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Studies on fungicide tolerance ability of some mutant isolates of *Trichoderma harzianum* and *Gliocladium virens*

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Fungicide tolerance ability of some Gamma-ray induced mutant isolates of *Trichoderma harzianum* and *Gliocladium virens* along with their wild biotypes was evaluated against benomyl, bavistin, thiram, captan, iprodione and mancozeb. The induction of fungicide tolerant strain was not target, through in some cases mutation altered the tolerance level either through enhancement or reduction. The performance of the mutant isolates of *T. harzianum* and *G. virens* was either more or less same against bavistin, thiram, iprodione and mancozeb or inferior against benomyl and captan to the wild isolates. The only exceptions were 50Th3VI against iprodione and 150GV1II against mancozeb.

Key words : Fungicide tolerance, mutant. *Trichoderma harzianum, Gliocladium virens*

INTRODUCTION

The indiscriminate use of potentially hazardous fungicides in modern agriculture system resulting in exclusive environmental pollution of resistant strains of the pathogens has been the course of worldwide concern. The possibility of controlling pathogenic fungi with antagonistic microorganisms introduced as a substitute for or in combination with sub-lethal doses of fungicides has long been considered to be an alternative approach as well as special interest of intergrated disease management (Papavizas, 1984). Vyas (1993) mentioned two phenomena involved in realistic approach for proper blending : (a) development of resistance in the antagonists for the fungicide to be used and (b) use of a selective fungicide does not become toxic to the antagonist, but at the same time effective against the pathogen. During last 20 years, several workers have reported the effective genetic manipulation of biocontrol agents for improving their tolerance to fungicides (Abd-El Moity *et al.*, 1982 ; Papavizas and Lewis, 1983 ; Papavizas *et al.*, 1982 ; Papavizas, 1987 ; Mukherjee and Mukhopadhyay, 1993). In the present investigation an attempt has been made to evaluate the fungicide tolerance ability of some gamma-ray induced mutant isolates of *Trichoderma harzianum* and *Gliocladium virens* along with their wild biotypes.

MATERIALS AND METHODS

Two wild isolates one each of *T. harzianum* (Th3) and *G. virens* (Gv1) were exposed to five different doses of gamma radiation viz, 50 KR, 75 KR, 100 KR, 125 KR and 150 KR. Several hundred mutants were isolated from the irradiated cultures and after extensive screening three gamma ray-induced mutants of *T. harzianum* and three of *G. virens* with enhanced *in vitro* biocontrol potential were selected for the present study along with their wild type isolates. The fungicide tolerance ability of the selected isolates towards six widely used fungicides viz., benomyl, bavistin, thiram, captan, iprodione and mancozeb was evaluated through poisoned food technique (Dhingra and Sinclair, 1995). For each fungicide four concentrations (eg., for benomyl and bavistin 0.5, 1.0, 2.0, 5.0 ppm, for thiram and iprodione 5.0, 10.0, 20.0, 50.0 ppm, for captan 1.0, 2.0, 5.0, 10.0 ppm and for mancozeb 20.0, 50.0, 100.0 and 250.0 ppm) were incoporated in sterilized PDA medium. The fungicide amended medium was

poured in 90 mm sterilized Petriplates and the plates were inoculated centrally with 6 mm diam. 3-4 day old mycelial discs of each isolate. Inoculated plates were incubated at 28±1°C for 4 days and the radial growth of the antagonists was measured. The ED_{50} value of the individual fungicide for each selected isolate was computed.

