 55.0, moderately resistant strains showed pathogenicity in between 53.3 to 65.0 while highly resistant strains gave pathogenicity from 58.6 to 72.4.

Material and Method

4) Pathogenicity Test

Ber fruits were surface sterilised with 0.01% $HgCl_2$ solution and washed 10 times with sterile distilled water. They were inoculated with spore suspension of *Aspergillus niger* isolates or mutants sensitive or resistant to carbendazim. Percentage control efficacy (PCE) was calculated (Cohen, 1989). In order to study the effect of carbendazim or other fungicides. Fruit wrapping technique by using tissue papers was used. After 12 days fruits were carefully handled and infection on fruit was classified in the following grades (Singh and Bedi, 1984) and PDA was calculated.

Numerical rating	Fruit area showing infection
0	Fruit healthy
1	1 to 25 % area infected
2	26 to 50 % area infected
3	51 to 75 % area infected
4	76 to 100 % area infection

Percentage control efficacy (PCE) was calculated by using following formula

PCE = 100 (1 – X/Y)

Where X = Diameter of the colony on the plates containing carbendazim / PDA on fruits

Y = Diameter of the colony on absolute control plates / PDA on fruits

13) Survival ability of resistant strain in the mixed population

To test survival ability of resistant *Aspergillsu niger,* spore suspension was prepared from the wild sensitive AN-9 and resistant strain AN EMS-9. The suspensions were mixed in the following proportions.

Sensitive		Resistant
90	:	10
75	:	25
50	:	50

Healthy Ber fruits were then inoculated with these mixtures of sensitive and resistant isolates in above mentioned ratio and wrapped with tissue paper and determined for the survival ability of resistant mutant in mixed population. The composition of resistant spores of each mixture was examined for five successive passages in the presence and absence of carbendazim (Horsten, 1979). The data were subjected to computer for statistical analysis.

Experimental Result:

Survival ability of resistant mutant in mixed population

The survival ability of carbendazim resistant mutant in mixed population was studied by inoculating *Zizyphus* fruits with a mixture of carbendazim sensitive (AN-9) isolate and carbendazim resistant mutant (EMS AN-9) of *Aspergillus niger*. The spore suspension from the sensitive isolate and resistant mutant were mixed in the following proportion.

	Sensitive	Resistant
1	90	10
2	75	25
3	50	50

Zizyphus fruits were inoculated according to the method described earlier for pathogenicity. After 10 days of inoculation of the fruit in the wrapped tissue paper infected portion was used for preparation of spore suspension. The spore suspension from each variant were used to inoculate second batch of *Zizyphus* fruits. For next passage prior to inoculation, 5 ml samples was taken from each spore suspension and adjusted to the desired concentration of 100 spores per ml. Approximately one ml of each suspension was pipetted onto the surface of 20 ml water agar plates. These plates were incubated for a week till the colonies of *Aspergillus niger* become visible. At least 100 colonies from each variant were transferred to plates of Czapek Dox agar medium containing 2.5 µg/ml a concentration lethal to sensitive isolate of *Aspergillus niger*. The plates were again incubated for a week. The surviving colonies were counted and their composition in each mixture was examined in treated and untreated with carbendazim. The results are presented in Table 261 and Fig. 19. It was observed that without carbendazim treatment resistant population in the mixture was reduced from passage. When the resistant population proportion was increased in the mixture still there was reduction from passage to passage. On the other hand in case of carbendazim treated passages resistant population was increased from passage to passage. This increase was also seen when its proportion was increased in mixture with the increase of passage number. Thus carbendazim treatment to the fruits of *Zizyphus* imposes such a selection pressure on the population that only few resistant colonies are able to survive at the fourth passage.

In order to determine the differences in fitness between carbendazim sensitive and carbendazim resistant mutant, the model given by Leonard (1969) was used. Accordingly the results in Table 27 were transformed and are again presented in the Fig. 20. The resulting values are for differences in fitness are presented in Table 27.2

$$\frac{p_n}{p_o} = (1 - S)^n \frac{q_n}{q_o} \qquad \ldots (1)$$

p_o = Initial relative proportion of the weaker genotype.

q_o = Initial relative proportion of stronger genotype.

n = Number of passages.

