



Induction of Carbendazim Resistance in *Aspergillus niger* through Mutagens

Purnima Sable^{1*} and L.V. Gangawane²

^{1*}Department of Botany, S.M.B.S.T. College, Sangamner, Maharashtra-422605, India.

²Soil microbiology and Pesticides Laboratory Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, Maharashtra, India.

Abstract: The sample of *Ziziphus* fruits infected with different pathogens was collected from the market at different places in Maharashtra. *Aspergillus niger* was found to be dominant all maximum fruits. Development of Carbendazim resistance in *Aspergillus niger* due to spontaneous mutation. Ultraviolet rays and chemical mutagens such as like bromyl uracil (Bu), ethyl methanesulfonate (EMS) and UV rays, same isolates were highly resistance, some were moderately resistance while few were resistance, highly resistance mutant were more pathogenic. Most sensitive isolates of *Aspergillus niger* A-9 was selected for this study. The spores of this isolate were treated with UV rays, bromyl uracil & ethyl methanesulfonate, it was seen that EMS treatment gave higher mutants followed by UV, Bu and spontaneous treatment in decreasing manner.

Keywords: Carbendazim, mutant, *Aspergillus niger*, Pathogen, Resistant, UV, Ethyl methanesulfonate.

1. Introduction

Ber is believed to originate in the Indo-China region. It is found growing wild semi-wild and in cultivated forms in almost all parts of India. The estimated area under Ber in India is about 12,000 hectares; it is mostly cultivated in Madhya Pradesh, Bihar, Uttar Pradesh, Haryana, Rajasthan, Punjab, Maharashtra, Gujarat and Andhra Pradesh.

The Ber (*Ziziphus mauritiana* Lamk) is an important fruit crop of arid and semiarid tropic in our country. It is xerophytes in nature and survives well under stress condition.

The Ber fruits have known for their flavors texture and they are highly nutritious and are available at low cost. Hence, it is really a poor man's fruit. The genus *Ziziphus* belongs to the family Rhamnaceae and consist of 40 species in tropical and subtropical regions of these *Ziziphus jujuba* and *Ziziphus mauritiana* are more common in Maharashtra. These 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 mould Alternaria leaf spot and phoma leaf spot.

The fruits are attacked by many pathogens at pre and postharvest condition and spoil the taste and market quality. According to WHO the fruits are supposed to be free from the diseases in order to export them abroad.

Therefore, it was considered to study the postharvest diseases of *Ziziphus* fruits in market storage condition in the Maharashtra work out the management strategies. Among the postharvest pathogens, *Aspergillus niger* was observed to be more common in Maharashtra state. There are reports that pre and postharvest diseases of various fruits can be managed through systemic and conventional fungicides including carbendazim.

However, during the last 20 years application of fungicides has plagued with several environmental problems and development of fungicides resistance. Carbendazim resistance was induced in *Aspergillus niger* through the treatment of UV rays, bromyl uracil and ethyl methanesulfonate (EMS). The EMS treatment gave higher mutant and they were also stable the mutants were moderately resistance and highly resistance the pathogenicity also increased with the increase of the resistance. Development of carbendazim

*Corresponding author:
E-mail: namjindal70@yahoo.co.in.

resistance in *Aspergillus niger* due to spontaneous mutation, ultraviolet rays and chemical mutagens such as like bromyl uracil (Bu) and ethyl methanesulfonate (EMS). Some isolates were highly resistance; some were moderately resistance while few were resistance. Highly resistance mutants were more pathogenic. This is possibly due to altered metabolism in the resistance isolate.

2. Materials and Methods

This was carried out with the help technique described by Horsten (1979). The sterilized Czapek Dox Agar (CDA) medium containing 3x MIC of carbendazim was poured in the plates and allowed to cool. One ml of the spore suspension was then uniformly distributed over Martins Rose Bengal Agar Surface with the help of L-Shaped sterile glass rod. Petri dishes thus prepared were incubated in dark at $26 \pm 3^\circ\text{C}$ for 10 days. The visible colonies were counted and percentage frequencies of resistant colonies were calculated from that of the control plates. Colonies showing different morphological character were isolated and maintaining on Czapek Dox Agar (CDA) medium without fungicide for further studies. These were considered as resistant mutant induced by spontaneous mutation (sp-mutants).

The spore suspension was exposed to UV light (30 minutes at 50mm distant) in inoculating chamber for induction of fungicide resistance. One ml of suspension

was spread over the Martins Rose Bengal agar medium containing carbendazim. The colonies showing different morphological character were then transferred on Czapek Dox Agar medium without fungicide for further studies. They were considered as UV mutants. The effect of chemical mutagens on the induction of fungicide resistance in *Aspergillus niger* was studied by treating spore suspension with ethyl methanesulfonate (EMS) and bromyl uracil (BU). Morphologically distinct colonies were isolated and maintained separately on CDA medium without fungicide. The mutants were designated UV mutant, EMS mutant and BU mutants for further studies (Gangawane and Shaikh, 1988a). The mutants were analyzed for persistence of resistance, resistance factor and pathogenicity.

3. Experimental Results

Again, the most sensitive isolate of *Aspergillus niger* A-9 was selected for this study. The spores of this isolate were treated with UV rays, bromyl uracil (BU) and ethyl methanesulfonate (EMS). One ml of the treated spore suspension was spread on the plates containing 3x of carbendazim (12g/ml). On the basis of phenotypic difference of colonies, mutants was considered and percentage frequency was calculated. The result is given in Table 1. It was seen that EMS treatment gave higher mutants following by UV, BU and spontaneous treatment in decreasing manner (Fig.1).

