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Induction of higher tolerance in *Trichoderma harzianum* against pesticides

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Trichoderma harzianum was subjected for induction of higher tolerance against fungicides (metalaxyl MZ, benomyl and propiconazole), nematicides (carbofuran and phorate) and weedicides (diuron and atrazin) at different concentrations. The higher tolerance in the bioagent was recorded against metalaxyl MZ upto 0.5 per cent. While, benomyl was found to be most toxic to the bioagent where tolerance could be induced upto 0.025%. Similarly for propiconazole the tolerance could be induced upto 0.025%. However, *Trichoderma harzianum* possessed tolerance against carbofuron 0.05% was further developed for higher tolerance of the same at 0.2% concentration. But in case of phorate tolerance could be induced upto 0.05%. Likewise, the isolates of the bioagent were developed to possess higher tolerance against diuron and atrazin each at 0.2%. Therefore, these fungicides, nematicides and weedicides can be integrated with *Trichoderma harzianum* for the integrated management of diseases, nematodes, weeds and soil borne plant pathogens, simultaneously.

Key words : *Trichoderma harzianum*, Fungicide

INTRODUCTION

The variable effects of pesticides mostly affect the bioagent by inhibiting its growth and sporulation. The need of hour is to develop tolerant strains against most commonly used pesticides. Abd-El Moity *et.al*. (1982) reported that tolerance could be developed in the antagonist *Trichoderma harzianum* against fungicides by prolonged and repeated exposure serially from lower to higher concentrations of fungicides.However, Papavizas (1987), Papavizas *et.al*. (1982) and Abd-El Moity *et.al*. (1982) reported the genetic manipulation for improving the effectiveness of the biocontrol agent for plant disease control.

MATERIALS AND METHODS

The sensitivity of *Trichoderma harzianum* against pesticides was evaluated by testing there effect on its radial growth with food poison technique (Grover & Moore, 1962). Further the isolates of *Trichoderma harzianum* having tolerance to sub-lethal doses of pesticides were subjected to a series of higher doses of the pesticides so as to develop even more tolerant isolates.

Pesticides *viz*., metalaxyl MZ (2000, 3000, 4000 and 5000µg ml^{-1}), benomyl (5, 10 and 25 µg ml^{-1}), propiconazole (25 and 50 µg ml^{-1}), carbofuran (500, 1000 and 2000 µg ml^{-1}), phorate (250, 500 and 1000 µg ml^{-1}), diuron (500, 1000 and 2000 µg ml^{-1}) and atrazin (500, 1000 and 2000 µg ml^{-1}) were used with PDA as basal medium for inducing higher tolerance in *Trichoderma harzianum.*

The tolerant isolates of *Trichoderma harzianum* against metalaxyl MZ (2000 µg ml^{-1}), benomyl (5 µg ml^{-1}), propiconazole (25 µg ml^{-1}), carbofuran (500 µg ml^{-1}), phorate (250 µg ml^{-1}), diuron (500 µg ml^{-1}) and atrazin (500 µg ml^{-1}) were selected as parent isolates for further enhancing the tolerance against higher doses of the chemicals. Mycelial disc of 5 mm diameter was taken from such tolerant culture and subjected to higher dose of the same chemical.

The isolates found growing well after 7 days of incubation were again transferred on higher graded concentration of medium. Gradual transfer of the isolate after 7 days of incubation up to 4-5 generations. Finally, the tolerant isolates developed were subjected at the same concentration to allow them to stabilize at the higher tolerance.

RESULTS AND DISCUSSION

Trichoderma harzianum was exposed to graded concentrations of seven selected pesticides so as to induce higher toleance. The isolate was subjected to increasing concentrations of metalaxyl MZ, benomyl, propiconazole, phorate, carbofuran, diuron and atrazin for 4-5 successive generations. The results are given in the Table 1.

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Table 1: Induction of higher tolerance in *Trichoderma harzianum* against pesticides

Sr. No.	Source of *T. harzianum*	Average radial growth (mm) Successive transfers at different concentrations µg ml^{-1}				
		I	II	III	IV	V
1	Metalaxyl MZ (2000 µg ml^{-1})	75.00* (2000)	83.33 (2000)	90.00 (3000)	90.00 (4000)	90.00 (5000)
2	Benomyl (5 µg ml^{-1})	27.00 (5)	67.33 (5)	52.66 (10)	51.00 (25)	-
3	Propiconazole (25 µg ml^{-1})	58.67 (25)	68.00 (25)	90.00 (25)	0.00 (50)	-
4	Carbofuran (500 µg ml^{-1})	73.67 (500)	55.67 (1000)	90.00 (2000)	90.00 (2000)	-
5	Phorate (250 µg ml^{-1})	69.33 (250)	75.00 (500)	90.00 (500)	0.00 (1000)	-
6	Diuron (500 µg ml^{-1})	63.00 (500)	90.00 (500)	72.33 (2000)	90.00 (2000)	-
7	Atrazin (500 µg ml^{-1})	57.67 (500)	63.33 (1000)	90.00 (2000)	90.00 (2000)	-

* Average of three replications.
Figures in the parenthesis are graded concentrations (µg ml^{-1}) of pesticides.

