**JBiopest 13**(1):97-102(2020)

# Dynamics of *Trichoderma* spp. against *Fusarium* wilt based on *in vitro* and *in silico*

**Preeti Sonkar* and Manisha Pritam**

**ABSTRACT**

Dynamics of *Trichoderma* spp., is considered as an ecofriendly process due to their prolific effect of secondary metabolite when used as biological control agent in agriculture for crop protection. *Fusarium* wilt is one of the most serious disease of crop which crop loss in short time due to vascular wilt with the support of secretary protein and enzymes i.e. SIX, Sg1, TOM, *pel* D. Here, we have selected two pathogens one causing the Tomato wilt and is the other Chickpea wilts for *in vitro* analysis. Moreover, we had been used of analysis approach as *in vitro* screening by dual technique and *in silico* by molecular docking based on AutoDock vina 4.0.*In-vitro* screening, the result was better with T2 (*Trichoderma virens*) in comparisontoT1, T5, T7, T12, T13,T14, Th, Tv and control in case of both pathogens. *In silico* screening, (101849747) ligand was found to the best when compared to 379 compound with SIX, Sg1, TOM, *pel*D based on binding energy as -15.5, -19.6,-19.7,-16.0.

**Key word:** *In vitro* screening, *In silico* screening, *Trichoderma* spp., *Fusarium* wilt, antagonistic, secondary metabolite.

**MS History:** 16.01.2020 (Received)-25.05.2020 (Revised)- 30.05.2020 (Accepted).

**Citation:** Preeti Sonkar and Manisha Pritam. 2020. Dynamics of *Trichoderma* spp. against *Fusarium* wilt based on *in vitro* and *in silico*. *Journal of Biopesticides*, **13**(1):97-102.

## INTRODUCTION

*Trichoderma* spp., have been used as an important biocontrol agents in agriculture fields against pathogen and crop improvement. The genus *Trichoderma* comparises green sporulating ascomycetes, filamentous, soil dwelling fungi that are found all over world. These fungi have been studied for their character of mycoparasitism and prolific of secondary metabolites with pharmaceutical and biotechnological importance that include non-ribosomal peptides (NRPs), siderophores, peptaibols, pyrones, poliketides, and volatile and nonvolatileterpenes which have used in crop protection (Contreras-Cornejo *et. al*. 2018). *Fusarium* wilt is one of the most serious diseases posing a threat to economic important cultivation due this ability develop a vascular wilt disease with the support of secretary protein and enzyme like SIX, Sg1, TOM, pelD (Roncero *et al*., 2000; Michielse and Rep, 2009; Sain and Rep, 2015). The use of beneficial microorganisms for plant disease management is an effective approach (Keswani *et al*., 2018).

To evaluated to the potentiality of native isolate *Trichoderma* spp. play a vital role in reducing the pathogenicity by using dual culture method and *in-silico* were employed. Application of the antagonistic fungi *in vitro* and *in silico*, was examined for their ability in reducing of *Fusarium* wilt disease and the performance of isolated strain of *Trichoderma* has been found to be superior over the others. Therefore, the experiment was carried out to assess the best antagonistic ability of native *Trichoderma* spp. in suppressing the populations of *Fusarium oxysporum* will pay the way for its applicability as a potential biocide in future.

## MATERIALS AND METHODS

### Fungal isolation

*Trichoderma* spp. were isolated from rhizospheric zone of healthy plant of chickpea and Tomato Chitrakoot (M.P. and U.P.) in this study for comparative efficacy of *Trichoderma* species on *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *ciceris*. The culture of *F. oxysporum* f. sp. *lycopersici* and *F.*

*oxysporum* f. sp. *ciceris* were received from biocontrol lab, C.S.A. and Technology, Kanpur (U.P.), India.

**In vitro assay**

In this experiment, a large number of isolates were examined to for their antagonistic activity based on the methods adopted by Saravanakumar *et al.* (2016) on potato dextrose agar plate. Here, each isolate *Trichoderma* was placed to opposite of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *ciceris*and a positive control was also kept without *Trichoderma* strains.

**In-silico assay**

This experiment was performed based on Mohd. *et. al.*,(2013)and Cosconati *et. al.* (2010) asdetailed given below:

The selected secondary metabolites of *Trichoderma* strain (379 compounds) was given in supplementary table 1 and wilt causing protein (SIX, TOM, Sg1, *pel* D) of *Fusarium oxysporum* with their accession number was also listed in table 1.

**PDB file formation of protein and ligand**

The fasta sequence of proteins (SIX1, TOM1, Sg1, *pel* D) was retrieved from NCBI by using accession number. After that we have used MGL Tools (http://mgltools.scripps.edu/downloads) for AutoDock vina download. AutoDock tools are a module within the MGL tools software package specifically for generating input (PDBQT file) for AutoDock vina. Phyre 2.0 was used for protein structure prediction. PDB file was downloaded and used as input file. However, MODELLER is used for homology modeling. In case of ligand compounds (secondary metabolites) we have retrieved the SDF file from Pubchem [http://www.pubchem.ncbi.nlm.nih.gov/]. These SDF file was converted into PDB file by using BABEL software.

