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INDUCTION OF *GLIOCLADIUM VIRENS* STABLE TOLERANT MUTANTS OF TOPSIN-M WITH BROAD TOLERANCE TO FUNGICIDES FOR INTEGRATED CONTROL

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ABSTRACT: ***Conidia of Gliocladium virens were exposed to Ethyl methansulphonate (EMS), 2-methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl) amino propylamino] acridine dihydrochloride (ICR-170) or UV radiation in order to select stable mutants tolerant to Topsin-M. The induced isolates were able to tolerate up to 20 µg (active ingredient) a.i. /ml Topsin-M. Wild type strain was completely inhibited at 5 µg a.i./ml Topsin-M. Seven mutants out at 12 isolates tested exhibited stability. Four out of 7 subcultured isolates of Gliocladium virens were stable; these isolates retained their tolerance to Topsin-M 50µg a.i. /ml even after five subcultures on Topsin-M free medium. The four stable tolerant isolates were acquired tolerance to four systemic fungicides of different groups (Tecto, Rizolex, Rovral and Vitavax) with different degrees of tolerances. In vitro, the stable tolerant mutants of Gliocladium virens were capable of inhibiting seven plant pathogenic fungi i.e. Botrytis cinerea; Fusarium culmorum; Macrophomina phaseolina; Pythium aphanidermatum; Sclerotinia sclerotiorum; Sclerotium rolfsii and Rhizoctonia solani as did the wild type. In some cases, growth inhibition was more manifested than those exhibited by the wild type. In greenhouse, the tested Gliocladium virens wild type and the Topsin-M tolerant mutants reduced the incidence of bean damping-off and root rot caused by S. rolfsii. Combination of stable tolerant mutants with the fungicide gave a markedly synergistic effect to each of them.***

Key words: ***Gliocladium virens, Topsin-M, Induce mutation, Biological control, Systemic fungicides.***

INTRODUCTION

Effective control of plant pathogenic fungi mainly depends on chemical fungicides. The positive contribution of fungicides in agriculture has been challenged by their negative environmental pollution and human health hazards, which overweight their benefits. Thus, the use of biological control is an alternative method for controlling fungal plant diseases (Cook and Baker, 1983). *Trichoderma* and *Gliocladium* species are the most widely used fungi as biological control agents (Elad *et al.* 1982 and Papavizas, 1985).

Integration of biological and chemical control methods was more effective than either of them alone (Strashnov *et al.* 1985 and Viyas, 1993). Thus

tolerance of biocontrol agent to the fungicide to be used could be the main approach for integrated plant disease control (Viyas, 1993). Papavizas *et al.* (1982) and Abd-El-Moity *et al.* (1982) and Papavizas (1987) reported the genetic manipulation for improving the effectiveness of the biological control agents for plant disease control. Mutant biotypes of *Trichoderma harzianum* and *T. viride* that tolerate high concentrations of fungicides of the benzimidazoles group (MBC fungicides) such as benomyl were induced by Ultraviolet (UV) irradiation (Papavizas and Lewis, 1983, Papavizas *et al.* 1982). Key and Stewart (1994) were able to isolate iprodione-tolerant mutants for *Ghaetomium globosum, T. harzianum, T. viride* and *Trichoderma sp.* by irradiation of conidia with UV light. They failed to generate benomyl-tolerant mutants.

The biotypes differed from their wild-types strains in growth characteristics, sporulation, survival in soil and suppression of several soil-borne plant diseases. They obtained benomyl-tolerant mutants by exposing conidia of *T. harzianum* to nitrosoguanidine mutagen (Ahmed and Baker, 1987) *Gliocladium virens* is a promising biocontrol agent of several plant diseases (Papavizas, 1985, Lewis and Papavizas, 1987 and Papavizas and Lewis, 1989). Fungicide tolerance in *G. virens* would be useful for the integrated use of chemical and biological control. Mutants of *G. virens* were induced by Howell, 1982, the mutants differed from wild type strain in their ability to produce the antibiotic gliovirin. Mukherjee *et al.* (1995) developed seven stable mutants of *G. virens* resistant to 20 *µ*g a.i./ml carboxin and none developed resistance to carbendazin at 5 *µ*g a.i./ml. No researches have been done to produce mutants of *G. virens* tolerant to Topsin-M (thiophanate-methyl fungicide), which it is the most commonly used and recommended systemic fungicide under Egyptian conditions for seed and soil treatments.