Table 1. Induction of resistance to Carbendazim in *Aspergillus niger* through various treatments.

Nature of mutant	Total mutant tested	Stable mutants	Stability percentage
Spontaneous (SP)	6	1	16.6
UV mutants	9	6	66.6
Bu mutants	7	2	28.5
EMS mutants	12	9	75.0

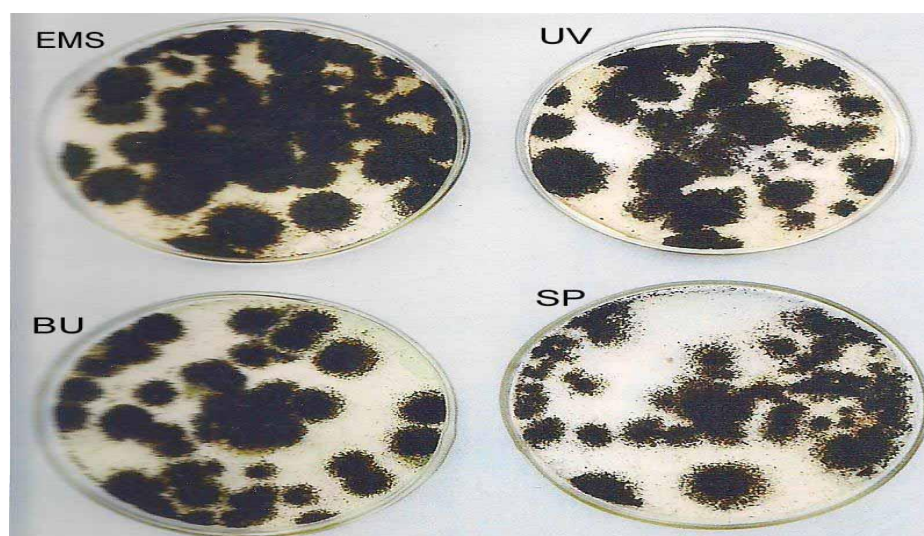


Fig. 1. Development of Carbendazim Resistance in *Aspergillus niger* through EMS, UV, BU and SP.

Table 2. Stability of *Aspergillus niger* mutants resistant to carbendazim.

Nature of mutant	Total mutant tested	Stable mutants	Stability percentage
Spontaneous (SP)	6	1	16.6
UV mutants	9	6	66.6
Bu mutants	7	2	28.5
EMS mutants	12	9	75.0

Table 3. Resistance factor and pathogenicity of carbendazim resistant mutants of *Aspergillus niger* obtained through induced mutations.

Mutants	Resistant factor	Resistant category	PDI
ANSP-1	3	R	49.0
ANUV-3	4	MR	65.0
ANUV-6	3	R	52.3
ANUV-8	4	MR	53.8
ANBU-2	4	MR	55.3
ANBU-4	6	HR	58.6
ANBU-5	5	MR	61.0
ANBU-8	3	R	55.6
ANEMS-3	5	MR	53.6
ANEMS-6	4	MR	63.0
ANEMS-7	8	HR	62.3
ANEMS-8	6	HR	72.4
ANEMS-9	6	HR	67.5
ANEMS-10	5	MR	53.3
Wild AN-4	4	MR	48.2
Sensitive	1	S	52.0

Representative mutants were isolated on Czapek Dox agar slants to test their stability. They were transferred for 10 times on plain Czapek Dox agar and again inoculated on this medium. Containing 3x MIC of carbendazim Result shown in Table 2 Indicated that maximum mutants obtained from EMS treatment were stable. This was followed by UV, and Bu mutants in decreasing manner. Mutants obtained spontaneously gave very less stable mutants.

Some mutant obtained through different mutagenic agents was 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 categorized as resistant, moderately resistant and highly resistant strains. It was noted that 3 strains were resistant, 8 were moderately resistant and 4 were highly (Table 3). Pathogenicity varied from 49.0 to 55.0, moderately resistant strains allowed pathogenicity in between 53.3 to 65.0 while highly resistant strains gave pathogenicity from 58.6 to 72.4.

4. Discussion

It is important to know that in the laboratory the extent fungicide resistant to pathogen can be achieved through the induction of resistance by various treatments. This may help in the management of fungicide resistance in a pathogen. In the present investigation, induction of carbendazim resistance in the sensitive *Aspergillus niger* isolate through the treatment of ultraviolet (UV), bromyl uracil (BU) and ethyl methanesulfonate (EMS) was studied. EMS treatment gave higher mutants and they were also

stable. The pathogenicity also increased with the increase of resistance. These results are in agreement with van Tuyl (1977); Ushiyama (1979); Davidse (1981); Dekker (1982); Gangawane and Reddy (1985).

van Tuyl (1977) induced benomyl and thiabendazole resistance in *Penicillium expansum*, *Aspergillus nidulans* and *Cladosporium cucumerinum*. Davidse and Flach, (1977) noted that allelic genes for resistance at chromosome locus in the pathogen. Reddy (1986) found that EMS, MMNG and UV treatment to *Aspergillus flavus* gave carbendazim and thiophanate-methyl resistant mutants. For this EMS was more powerful. Gangawane *et al.*, (1988) noted that emergence of resistance in *Phytophthora drechsleri* f.sp. *cajani* and *Puccinia arachidis* due to treatment of UV, gamma and SA.

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