Induction of tolerance to metalaxyl MZ :
Trichoderma harzianum which scored 51.33 mm radial growth at 2000 µg ml^{-1} was used as parent isolate. The same isolate was again subjected to the same dose (2000 µg ml^{-1}) of metalaxyl MZ, wherein, it gained75.00 mm radial growth. In successive transfers from second and third each at 3000 µg ml^{-1}, it grew to 83.33 mm and to 90.00 mm, respectively. In fourth and fifth successive transfers at 4000 and 5000 µg ml^{-1} respectively the bioagent grew profusely. Thus, the higher tolerance up to 5000 µg ml^{-1} could be induced in *Trichoderma harzianum* against metalaxyl MZ.

Induction of tolerance to benomyl :
Initially, *Trichoderma harzianum* was found to be most sensitive to benomyl as no growth was recorded at 25 µg ml^{-1}. Therefore, the bioagent was subjected to the lowest dose of 5 µg ml^{-1} where it showed 27.00 mm radial growth. In second generation at the same concentration, it grew to 67.33 mm. Further, in third and fourth transfers of 10 and 25 µg ml^{-1} it grew to 52.66 and 51.00 mm respectively. Thus the bio agent slowly adopted the tolerance for 25 µg ml^{-1} of benomyl.

Induction of tolerance to propiconazole :
The isolate of *Trichoderma harzianum* growing at 25 µg ml^{-1} concentration of propiconazole was selected for induction of tolerance to higher doses of propiconazole. In first transfer at 25 µg ml^{-1}, the radial growth of the test fungus was restricted to 58.67 mm. However, in second and third transfer to the same dose, the growth was enhanced to 68.00 mm and 90.00 mm, respectively. While in fourth transfer at 50 µg ml^{-1} the antagonist failed to grow. Therefore *Trichoderma harzianum* could express its tolerance against propiconazole up to 25 µg ml^{-1} only.

Induction of tolerance to carbofuran :
The antagonist growing in 500 µg ml^{-1} of carbofuran gave 73.67 mm radial growth when subjected at the same concentration of carbofuran. Further, in second transfer at 1000 µg ml^{-1} as well as third and fourth transfer each at 2000 µg ml^{-1} it gained 55.67 mm and full growth (90.00 mm), respectively. Thus, the bioagent acquired tolerance easily upto 2000 µg ml^{-1} of carbofuran.

Induction of tolerance to phorate :
Trichoderma harzianum in first transfer at 250 µg ml^{-1}

of phorate recorded 69.33 mm radial growth. While, in second and third transfer each at 500 µg ml^{-1}, it acquired 75.00 mm and full growth i.e. 90.00 mm, respectively. But at fourth transfer at 1000 µg ml^{-1} phorate it failed to grow. Thus the bioagent could acquired the tolerance against 500 µg ml^{-1} of phorate successfully.

Induction of tolerance to diuron :

First transfer of *Trichoderma harzianum* at 500 µg $ml^{-1}$of diuron recorded 63.00 mm radial growth. Further in second transfer, it recorded full and profused growth (90.00 mm) while in third transfer at higher dose of 2000 µg ml^{-1} it gave 72.33 mm radial growth. Finally, at the fourth transfer of same dose full and profused growth of the bioagent was obtained. Thus, the bioagent could acquire the tolerance upto 2000 µg ml^{-1} of diuron.

Induction of tolerance to atrazin :

The antagonist was tried against atrazin at 500 µg ml^{-1} wherin 57.67 mm radial growth was recorded in first transfer. In second transfer at 1000 µg ml^{-1} as well as third and fourth transfers each at 2000 µg ml^{-1} it recorded 66.33, 90.00 and 90.00 mm radial growth, respectively. Thus, like diuron, atrazin also permitted *Trichoderma harzianum* to acquire higher tolerance upto 2000 µg ml^{-1}.

According to Georgopoulos (1977), benzimidazoles could enhance resistance even if used on a small quantity because they accelerated recombination and gene mutation and have mutagenic effect.

However, Papavizas *et. al.* (1982) developed tolerant biotypes of wild isolates of *Trichoderma harzianum* by transforming it from lower to higher doses of benomyl. Similarly, Vyas and Khare (1986) developed tolerant strains of *Trichoderma harzianum* and *Trichoderma viridae* by serially transforming them from sub lethal to higher concentrations of carbendazim.

The fungicides metalaxyl MZ, benomyl and propiconazole, nematicides carbofuran and phorate and weedicides diuron and atrazin can be integrated along with the tolerant biocontrol agent *Trichoderma harzianum* in the Integrated pest disease management modules as per the need of time.

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