RESULTS AND DISCUSSION

The results on fungicide tolerance ability of gamma ray induced mutant isolates of *Trichoderma harzianum* and *Gliocladium virens* and their wild type isolates revealed that all the isolates were very sensitive to benomyl with ED_{50} values ranging from 0.69 ppm (for 50Gv1V) to 1.97 ppm (for 50Th3II) and the tolerance due to mutation slightly increased in 75GV1VI and 50Th3II over wild isolates (Fig. 1). Bavistin, being the fungicide of same chemical group, was found to be inhibitorier than benomyl to mycelial growth. The colony growth sharply checked at the concentration as low as 0.5 ppm. However, no remarkable variation in tolerance to bavistin was observed (Fig. 2). Thiram was less effective to restrict the mycelial growth of wild and mutant isolates *in vitro*. Though isolates fluctuated in their response towards some concentration, no detectable variation was noticed finally and ED_{50} values were more or less same (between 33.50 to 41.89 ppm) which revealed that mutation did not

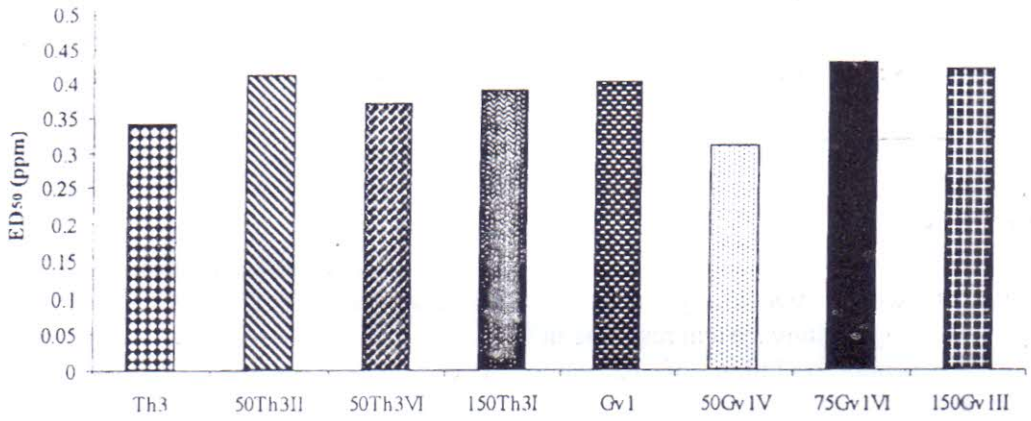

Fig. 1 : Tolerance to benomyl by wild and mutant isolates of *T. harzianum* and *G. virens*

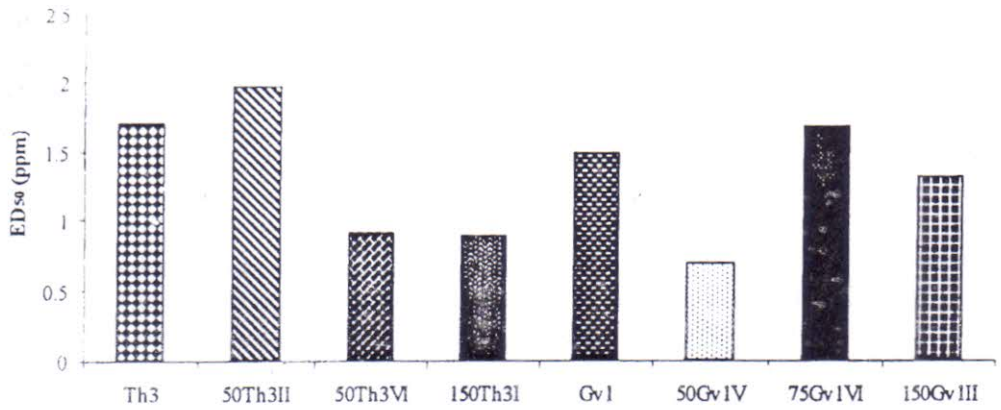

Fig. 2 : Tolerance to bavistin by wild and mutant isolates of *T. harzianum* and *G. virens*

have any effect on tolerance level to thiram (Fig. 3).