The objectives of this study were to induce new biotypes of *G. virens* tolerant to Topsin-M, and test their stability, and test their cross tolerance to some systemic fungicides of different groups, and to test their capabilities as biological control agents *in vitro* and in greenhouse.

MATERIALS AND METHODS

Strains: A culture of *Gliocladium virens* was isolated from the rhizosphere of faba bean plants grown in Behaira governorate, Egypt. The plant pathogens cultures i.e., *Pythium aphanidermatum; Fusarium culmorum; Macrophomina phaseolina; Rhizoctonia solani; Sclerotinia sclerotiorum* and *Botrytis cinerea*, were supplemented from collection of agricultural Botany, Dept. Fac. of agriculture, Minufiya University, Shibin El-Kom, Egypt. *Sclerotium rolfsii* was freshly isolated from bean infected roots. All the cultures were maintained on 20% glycerol in refrigerator under freezing, when required, cultures were grown on PDA plates at 25 $\pm$ 2°C.

Conidia of *G. virens* were produced by growing the wild type isolate on PDA for 6 days under continuous fluorescent light. Conidia were removed

from the agar surface by pipetting 5 ml of sterile distilled water onto the surface and gently rubbing the surface with sterile cotton tipped applicator.

Conidia were counted with a haemocytometer and the suspension was adjusted with sterile distilled water to provide the desired concentration of conidia to contain $1x10^8$ conidia/ml approx.

Induction and selection of Topsin-M tolerant mutants:

Mutation treatment was carried-out following the method described by Papavizas *et al*. (1990), the aqueous suspensions of conidia were centrifuged twice at 5000 rpm for 15 min. and washed in 0.067 M phosphate buffer (pH 7.0). After the second washing, the pellets were resuspended in 4.0 ml phosphate buffer and conidial concentrations were adjusted with sterile distilled water to around 4×10^7 conidia/ml. The 0.067 M phosphate buffer conidial suspension was treated with either Ethyl methansul phonate (EMS) or 2-methoxy-6-chloro -9- [3- (ethyl-2- chloroethyl) amino propylamino] acridine dihydrochloride (ICR-170). Mutagens were added to conidial suspension at the desired concentrations as fellow:

EMS treatment: one ml of conidial suspension was added to a sterile 200 ml flask and 41.8 µl of 0.2 M, EMS in 1.0 ml of 0.067 phosphate buffer (0.04 EMS) was added to the spore suspension for a final conidial concentration of 2×10^7 conidia/ml. The mixture was incubated at 25 ± 2 °C in a water-bath shaker (150 rpm) for 1 hr. After incubation, 2.0 ml of 0.3 M, $Na_2S_2O_3$ was added to the conidia-EMS mixture to denaturize the mutagen.

ICR-170 treatment: ICR-170 was added at the rate of 100 µg/ml in the dark.

A 50 µl aliquots of EMS or ICR-170 treated conidia was spread on the surface of each Petri dish containing PDA supplemented with 20 µg a.i. /ml Topsin-M, 10 plates were inoculated.

UV-treatment: the fungicidal (20 µg a.i./ml) amended plates were lids removed, then exposed to UV radiation for 20 min at a distance of 25 cm of plate surface. The irradiated plates were recovered. The seeded plates of all treatments were incubated at 25 ± 2 °C under continuous fluorescent light, or in the dark for UV treatment. Each developing colony had resulted from conidia and each conidia receives only a single nucleus from the conidiophores (Picataggio *et al.* 1983).

Stability and selection of tolerant mutants:

Selection of tolerant mutants was based on their stability and colonies diameter on PDA amended with 50 µg a.i./ml Topsin-M.

The original mutants were sub cultured five times on fungicide free medium. Radial growth of the fifth subculture on PDA amended with 50 µg a.i./ml Topsin-M was measured and compared to that of the same isolate before selection.

Cross tolerance to other systemic fungicides:

The ability of the tolerant mutants to tolerate a range of chemicals representing four systemic fungicide groups was tested by poisoned food

technique. Appropriate amount of the fungicide was first suspended in 95% ethanol, then 1 ml of the suspension was added to 100 ml of PDA to obtain the desired active ingredient concentrations. Fungicides used were, Tecto (Benzimidazoles), Rizolex (Aromatic hydrocarbon fungicides), Rovral (Dicarboximides) and Vitavax (Carboxamides). Fungicides amended plates were centrally inoculated with 6 mm mycelial disc of the actively growing mycelial mat of the mutants. The inoculated plates were incubated at 25 ± 2°C and observations on radial growth were recorded periodically. Unamended inoculated PDA plates served as control.

