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# Compatibility of *Trichoderma* strains and their mutants with common agrochemicals

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**Abstract**
A study was conducted to evaluate compatibility of two *Trichoderma* strains, *T. asperellum* (Th 14) and *T. harzianum* (Th 3) and their mutants (6 nos.) with seven common agrochemicals (captan, carbendazim, tebuconazole, imidacloprid, thiamethoxam, glyphosate and quizalofop ethyl) *in vitro* at two concentrations, *i.e.* 100 and 250 µg $ml^{-1}$. Results revealed that the parent strains were fully compatible (100%) with captan, imidacloprid, thiamethoxam and glyphosate, partially compatible with quizalofop ethyl and incompatible (0%) with carbendazim and tebuconazole, at both concentrations. All the mutants were found incompatible with carbendazim at both the concentrations. But compatibility was induced in mutants Th 3 M1 and Th 3 M6 with tebuconazole at 100 and 250 µg $ml^{-1}$(76.1 and 57.8%; 45.9 and 36.2%, respectively) as compared to their incompatible parent strain (Th 3). However, mutants Th 14 M2, Th 3 M2 and Th 3M6 exhibited lower compatibility (89.6, 83.3 and 94.4%, respectively) with captan at 250 µg $ml^{-1}$ as compared to their parent strains (Th 14 and Th 3). On the contrary, mutants Th 3 M1 and Th 3 M2 showed enhanced compatibility with quizalofop ethyl at 100 and 250 µg $ml^{-1}$ (58.4 and 47.0 %; 58.1 and 37.0%, respectively) as compared to their parent strain (Th 3). These findings indicated that the mutants varied in their interaction with the agrochemicals, yielding higher, similar or lower compatibility as compared to their parent strains. Mutants Th 3 M1 and Th 3 M6 were found most promising exhibiting compatibility with all the test chemicals except carbendazim.

**Keywords:** *Trichoderma,* mutation, compatibility, agrochemicals

## Introduction

Agrochemicals have been an indispensable component in agriculture for plant protection and maintenance of general health of the crops. However, an indiscriminate use of agrochemicals in past few decades has started taking its toll on the environment, casting multifaceted detrimental effects on all life forms including humans (Dutta *et al*., 2014) [4]. This has prompted the investigators to search for new strategies of pest control to contain the abuse of these chemicals. Biological control has been looked upon as a promising alternate approach for plant disease management under the persistent conditions of climate change, food safety and commercialization in agriculture (Manczinger *et al*., 2002) [11]. *Trichoderma* has been regarded as one of the most successful fungal biocontrol agents for past few decades owing to its properties of antagonism of phytopathogens, growth promotion, induction of systemic and localized resistance in plants and capability to survive in different environments (Dwivedi and Tewari, 2017) [5]. In integrated pest management (IPM), biocontrol agents are considered safer alternatives though rather less-reliable and less-efficient than their chemical counterparts. Hence, replacement of chemical pesticides with biocontrol agents appears rather unreasonable at present (Monte, 2001) [12]. Both biocontrol agents and chemicals are integral part of IPM strategies. So, the only option is to integrate them with low doses of chemical pesticides in order to chalk out an effective IPM strategy providing holistic plant protection which is effective, long lasting and eco-friendly. Agro-chemicals applied by any of the methods (seed treatment, soil application or foliar spray) ultimately reach the soil where they interact with biocontrol agents. These chemicals drastically affect the growth, reproduction and survivability of biocontrol agents and thereby their bio-efficacy potential (Patibanda and Rangaswamy, 2018) [13]. This explains the incompatibility of *Trichoderma* spp. with many agrochemicals (fungicides, pesticides and herbicides) (Bhowmick *et al*., 2015) [1]. Addressing the issue, it is important to develop stable pesticide resistant strains of biocontrol agents which could be done by using techniques like mutation (Villena and Guttierrez-Correa, 2006) [19]. It is also imperative to assess the compatibility of potential biocontrol agents with major agrochemicals used in modern agriculture (Krediks *et al*., 2003) [8]. It might enhance the efficiency with which biocontrol agents can be included in IPM strategies and might also broaden their applicability.

Keeping above in view, present study was conducted to evaluate compatibility of *Trichoderma* strains and their mutants with common agrochemicals for management of plant health.

## Materials and Methods

### *Trichoderma* strains and mutants used in the study

Two strains of *Trichoderma* used in the present study were *T. asperellum* (Th 14) procured from Department of Plant Pathology, Gobind Ballabh Pant University of Agriculture and Technology, Pantnagar and *T. harzianum* (Th 3) procured from Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi.
Mutants were developed by exposing these strains to chemical (ethyl methanesulphonate) and physical (ultraviolet and gamma irradiations) mutagens. List of parent strains and selected mutants is given in Table 1.