**Molecular docking based on AutoDock vina**

Prepared PDBQT format for protein and ligand (protein.pdbqt and ligand.pdbqt) file was used as input in molecular docking of Autodock vina tool.

**Data analysis**

The analysis of data was done by XLSTAT software, measure to Average value, standard derivation, standard error, also using to ANOVA-single factor which is represented in result.

**RESULT**

The list of isolates strain from rhizosphere and rhizoplaneare also included to species identification accession along with Their identification are presented here under Table 1.

Table 1. List of *Trichoderma* spp. isolated from Rhizosphere

| Strain code | Fungi | ID. NO. |
|---|---|---|
| T-1 | *T. longibranchiatum* | 10,319.16 |
| T-2 | *T. longibranchiatum* | 10,320.16 |
| T-3 | *T. longibranchiatum* | 10,321.16 |
| T-4 | *T. longibranchiatum* | 10.322.16 |
| T-5 | *T. longibranchiatum* | 10.323.16 |
| T-6 | *T. longibranchiatum* | 10,324.16 |
| T-7 | *T. longibranchiatum* | 10,325.16 |
| Th | *T. asperellum* | Molecular identification only |
| Tv | *T. asperellum* | Molecular identification only |

List of Table 1, was isolated total nine from rhizosphere zone and not found from rhizoplane zone of healthy plant of chickpea and tomato. This result indicated that mostly *Trichoderma* spp. presented in rhizosphere zone and rare in rhizoplane zone. Species conformed from ITCC, New Delhi which available in table 1.

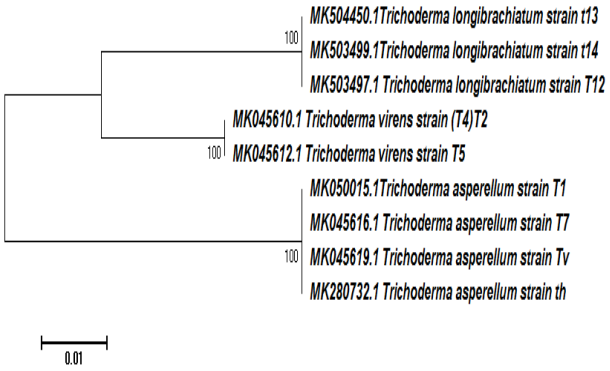


**Figure 1.** Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [1]. The tree with the highest log likelihood (-1023.0261) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the

Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 9 nucleotide sequences. Codon positions included were $1^{st} + 2^{nd} + 3^{rd} +$ Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were A total of 528 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [2].

**N**ext experiment was performed to molecular identification based on ITS5 and ITS4 marker and species variation and similarity represented in dendogram form in fig. 1. Number of isolate species was divided in 3 group.

**Table 2(A).** Growth of *Fusarium oxysporum* f. sp. *ciceri* (FOC) in dual culture

| Strain code | Mean | S.E. |
|---|---|---|
| **T1** | 1.133 | 0.0666 |
| **T2** | 0.8 | 0.1527 |
| **T5** | 1.2 | 0.0577 |
| **T7** | 1.266 | 0.08819 |
| **T12** | 1.233 | 0.08819 |
| **T13** | 1.266 | 0.12018 |
| **T14** | 1.266 | 0.0333 |
| **Th** | 1.133 | 0.08819 |
| **Tv** | 1.233 | 0.0333 |
| **FOC(Control)** | 2.4 | 0.0577 |

Growth of *Fusarium oxysporum* f. sp. *ciceri* in dual culture technique was range of average value as T1 1.133, T2 0.8, T5 1.2, T7 1.266, T12 1.233, T13 1.266, T14 1.266, TH 1.133, Tv 1.233, Foc (Control) 2.4 where T2 was presented 0.8 A.V. minimum growth of *F.oxysporum* f. sp. *ciceri*other hand T7, T13, T14 were equal in average value as 1.266 but higher growth to T2. ANOVA-single factor analysis, F>F critical as 79.85944 >3.354131, we rejected the null hypothesis. Test of significant are available.

Results of per cent inhibition of FOC showed that maximum percentage of inhibition was T2 (66.66%) in comparisons to other strain as T1 (52), T5 (50), T7 (47.25), T12 (48.62), T13 (47.25), T14 (47.25), TH (52.79), Tv(48.62) and Foc (control) (100).