(1-S) = Differences in fitness between the weaker and stronger genotype defined as the relative reproduction ratio between two genotypes.

The number of passages N needed to decrease the proportion. Po to the proportion Pn is given by

$$n = \frac{\text{In} \dfrac{Pn\ (1 - Po)}{Po\ (1^{-} Pn)}}{Io\ (1 - S)} \quad \ldots (2)$$

The curve obtained in this way can be transferred to a straight line by the following equations:

$$\ln \frac{p_n}{1 - p_n} = \ln \frac{q_n}{1 - q_n} + n \ln (1 - S) \qquad (3)$$

In this way, the differences in fitness (1–S) can be estimated if ln (P/1-P) is plotted against the number of passages. Using the last equation (3), the results presented in Fig. 19 were transformed and again presented in Fig. 20. The results values for differences in fitness are given in table 283. Table 28: Relative reproduction ratio of carbendazim resistant

Table 28 3. *Aspergillus niger* strain in mixed population during four Successive passages on *Zizyphus* fruit

Original ratio S : R	**Relative reproduction ratio**	
	Untreated	**Treated**
90 : 10	0.4627	1.0862
75 : 25	0.4278	1.3449
50 : 50	0.4784	1.5840

The population from the untreated fruits of *Zizyphus*, the reproduction ratio of the conidia is always smaller. This shows that resistant strain has a smaller reproduction than the sensitive one. Hence, the resistant strain will slowly disappear from the mixed population. The fruits of sweet orange treated with carbendazim reproduction ratio of the resistant mutant (AN EMS-9) are greater than that of the sensitive one (AN – 9). Here, the resistant mutant will finally dominate completely over the sensitive one and hence it is most fit for its survival under selection pressure of carbendazim.

Survival ability of resistant mutant in mixed population

The survival ability of carbendazim resistant mutant in mixed population was studied by inoculating *Zizyphus* fruits with a mixture of carbendazim sensitive (AN-9) isolate and carbendazim resistant mutant (EMS AN-9) of *Aspergillus niger*. The spore suspension from the sensitive isolate and resistant mutant were mixed in the following proportion.

	Sensitive	Resistant
1	90	10
2	75	25
3	50	50

Zizyphus fruits were inoculated according to the method described earlier for pathogenicity. After 10 days of inoculation of the fruit in the wrapped tissue paper infected portion was used for preparation of spore suspension. The spore suspension from each variant were used to inoculate second batch of *Zizyphus* fruits. For next passage prior to inoculation, 5 ml samples was taken from each spore suspension and adjusted to the desired concentration of 100 spores per ml. Approximately one ml of each suspension was pipetted onto the surface of 20 ml water agar plates. These plates were incubated for a week till the colonies of *Aspergillus niger* become visible. At least 100 colonies from each variant were transferred to plates of Czapek Dox agar medium containing 2.5 µg/ml a concentration lethal to sensitive isolate of *Aspergillus niger*. The plates were again incubated for a week. The surviving colonies were counted and their composition in each mixture was examined in treated and untreated with carbendazim. The results are presented in Table 26 and Fig. 19. It was observed that without carbendazim treatment resistant population in the mixture was reduced from passage. When the resistant population proportion was increased in the mixture still there was reduction from passage to passage. On the other hand in case of carbendazim treated passages resistant population was increased from passage to passage. This increase was also seen when its proportion was increased in mixture with the increase of passage number. Thus carbendazim treatment to the fruits of *Zizyphus* imposes such a selection pressure on the population that only few resistant colonies are able to survive at the fourth passage.

In order to determine the differences in fitness between carbendazim sensitive and carbendazim resistant mutant, the model given by Leonard (1969) was used. Accordingly the results in Table 27 were transformed and are again presented in the Fig. 20. The resulting values are for differences in fitness are presented in Table 27.2

$$\frac{p_n}{p_o} = (1 - S)^n \frac{q_n}{q_o} \quad \ldots (1)$$

p_o = Initial relative proportion of the weaker genotype.

q_o = Initial relative proportion of stronger genotype.

n = Number of passages.

(1-S) = Differences in fitness between the weaker and stronger genotype defined as the relative reproduction ratio between two genotypes.