Antagonistic ability of stable tolerant mutants:

The biological control performance of *G. virens* fungicide-tolerant stable mutants to some fungal plant pathogens was assessed *in vitro* in relation to the corresponding wild type strain, and against bean damping-off and root rot caused by *S. rolfsii* was assessed under greenhouse conditions.

***In vitro*: One week old PDA cultures of mutants, wild type and pathogens were used as source of inocula.**

One agar disc of *G. virens* wild type and the mutants were separately co-inoculated with the test pathogens on 90 mm diameter PDA plates 20 mm from the edge of the PDA plate opposite to each other. The inoculated plates were incubated at 25 ± 2 °C and observed daily. Radial growth of the tested pathogens was measured when the control plates attained a linear growth of 90 mm. Radial growth inhibition percentages of the pathogens were calculated following the formula of Zhou and Reeleder (1990), as follows: IP= (R1-R2)/R2 x 100.

Where, IP stands for inhibition percentage, R1 is the maximum radius of the colony of the pathogen and R2 is the radius of that part of the colony of the pathogen directly opposite the colony of the biological control agent.

In greenhouse: Antagonistic ability of wild type isolate of *G. virens* and selected tolerant mutants on damping-off and root rot of bean caused by *S. rolfsii* was assessed under greenhouse conditions.

Three parts of field soil and one part of well decomposed farm yard manure were mixed together and filled in plastic pots of 20 cm diameter (2 kg/pot).

The inoculums of *G. virens* wild type, selected mutants and *S. rolfsii* were prepared on wheat bran-vermiculite (1:1 w/w) in polyethylene bags. The multiplied inoculate of different fungi were applied at the rate of 20 gm/kg of pot soil one week before sowing for pathogen and at the time of sowing for antagonists. Pots infested with the pathogen only and uninfected soil were kept as control treatment.

Bean seeds CV Giza-6 were treated with Topsin-M (2.5 gm/kg) and air dried overnight and used as comparison treatment. Untreated seeds were kept as a control treatment. Ten seeds were sown in each pot, and then pots were kept under greenhouse conditions. The percentages of damping-off and root rot were recorded after 30 and 45 days of sowing, respectively.

RESULTS

Mutagenesis: A series of mutagenesis experiments were conducted to obtain Topsin-M stable tolerant mutants of *G. virens*. 100 µg a.i. /ml of ICR-170/ml, 0.04 M EMS and a single dose of UV radiation were used to mutaginize *G. virens* conidia. These concentrations were chosen based on concentration series experiments for each mutagen, where concentrations giving 20% survival for ICR-170, 10% survival for EMS or UV were chosen. Surviving colonies appeared 5 days after incubation at 25 ± 1 °C on PDA medium supplemented with 20 µg a.i. /ml Topsin-M. Wild type strain was completely inhibited at 5 µg a.i. /ml Topsin-M.

Treatment with ICR-170 resulted in 7 Topsin-M tolerant isolates, EMS treatment resulted in a relatively lower number of *G. virens* tolerant isolates i.e. 4 isolates. Uv treatment, resulted in the lowest frequencies of the fungicide Topsin-M tolerant isolates (one isolate) (Table 1).

Table (1): Number of *Gliocladium virens* colonies tolerant to Topsin-M induced by each of ICR-170, EMS or UV radiation.

Mutagen	Conidial survival (%)	Number of mutagenized conidia *	Tolerant colonies number per 10^7 survivors
100 µg ICR-170/ ml	20	3.1×10^7	7
EMS	10	2×10^7	4
UV	10	4×10^7	1
Control	95	4×10^7	0

*** Number of mutagenized conidia that provide 10^7 conidia/ml at all treatments.**

Topsin-M tolerant capacity of the selected mutants of *G. virens*:

The fungicide tolerant capacity of isolated colonies was initially determined. The induced isolates responded differently to Topsin-M concentrations. All GvTR isolates (*G. virens* tolerant to ICR) were not greatly affected by concentration 30 µg a.i./ml, Gv TR-1 was the most tolerant one at this concentration and gave 80% radial growth, while GvTR-4 and Gv TR-7 were the least tolerant ones and gave 64% radial growth. Also GvTE isolates (*G. virens* tolerant to EMS) were all tolerant at concentration 30 µg a.i./ml. GvTE-2 was the most tolerant isolate, while GvTE-4 was the least tolerant one. On the other hand, GvTU-1 (*G. virens* tolerant to UV) did not tolerate all tested concentrations and stopped growth completely (Fig 1).