**Table 1:** List of the parent *Trichoderma* strains and their mutants used in the study

| Parent strains | Mutants |
|---|---|
| *Trichoderma asperellum* (Th 14) | Th 14 M1 |
| | Th 14 M2 |
| *Trichoderma harzianum* (Th 3) | Th 3 M1 |
| | Th 3 M2 |
| | Th 3 M3 |
| | Th 3 M6 |

### Agrochemicals used in the study

Compatibility of *Trichoderma* strains (Th 14 and Th 3) and their mutants was tested with seven agrochemicals. Details are given in table 2.

**Table 2:** List of agrochemicals used in the study

| Group | Common Name | Trade name | Manufacturer |
|---|---|---|---|
| Fungicide | Tebuconazole | Folicur 25.9 EC | Bayer Crop Science India |
| | Captan | Captaf 50 WP | Rallis India Ltd. |
| | Carbendazim | Bavistin 50 WP | Crystal Crop Protection |
| Insecticide | Imidacloprid | Confidor 17.8 SL | Bayer Crop Sciences |
| | Thiamethoxam | Actara 25 WG | Syngenta India Ltd. |
| Herbicide | Quizalofop ethyl | Targa Super 5 EC | Monsanto India Ltd. |
| | Glyphosate | RoundUp 41 SL | Dhanuka Agritech Ltd. |

### Compatibility estimation of *Trichoderma* strains and mutants with agrochemicals

The compatibility of *Trichoderma* strains and their mutants with agrochemicals was evaluated *in vitro* by 'Poisoned food technique' (Schmitz, 1930) [15]. Both the strains and their mutants were assayed against seven agrochemicals at two concentrations *viz.* 100 and 250 µg $ml^{-1}$. The required volume of stock solution of the chemicals was incorporated aseptically into molten Potato Dextrose Agar (PDA) medium before plating to obtain desired concentration of 100 and 250 µg $ml^{-1}$ of each chemical. Five mm disc of parent *Trichoderma* strains as well as their mutants were cut from the margins of 7-day old cultures and placed at the centre of Petri plates seeded with poisoned media. Plates were incubated at 26± 2˚C for 7 days. The strains and their mutants grown on non-poisoned PDA plates served as check. Experiment was laid out in completely randomized design while maintaining five replications for each treatment. Per cent mycelial inhibition was calculated using formula of Vincent (1947) [20], *i.e.* per cent mycelial inhibition= (X-Y)/X * 100, where, X= colony diameter (cm) in check; Y= colony diameter (cm) in treatment. Per cent compatibility was calculated as per the formula used by Khirallah *et al*. (2016) [7], *i.e.* Per cent compatibility = 100- (per cent mycelial inhibition).

### Statistical analysis

The data were analyzed using ICAR-CCARI statistical software (WASP 2.0). ANOVA (Analysis of Variance) of CRD was followed for treatment comparison.

## Result and Discussion

### Compatibility with fungicides

The results given in table 3, highlighted incompatibility of the parent strains (Th 14 and Th 3) with tebuconazole at 100 and 250 µg $ml^{-1}$. Similar results were obtained in the study of Rai *et al*. (2016) [14] where *T. harzianum* (Th 14) was found 100% incompatible with carbendazim and tebuconazole at 100 µg $ml^{-1}$. However, mutants Th 3 M1 and Th 3 M6 showed compatibility with tebuconazole at 100 µg $ml^{-1}$ (76.1% and 45.9%, respectively) and at 250 µg $ml^{-1}$ (57.8% and 36.2%, respectively) (Table 3). Rest of the mutants were found incompatible with the fungicide tebuconazole. In a study conducted by Hatvani *et al*. (2004), mutants of *T. harzianum* and *T. atroviride* tolerant to 100 µg $ml^{-1}$ tebuconazole were developed by UV mutagenesis. However, reports for the tolerance in *Trichoderma* beyond 100 µg $ml^{-1}$ concentration of tebuconazole are not present. Hence, compatibility of mutants Th 3 M1 and Th 3 M6 recorded in the present study with tebuconazole at 250 µg $ml^{-1}$ is novel in this context.
The results indicated in Table 3 also indicated no compatibility (0%) of the strains and their mutants with carbendazim at both concentrations *viz.* 100 and 250 µg $ml^{-1}$. Results also showed that both the strains and their mutants were highly compatible (100%) with captan at 100 µg $ml^{-1}$ as well as 250 µg $ml^{-1}$. Although, mutants Th 14 M2, Th 3 M2 and Th 3 M6 showed 100 per cent compatibility with captan at 100 µg $ml^{-1}$, however, they were found comparatively less compatible (89.6, 83.3 and 94.4%, respectively) at 250 µg $ml^{-1}$ (Table 3). These findings are in accordance with Singh *et al*. (2015) [17] and Singh *et al*. (2016) [18] where 100 per cent compatibility of *T. harzianum* and *T.viride* with captan was reported. It is in accordance with the study of Malathi *et al*. (2002) [10], where radial growth of *Trichoderma* species was completely inhibited by carbendazim even at 1 µg $ml^{-1}$.