The number of passages N needed to decrease the proportion. Po to the proportion Pn is given by

$$n = \frac{\text{In} \dfrac{Pn\,(1 - Po)}{Po\,(1^{-} Pn)}}{Io\,(1 - S)} \quad \ldots (2)$$

The curve obtained in this way can be transferred to a straight line by the following equations:

$$\ln \frac{p_n}{1 - p_n} = \ln \frac{q_n}{1 - q_n} + n \ln (1 - S) \qquad (3)$$

In this way, the differences in fitness (1–S) can be estimated if ln (P/1-P) is plotted against the number of passages. Using the last equation (3), the results presented in Fig. 19 were transformed and again presented in Fig. 20. The results values for differences in fitness are given in table 28.3

The population from the untreated fruits of *Zizyphus*, the reproduction ratio of the conidia is always smaller. This shows that resistant strain has a smaller reproduction than the sensitive one. Hence, the resistant strain will slowly disappear from the mixed population. The fruits of sweet orange treated with carbendazim reproduction ratio of the resistant mutant (AN EMS-9) is greater than that of the sensitive one (AN – 9). Here, the resistant mutant will finally dominate completely over the sensitive one and hence it is most fit for its survival under selection pressure of carbendazim.

Table 27 .2: Survival of carbendazim resistant *Aspergillus niger* strain in mixed population four successive passages in Ber fruit

Original ratio S : R	Mixture	Regression constant	Regression coefficient	Correlation
90 : 10	Untreated	1.3166	0.0827	0.8597
75 : 25	Untreated	1.2770	0.2963	0.9487
50 : 50	Untreated	0.3682	0.4599	0.9036
90 : 10	Treated	0.8922	- 0.7706	0.9005
75 : 25	Treated	0.8160	- 0.8492	0.9834
50 : 50	Treated	-0.4567	- 0.7373	0.9519

Table 26 .1: Survival of carbendazim resistant *Aspergillus niger* strain in mixed population during four successive passages on *Zizyphus* fruits

Original mixture ratio	Untreated fruits passage				Treated fruits passage			
	I S : R	II S : R	III S : R	IV S : R	I S : R	II S : R	III S : R	IV S : R
90 : 10	80 : 20	80 : 20	82 : 18	85 : 15	30 : 70	45 : 55	20 : 80	10 : 90
75 : 25	86 : 14	87 : 13	89 : 11	92 : 8	40 : 60	30 : 70	14 : 86	8 : 92
50 : 50	75 : 25	85 : 15	82 : 18	89 : 11	15 : 85	12 : 88	6 : 94	4 : 96