By increasing the concentration, both GvTR and Gv TE isolates showed gradual decrease in their tolerances. All these isolates showed tolerances to concentration up to 100 µg a.i../ml. GvTE-3 was the most tolerant isolate at 100 µg a.i./ml and gave the highest radial growth (27%) followed by GvTE-2 and GvTR-3 (25% and 24%, respectively). On the other hand, GvTR-4, GvTR-7, GvTE-1 and GvTE-4 were the least tolerant isolates at 100 µg a.i./ml (10% radial growth) (Fig 1) compared to control (fungicide free medium).

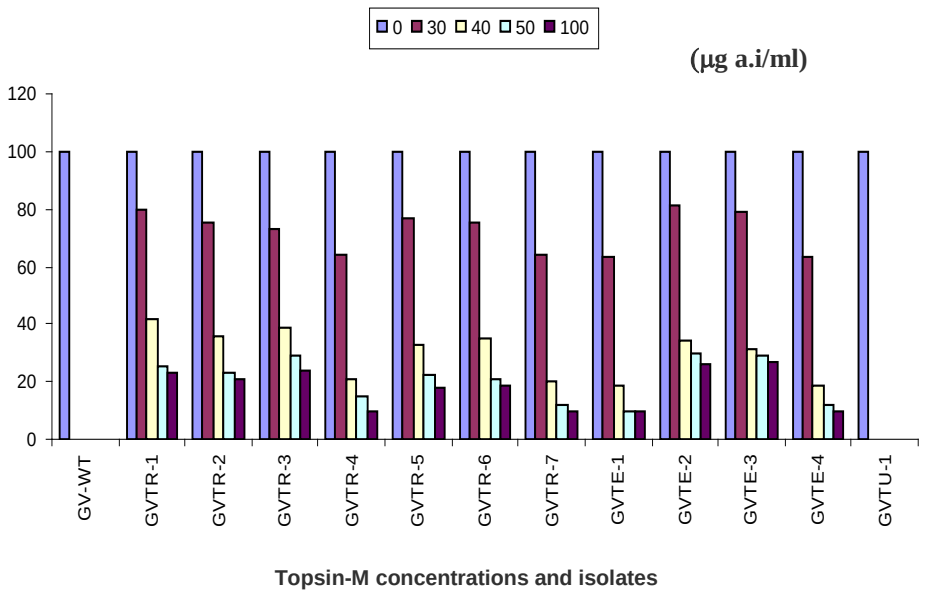


Fig (1): Topsin-M tolerance of *Gliocladium virens* (Gv) tolerant induced isolates expressed as radial growth (%),TR, Topsin-M tolerant isolates; TR, ICR-170 isolates; TE, EMS isolates and TU, UV isolates. Radial growth percent was calculated by 100 x X/Y, where X is colony radius (mm) of tolerant isolate and Y is the radius of corresponding wild type (WT) colony grown on Topsin-M free medium.

Stability of Topsin-M tolerant isolates:

Stability of tolerant isolates to the fungicide Topsin-M was tested for *G. virens* isolates which were able to tolerate high Topsin-M concentrations and showed the same growth on fungicide free medium as did the wild type, four isolates out of 7 isolates were able to grow and their conidia germinated on Topsin-M supplemented medium (50 µg a.i. /ml) after five subcultures without Topsin-M. The stable tolerant isolates were GvTR-2, GvTR-3, GvTR-6 and GvTE-3. On the other hand, the three isolates GvTR-1, GvTR-5 and GvTE-2 failed to retain their tolerance to Topsin-M through sub culturing on fungicide free medium (Table 2).

Table (2): Differential effect of Topsin-M (50 µg a.i/ml) on radial growth and conidial germination of original isolates and fifth isolation subcultures of *Gliocladium virens*.