### Compatibility with insecticides

Results indicated in Table 3 highlighted 100 per cent compatibility of *Trichoderma* strains (Th 14 and Th 3) and their mutants with the insecticides *viz.* imidacloprid and thiamethoxam at both concentrations, *i.e.* 100 and 250 µg $ml^{-1}$. Work of Dhanya *et al*. (2016) [2] and Sharma and Singh (2016) [18] support the findings of the study where *T. viride* and *T.* harzianum, respectively, exhibited 100 per cent compatibility with imidacloprid and thiamethoxam. Similar findings were reported by Dutta and Das (2017) [3] where 100 per cent compatibility in *T. harzianum*, *T. asperellum*, *T. viride*, and *T. pseudokoningii* was found with thiamethoxam (25 % WG).

### Compatibility with herbicides

Both the strains and their mutants exhibited 100 per cent compatibility of the strains and mutants with glyphosate at 100 and 250 µg $ml^{-1}$ (Table 3). Similar findings were reported by Dutta and Das (2017) [3] where *T. viride* showed 100 per cent compatibility with glyphosate. *Trichoderma* strains and their mutants exhibited varying compatibility with quizalofop ethyl. Per cent compatibility recorded for strain Th 14 at 100 and 250 µg $ml^{-1}$ was 35.7 and 27.0 per cent, respectively, while for strain Th 3 it was 49.2 and 35.9 per cent, respectively. Compatibility was enhanced in mutants Th 3 M1 at 100 µg $ml^{-1}$ (58.4%) and 250 µg $ml^{-1}$ (47.0%), which was at par with mutant Th 3 M2 showing compatibility of 58.1 and 37.0 per cent at 100 and 250 µg $ml^{-1}$, respectively as compared to parent strain Th 3. Compatibility was diminished in mutants Th 3 M6 (37.8 and 27.3 percent) followed by Th 14 M1 (25.9 and 15.0 percent), and Th 14 M2 (26.7 and 22.9 percent) at 100 and 250 µg $ml^{-1}$, respectively as compared to their parent strains. Study conducted by Kurumuri and Singh (2015) [9] showed 57.33 per cent compatibility of *T. harzianum* (strain Ts6) to 500 µg $ml^{-1}$ of quizalofop ethyl.

**Table 3:** Percent compatibility of *Trichoderma* strains and their mutants with major agrochemicals

| ***Trichoderma* strains/ mutants** | **Concentration (µg $ml^{-1}$)** | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Tebuconazole** | | | **Carbendazim** | | | **Captan** | | | **Imidacloprid** | | | **Thiamethoxam** | | | **Glyphosate** | | | **Quizalofop ethyl** | | |
| | **C*** | **100** | **250** | **C** | **100** | **250** | **C** | **100** | **250** | **C** | **100** | **250** | **C** | **100** | **250** | **C** | **100** | **250** | **C** | **100** | **250** |
| Th 14 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 35.7 | 27.0 |
| Th 14 M1 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 25.9 | 15.0 |
| Th 14 M2 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 89.6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 26.7 | 22.9 |
| Th 3 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 49.2 | 35.9 |
| Th 3 M1 | 100 | 76.1 | 57.8 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 58.4 | 47.0 |
| Th 3 M2 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 83.3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 58.1 | 37.0 |
| Th 3 M3 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 52.9 | 36.7 |
| Th 3 M6 | 100 | 45.9 | 36.2 | 100 | 0 | 0 | 100 | 100 | 94.4 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 37.8 | 27.3 |
| cd (0.05%) | - | 1.20 | 1.24 | - | - | - | - | - | 2.32 | - | - | - | - | - | - | - | - | - | - | 1.44 | 1.42 |
| Cv | - | 3.13 | 3.17 | - | - | - | - | - | 2.11 | - | - | - | - | - | - | - | - | - | - | 3.58 | 2.82 |

*C stands for check

Any two means having common letters are not significantly different at the 5% level of significance

### Conclusion

Mutation has been proved to be a promising technique in developing pesticide compatibility in bio-efficacious *Trichoderma* strains. The present study highlighted that interaction of the mutants with the agrochemicals might yield lower, similar or higher compatibility as compared to the parent strains from which they are derived. Mutants Th 3 M1 and Th 3 M6 were found compatible with fungicide tebuconazole at 250 µg $ml^{-1}$ as compared to the parent strain Th 3, which is the first report of its kind. Mutants Th 3 M1 and Th 3 M6 were found compatible with all the other agrochemicals as well, except carbendazim which highlights their broad range of applicability. Biocontrol potential of these mutants is under evaluation and if found competent, they could become a very crucial component of IPM strategies.

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