The tolerance level of all mutants decreased toward captan due to mutation as compared to their wild counterparts (Fig. 4). The most interesting result was noticed in tolerance to iprodione. The albino mutant of *T. harzianum*, 50Th3VI towards iprodione was remarkably high having ED_{50} value 56.49 ppm while the ED_{50} value of the wild type isolate was 22.45 ppm. On the other hand, in case of mutant isolates of *G. virens* the tolerance level decreased toward iprodione and the values were less than half of the wild isolate (Fig. 5). Aganist mancozeb, all the isolates of *T. harzianum* and *G. virens* exhibited extremely high level of tolerance. In case of *T. harzianum* the ED_{50} values ranged

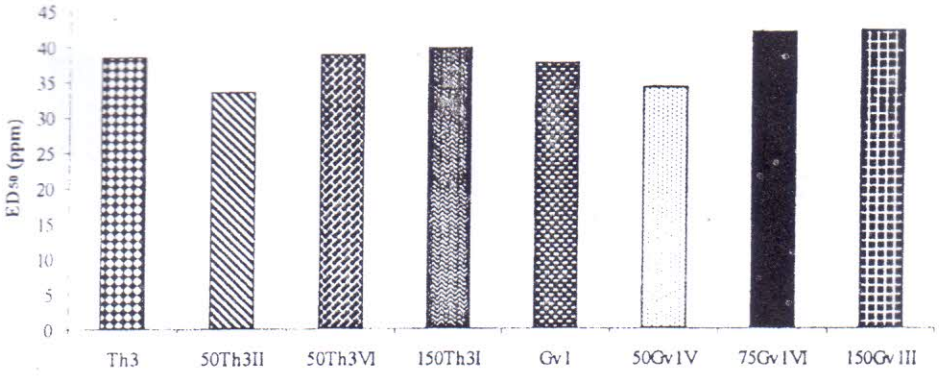

Fig. 3 : Tolerance to thiram by wild and mutant isolates of *T. harzianum* and *G. virens*

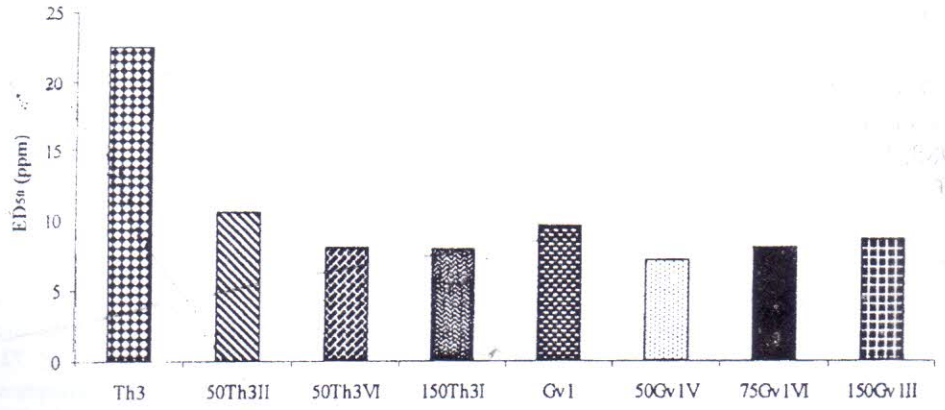

Fig. 4 : Tolerance to captan by wild and mutant isolates of *T. harzianum* and *G. virens*