Fungal isolates	Radial growth (%)			Conidial germination (%)		
	Original isolates	Fifth subculture	differences	Original isolates	Fifth subculture	differences
GV-WT	00.0	00.0	00.0	00.0	00.0	00.0
GVTR-1	50.3	00.0	50.3*	95.6	00.0	95.6*
GVTR-2	85.6	83.2	2.4	96.7	95.4	1.3
GVTR-3	82.5	81.9	0.6	95.1	94.2	0.9
GVTR-5	63.8	00.0	63.8*	95.8	11.1	84.7*
GVTR-6	83.7	81.4	2.3	95.2	94.6	0.6
GVTE-2	60.4	00.0	60.4*	92.3	8.5	83.8*
GVTE-3	63.4	61.7	1.5	93.4	92.7	0.7

Radial growth percent was calculated by 100 x X/Y, where X is colony radius (mm) of tolerant isolate and Y is the radius of corresponding wild type (WT) colony grown on Topsin-M free medium; T, Topsin-M tolerant isolates; R, ICE-170 isolates and E, EMS isolates.
*** Significant at 5% level according to the least significant difference test (Snedecor and Cochran, 1967).**

Cross tolerance of *G. virens* mutants to different systemic fungicides:

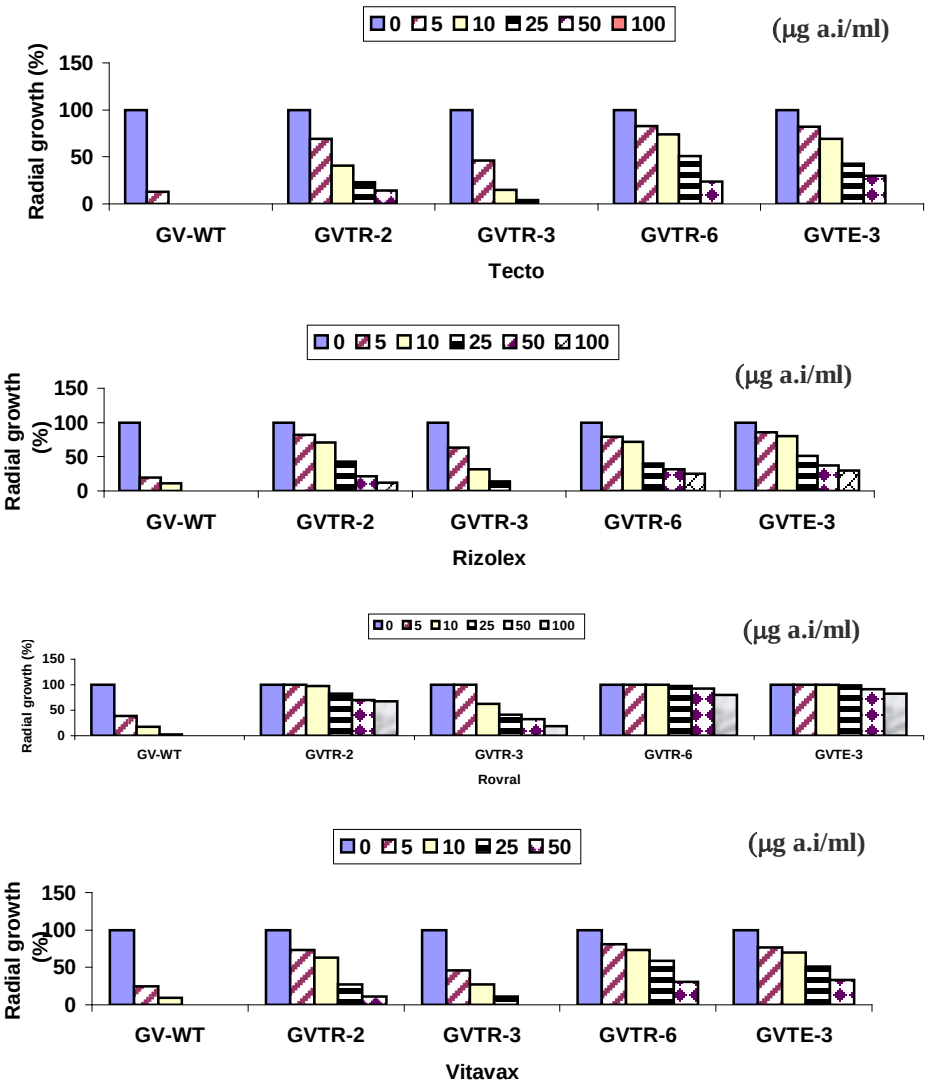
Four stable tolerant mutants of *G. virens* to Topsin-M i.e. GvTR-2, GvTR-3, GvTR-6 and GvTE-3 were tested for their tolerances to four systemic fungicides belong to different groups (Fig. 2).

All tested isolates were tolerant to the fungicide Tecto up to 25 µg a.i./ml. GvTR-2, GvTR-6 and GvTE-3 were tolerant to Tecto up to 50 µg a.i./ml. GvTR-6 and GvTE-3 were relatively more tolerant to Tecto than GvTR-2, whereas, GvTR-3 was the least tolerant one to Tecto.