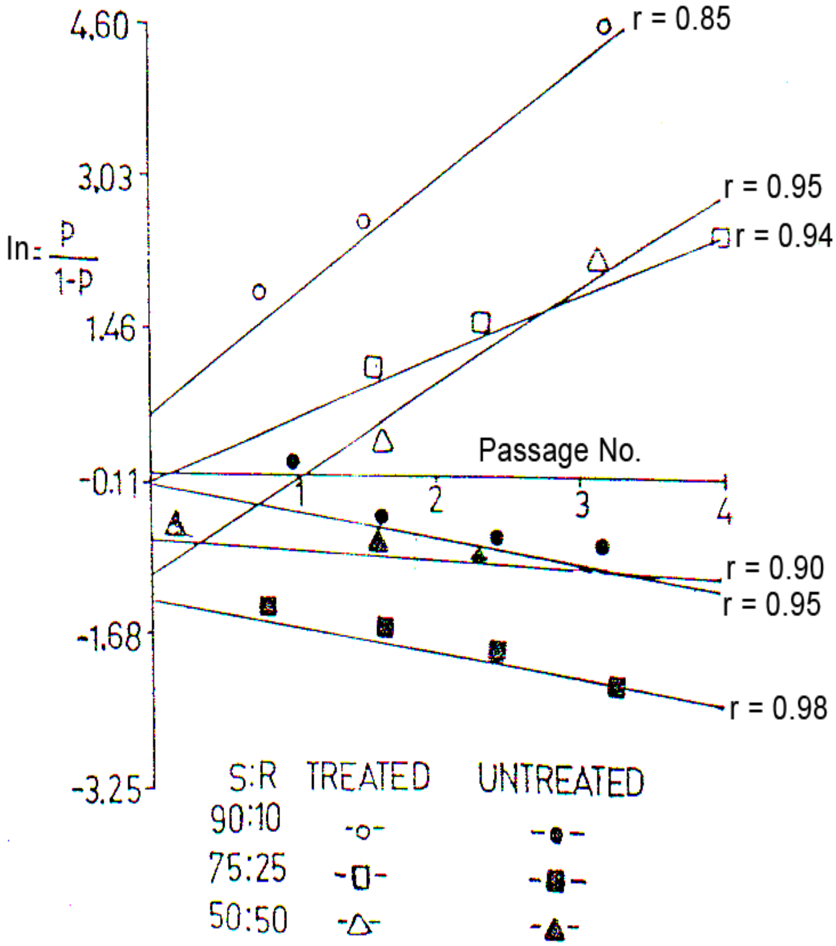

Fig N o 19

Disscussion

There is potential danger of the development of fungicide resistance in the pathogen in the management of a disease. The resistant mutants are released into the population of sensitive population of pathogenic fungi. Therefore it is necessary to study the ability of such resistant mutant whether it can survive under natural conditions. Such information on the fitness or survival ability of fungicide resistant mutant would be highly valuable in the management of a disease. In this regard the present investigation shows that without carbendazim treatment the resistant population was reduced from passage to passage. But in the presence of carbendazim the resistant mutant of *Aspergillus niger* was most fit for its survival. Reproduction ratio was also higher in resistant mutant under selection pressure of carbendazim. These results are in agreement with other workers in case of many pathogens resistant to various fungicides (Dovas *et. al.*, 1976; Hollomon, 1978; Gullino and Garibaldi, 1981; Gangawane and Reddy, 1987). Horsten (1979) found that carbendazim resistant strain of *Septoria nodorum* was fit to survive in the mixed population of sensitive strain under selection pressure. According to Wolfe (1973) selection pressure of a fungicide should not be increased since it improves the fitness or resistant isolate. Gangawane and Reddy (1987) showed that at equal proportion carbendazim sensitive population of *Aspergillus flavus* was completely eliminated from the rhizosphere of groundnut under selection pressure. This suggests that discontinuation use of carbendazim for certain period may eliminate the resistant population in the storage of the *Zizyphus* fruits.

Summery

1) Survival ability of carbendazim resistant strain in the mixed population was studied. It was seen that without carbendazim treatment resistant population was reduced from passage to passage. This increase was also seen with the increase of its proportion in the sensitive population.

Reference

Cohen, E. *(1989). Evaluation of fenpropimorph and flutriafol for control of sour rot, blue mold and green mold in lemon fruit. Plant. Dis.* ***73****: 807-80*

Dovas, C., G. Skylakakis, S.G. Georgopoulos *(1976). The adaptability of the benomyl resistant population of Cercospora beticola in northern Greece. Phytopathol.* ***66****:1452-1456.*

Gullino M. *(1983). Resistance of Botrytis cinerea to dicarboximide fungicides: Situation in Italy. ISPP Chemical control Newsletter No.2, P.5.*

Gullino, M.L. and A. Garibaldi *(1981). Competition in vitro and in vivo between strains of Botrytis cinerea sensitive and resistant to dicarboxymides. Proc. Resistance to fungicides in plant pathogens, Wageningen, Neth. 28, July-6 Aug, 1981.*

Gangawane, L.V. and B.R.C. Reddy *(1987). Development and management of carbendazim resistance in Aspergillus flavus. Ind. Phytopath.* ***40****(2): 297.*

Hollomon, D.W. *(1978). Competitive ability and ethirimol sensitivity in strains of borley powdery mildew. Ann. appl. Biol.* ***90****: 195-204.*

Horsten, J.A., H.M. *(1979). Acquired resistance to systemic fungicides of septoria nodorum and cercosporella herpotrichoides in cereals dissertation, Agric. Univ. Wageningen, Netherlands, pp. 107.*

Singh, P.P. and P.S. Bedi *(1984). Aflatoxin producing potential of toxigenic culture of Aspergillus flavus isolated from Karnal bunt infected wheat grains. Ind. Phytopath.* ***37****: 520-523.*

Wolfe M.S. *(1973). Changes and diversity in population of fungal pathogens. Ann. Appl. Biol.* ***75****: 132-136.*