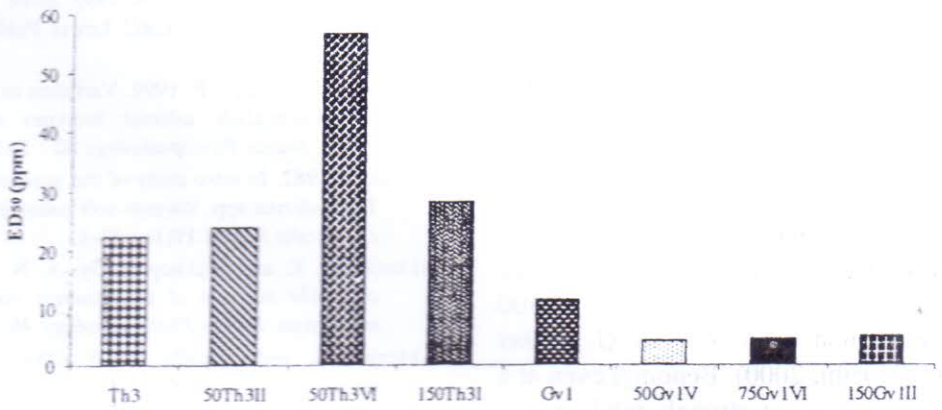

Fig. 5 : Tolerance to iprodione by wild and mutant isolates of *T. harzianum* and *G. virens*.

from 1188.50 to 1324.0 ppm and the all mutant isolates showed better tolerance potential in comparison to the wild biotype. Whereas, in case of *G. virens* the ED_{50} range from 331.13 to 758.58 ppm and the mutant isolate 150Gv1II was highly tolerant to mancozeb than its wild counterpart (Fig. 6).

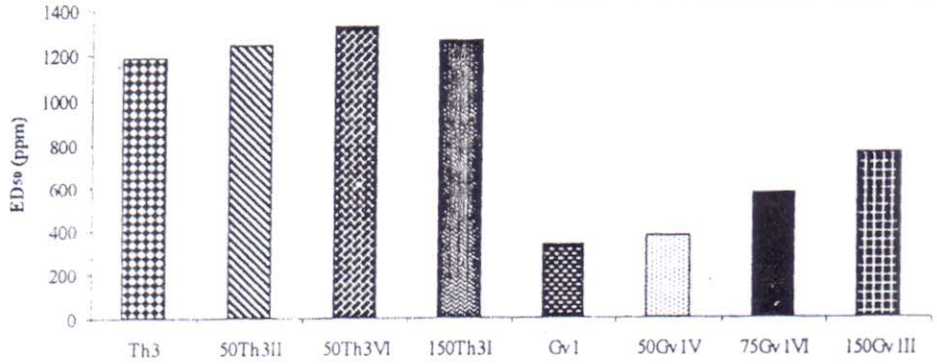

Fig. 6 : Tolerance to mancozeb by wild and mutant isolates of *T. harzianum* and *G. virens*

In the present investigation, the induction of fungicide tolerant strain was not the target, though in some cases mutation altered the tolerance level through enhancement or reduction. The modification in non-targeted desirable characters during mutational approach was reported (Ahmed and Baker, 1988, Kumar and Gupta, 1999). In this study, the performance of the mutant isolates of *T. harzianum* and *G. virens* was either more or less same against bavistin, thiram, iprodione and mancozeb or inferior against benomyl and captan to the wild isolates. The only exceptions were 50Th3VI against iprodione and 150Gv1II against mancozeb. Earlier it was reported that thiram, captan, iprodione and mancozeb were less inhibitory to *Trichoderma* (Abd-El Moity *et al.*, 1982, Papavizas, 1985), whereas benzimidazole group of fungicides i.e., benomyl and bavistin were toxic to *T. harzianum* and *G. virens* (Viji *et al.*, 1997). Pant and Mukhopadhyay (2001) found that thiram at 25 ppm inhibited the growth of both *T. harzianum* and *G. virens* while bavistin was found inhibitory to both the antagonists and more than 90% inhibition was observed at 1 ppm concentration. Mancozeb at concentration of 100 ppm was reported non inhibitory to *G. virens* (Mukherjee and Tripathi, 2000). Benomyl even at a concentration of 0.5 ppm was strongly inhibitory to *Trichoderma* spp. (Mirkova, 1982, Papavizas *et al.*, 1982). However, Papavizas *et al.* (1990) successfully developed stable mutants of *G. virens* tolerant to 10 ppm benomyl. Therefore, it can be concluded that induced mutation sometimes affect other traits along with the targeted one which may be desirable or undesirable for an antagonist.

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