The three isolates GvTR-2, GvTR-6 and GvTE-3 were tolerant to Rizolex up to concentration 100 µg a.i./ml, but isolate GvTR-3 was less tolerant to Rizolex and tolerated up to 25 µg a.i./ml. Also, GvTR-6 and GvTE-3 were more tolerant than GvTR-2.

All the four tested mutants were highly tolerant to the fungicide Rovral and showed tolerances up to 100 µg a.i./ml. GvTR-2 and GvTR-6 were most tolerant and GvTR-3 was the least tolerant. The three isolates GvTR-2, GvTR-6 and GvTE-3 were tolerant to concentration up to 50 µg a.i./ml of Vitavax, while isolate GvTR-3 tolerated up to 25 µg a.i./ml.

In general, all the tested mutants showed the most tolerances to the fungicide Rovral then Rizolex. GvTR-6 and GvTE-3 mutants showed the most tolerances to all tested fungicides.



(Fig. 2): Cross tolerance of *G. virens* Topsin-M tolerant mutants to some systemic fungicides of different groups compared to wild type, expressed as radial growth percent. T, Topsin-M tolerant isolates; R, ICR-170 isolates; E, EMS isolates and U, UV isolates. Radial growth percent was calculated by 100 x X/Y, where X is colony radius (mm) of tolerant isolate and Y is the radius of corresponding wild type colony grown on Topsin-M free medium.

Antagonistic effect of Topsin-M tolerant mutants of *G. virens* on radial growth of some pathogenic fungi in dual culture:

All tested mutants as well as the wild type of *G. virens* were able to inhibit the radial growth *in vitro* in dual culture of several pathogenic fungi i.e. *Botrytis cinerea, Fusarium culmorum, Pythium aphanidermatum, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Sclerotium rolfsii*. In some cases, growth inhibition was more manifested than that exhibited by the wild type. GvTR-2 was superior to inhibit *F. culmorum.* GvTR-6 was the most inhibitory mutant to *M. phaseolina* and resulted the highest growth reduction comparing to the other tested mutants and the wild type *G. virens*. Also, GvTR-6 was the most inhibitory mutant to *S. sclerotiorum*. On the other hand, some mutants were less inhibitory than the wild type of *G. virens.* GvTR-2 and GvTR-3 resulted in lower inhibition of radial growth of *P. aphanidermatum* than did the wild type. All the tested mutants were similar in their effect to the wild type in growth inhibition of *Botrytis cinerea* (Table 3).

Table (3): Percent inhibition of radial growth of some fungal pathogens by Topsin-M tolerant mutants of *Gliocladium virens*.

Fungal mutants	Radial growth inhibition (%)						
	Botrytis cinerea	*Fusarium culmorum*	*Macrophomina phaseolina*	*Pythium aphanidermatum*	*Sclerotinia sclerotiorum*	*Rhizoctonia solani*	*Sclerotium rolfsii*
G. virens (WT)	60.5 C	70.1 D	43.9 E	51.8 C	33.9 D	34.7 C	55.8 C
GV TR-2	66.9 A	83.1 A	52.3 C	42.9 D	44.8 C	36.1 B	56.1 C
GV TR-3	61.9 C	71.5 D	45.9 D	40.3 E	32.2 E	31.7 D	52.03 D
GV TR-6	67.4 A	75.2 C	63.8 A	57.03 A	69.3 A	38.3 A	57.2 B
GV TE-3	65.4 b	77.3 B	57.8 B	54.7 B	52.8 B	38.9 A	58.6 A

Percent inhibition of radial growth was calculated by (R1-R2)/R1x100, where R1 is maximum radius of the colony of the pathogen and R2 is the radius of that part of the colony of the pathogen directly opposite the colony of the biological control agent (Zhou and Reeleder, 1990).
Means within a column followed by the same litter(s) are not significantly different at the P=0.05 level according to the Least Significant Difference Test (Snedecor and Cochran, 1967).

Integration of stable tolerant mutants of *G. virens* with the fungicide Topsin-M for the control of damping-off and root rot of bean under greenhouse conditions:

All the tested mutants as well as the wild type of *G. virens* significantly reduced the incidence of bean damping-off and root rot caused by *S. rolfsii* under greenhouse conditions (Table 4). Combination of the wild type of *G. virens* with Topsin-M resulted in an effect similar to the fungicide separately. On the other hand, combination of stable tolerant mutants with Topsin-M gave markedly synergistic effects. GvTE-3 combined with Topsin-M was the most effective one for controlling damping-off comparing to the other combination. GvTR-6 and GvTE-3 were the most effective combination in reducing root rot caused by *S. rolfsii.*

Table (4): Integrated control of been damping-off and root rot caused by *S. rolfsii* by Topsin-M and its tolerant isolates of *G. virens* under greenhouse conditions.

Treatments	Damping-off %		Root rot %	
	Incidence	Disease reduction	Incidence	Disease reduction
G. virens (WT)	45.1 b	38.5	40.7 b	49.7
WT-Topsin-M	35.3 d	52.7	32.7 cd	59.6
GVTR-2	39.2 c	47.5	31.2 d	61.1
GVTR-2 + Topsin-M	23.7 ef	68.2	15.5 f	80.8
GVTR-6	41.8 c	44.0	33.2 c	59.0
GVTR-6 + Topsin-M	26.1 e	65.0	14.4 f	82.2
GVTE-3	39.1 c	47.6	32.3 cd	60.1
GVTE-3 + Topsin-M	22.03 f	70.5	14.9 f	81.6
Topsin-M	34.03 d	54.4	23.6 e	70.8
Control (untreated)	74.6 a	-	80.9 a	-

***Disease reduction in relation to untreated control = (untreated control – treatment)/ untreated control.**

Means within a column followed by the same litter(s) are not significantly different at the P=0.05 level according to the Least Significant Difference Test (Snedecor and Cochran, 1967).

DISCUSSION

Fungicide-tolerant mutants of biological control fungi provide powerful means to minimize fungicides use, as they can be integrated with fungicide in plant disease control programs. The present study showed that it is possible to induce *G. virens* mutants which tolerate Topsin-M concentration higher than the recommended field concentrations. The mutants were able to tolerate about 20 times more Topsin-M concentration than the respective wild type strain.

The tolerant isolates responded differentially to Topsin-M concentration. Since it was shown that Topsin-M conversion products are similar to benzimidazoles which bind tubulin in fungi, inhibit DNA synthesis or closely related process. Although strongly inhibit RNA and protein synthesis; and act as anti-metabolites of purines (Davids and Flash, 1977; Baum *et al.* 1978 and Kilmartin, 1981). Consequently it is possible that such an effect is due to different degree of binding affinity of Topsin-M to the altered tubulin proteins in the different mutants.

The present study showed that the Topsin-M stability of the induced tolerant mutants is affected by the Kind of mutagen used. The number of tolerant mutants of ICR-170 mutagen was more (3 isolates) than that of EMS mutagen (1 isolate) or UV radiation which doesn't result any stable mutants. Such results could be attributed to different response of *G. virens* to the

mutagen used and/or its repair. Notably (Key and Stewart, 1994) failed to induce *T. harzianum* biotype having benomyl tolerant stability. However in *T. viride,* Papavizas and Lewis, (1983) were able to obtain benomyl tolerant biotypes.

Furthermore, chemicals used as mutagen could have some effect on the outcome of mutant stability. Results of the present study tend to favor ICR-170 (a frame shift mutagen) for inducing a relatively higher frequency of stable mutants than EMS (chromosome breaker and point mutation inducer) or UV (Cyclobutane dimmer and DNA-protein cross-link).

The present study was capable to obtain stable Topsin-M tolerant mutants of *G. virens.* These mutants were able to develop a cross tolerance to some systemic fungicides of four different groups. The mutants were able for some what more or less to tolerate the fungicide Tecto (benzimidazole) up to 25 *µ*g a.i. /ml, and this could be attributed to the mode of action of Tecto which is antimitotic agent that specifically interfere with nuclear division and related process (Papavizas *et al.* 1990).

The mutants tolerated the fungicide Rizolex (aromatic hydrocarbons) up to 100 *µ*g a.i. /ml, since the fungicide induce a lipid peroxidation of mitochondrial and endoplasmic reticulum membrane and inactivate the enzyme of cytochrome-c-reductase (Vantuyl, 1977). Also, the mutants tolerated Rovral (Dicarboximides) up to 100 *µ*g a.i. /ml, the fungicide causes mitotic instability in cell division, DNA and RNA synthesis, also cause an increased frequency of mitotic recombination.

The mutants were able to tolerate up to 50 *µ*g a.i./ml, of Vitavax (carboximides), the fungicide act on inhibition of mitochondrial respiration, ribosomal function and synthesis of protein.

The mutation for tolerance to Topsin-M fungicide which have a broad spectrum of antifungal actively as well as the mutagens used resulted in a broad tolerance of the stable mutants to other fungicides (Davids, 1986; Papavizas *et al.* 1990; Salama and Amer, 1996 and Salama and Khalifa, 1997).

The present investigation was capable to obtain stable Topsin-M tolerant mutants of the biological control fungus *G.virens*. *In vitro* test of antagonistic ability in dual culture demonstrated that, most of the stable Topsin-M tolerant mutants did not lose their inhibiting growth of seven plant pathogenic fungi. Furthermore, the ability to inhibit *F. culmorum, M. phaseolina* and *S. sclerotiorum* was improved for the same of tolerant mutants. The mutants were similar or less effective than did the wild type strain against *P. aphanidermatum* or *B. cinerea*. It is important to consider *In vitro* test in selecting strains for use in the greenhouse and field as antagonists (Papavizas, 1985; Zhou and Reeleder, 1990; Burges, 1998; Amer, 2000 and Amer and El-Shennawy, 2007).

All the tested mutants and the wild type isolates of *G. virens* alone or combined with Topsin-M reduced the incidence of been damping-off and root rot caused by *S. rolfsii* under greenhouse conditions. Integration of Topsin-M

with *G. virens* wild type resulted in an effect similar to that of fungicide separately. Integration of stable tolerant mutants with Topsin-M gave markedly synergistic effect. GvTE-3 combined with Topsin-M was the most effective for controlling damping-off compared with other combinations. GvTR-6 and GvTE-3 combined with Topsin-M were the most effective combination in reducing root rot. Such synergistic effect could be due to the tolerance of the stable mutants to Topsin-M, whereas the wild type was sensitive. Biological control improvement has been reported for fungicide tolerant mutants of *T. viride, T. harzianum* and *G. virens* (Papavizas and Lewis, 1989; Papavizas and Collins, 1990; Key and Stewart, 1994; Viyas, 1993 and Amer and Salama, 2000).

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استحداث طفرات من فطر جليوكلاديوم فيرنس ثابتة التحمل للمبيد توبسن– م لاستخدامها في المقاومة المتكاملة

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الملخص العربى:

أمكن استحداث طفرات من فطر الجليوكلاديوم فيرنس يمكنها تحمل المبيد الفطرى توبسن–م عن طريق معاملة الجراثيم الكونيدية بأحد المطفرات . ايثايل ميثان سلفونات أو الاكريدين –١٧٠ أو الأشعة فوق البنفسجية وذلك لاستخدامها في برامج المقاومة المتكاملة لتقليل الجرعات العالية من المبيدات المستخدمة. تم الحصول على سبعه طفرات يمكنها تحمل تركيز يصل إلى ٥٠ ميكروجرام مادة فعالة من التوبسن– م مقارنة بالسلالة الأصلية والتى لم يمكنها النمو عند التركيز ٥ ميكروجرام مادة فعالة فقط. ولقد أظهرت أربعة طفرات من سبعة طفرات من الفطر جليوكلاديوم فيرنس ثباتاً لمقاومتها للتوبسن–م وذلك بعد تنميتها لعدد ٥ مرات على بيئة خالية من المبيد .

ولقد وجد أن الأربعة طفرات ثابته التحمل للمبيد توبسن– م كانت قادرة على تطوير مقدرتها على تحمل أربعة مبيدات جهازيه أخرى من أربعة مجموعات مختلفة وهى (التكتو ، الريزولكس ، الروفرال والفيتافاكس) وبدرجات مختلفة من التحمل .

وجد أن الطفرات ثابتة التحمل للمبيد ذات قدرة عالية على تثبيط نمو سبعة من الفطريات الممرضة للنبات تحت ظروف المعمل مثلها مثل العزلة الأصلية للفطر . وكانت بعض الطفرات أكثر تثبيطا للفطريات الممرضة عن العزلة الأصلية . ولقد وجد أن الطفرات ثابتة التحمل للمبيد كانت لها القدرة على مقاومة مرض موت البادرات وعفن الجذور في الفاصوليا تماماً مثل العزلة الأصلية من الفطر تحت ظروف الصوبة .

عند استخدام هذه الطفرات مجتمعه مع المبيد وجد أن نسبة مقاومة المرض كانت بدرجة أكبر عن أى منها منفرده.