**Table 3(A).** Growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in Dual culture technique

| S. NO. | AV | SE |
|---|---|---|
| 1 | 1.6 | 0.208167 |
| T2 | 0.966667 | 0.120185 |
| T5 | 1.866667 | 0.185592 |
| T7 | 1.233333 | 0.145297 |
| T12 | 1.1 | 0.057735 |
| T13 | 1.166667 | 0.088192 |
| T14 | 1.133333 | 0.088192 |
| Th | 1.2 | 0.057735 |
| Tv | 1.266667 | 0.088192 |
| FOL | 4.066667 | 0.296273 |

Data of Table 3 (A) represented to Growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in Dual culture technique. Minimum growth of FOL was in T2 (0.966667) in comparison to T1 (1.6), T5(1.866667), T7(1.233333), T12(1.1), T13(1.166667), T14(1.133333), Th (1.2), Tv(1.266667), Fol (4.066667). After statistical analysis, and Standard error value (S.E.) value are presented in Table 3 (A). F>F critical value as 21.94308>3.354131represented reject to null hypothesis as well as proof test of significant are available.

**P**ercentage of inhibition of FOL was revelas that maximum percentage of inhibition 75.85 was observed in T2 in comparison to other i.e. T1 (60), T5 (53.5), T7 (69.25), T12 (72.5), T13 (71), T14 (71.75), TH (70), Tv (70), and FOL (100-Control). *In vitro*, the dual culture technique was measurement according to Barari (2016). Each species represented vary degree of inhibition to FOL and FOC (Rahman *et al.*, 2009).

**In-silico analysis**

In this experiment, 379 volatile compounds of *Trichoderma* spp. were performed to interaction with*Fusarium oxysporum* sp. releasecompounds*in silico* as well as we was gained best 101849747 as ligand which are presented in table 4. Ligand 101849747 was showed best interaction with TOM in comparison to SIX, pel D, Sg1 which is macromolecule of Wilt causing interaction. **A** ribbon like virtual interaction observed between TOM and 3, 3, 4, 4, 5, 5-hexamethyl, 6-(5-hydroxy) pentyl-24-Pyran-2-one. Also another order of interaction as TOM >Sg1>SIX> pel D based on binding energy by using Autodock vina 4.0.

**Table 4. Interaction of bio-molecule *in-silico***

| Wilt causing protein (Macromolecule) | *Trichoderma* compound (Ligand) | Binding Energy (Kcal/mol.) |
|---|---|---|
| SIX | 101849747 | -15.5 |
| TOM | 101849747 | -19.7 |
| pel D | 101849747 | -16.0 |
| Sg1 | 101849747 | -19.6 |

## DISCUSSION

*F. oxysporum* is as a tomato wit agent and chickpea wilt agent which is considered as a disease in Tomato and Chickpea. Therefore, *Fusarium* wilts are evaluated a limiting factors for production of tomato and chickpea. Here, we focused on management of *Fusarium* wilt in tomato and chickpea through *Trichoderma* spp. The interaction between *Trichoderma*-pathogen placed on PDA plates .*Trichoderma* spp., mostly are presence in soil of plant root (Ranasingh, 2006). The result of native isolate *Trichoderma* spp. revealed based on morphology and micrography by light microscope (Riafi,1999) and conformed by molecular identification from nfcc, pune.

We determined the efficacy of each *Trichoderma* spp. against FOL and FOC *In-vitro* and *In-silico*. *In-vitro,* the dual culture technique (Barari, 2016) was followed, represented different degree of inhibition to FOL and FOC ((Rahman*et. al*., 2009) and varied average range of FOC and FOL growth were as 2.4, 1.133, 0.8, 1.2, 1.266, 1.233, 1.266, 1.266, 1.133, 1.233 and 4.066, 1.6, 0.966667, 1.866667, 1.23333, 1.1, 1.166, 1.133, 1.2, 1.266667. Antagonist character of each species is varied degree. Therefore, T2 has inhibited maximuman growth of FOL and FOC in comparision to other plates. Therefore, T2 (or T4) represented as *T. asperellum*MK045610 based on molecular identification (Wu *et al.*, 2017). FOL and FOC was minimum radial mycelium growth in $T_2$ treatment in comparision to control and others treatment. As well as *T. asperellum*MK045610 was showed best bioconol agent for inhibition of FOL and FOC by *in vitro*. Hernandez *et. al.* (2011) reported that *T. asperellum* and *T. longibranchiatum*was best in antagonistic compare to other *in vitro*. Further, Antagonist *Trichoderma* has reduced growth of FOL and FOC by different mechanisms like competition, hyphal interaction, antibiosis, mycoparasitism, and enzyme secretion, according to Ranasingh (2006). Similarly, Benitez (2004) has reported that *Trichoderma harzianum* CECT2413 was protected from *Rhizoctonia*. The efficacy of different isolate *Trichoderma* spp. was determined against Fol and Foc based on dual technique in which T2 was the best for both cases (Fol and Foc), % of inhibition was 66.66 in Foc and 75.85 in Fol respectively.

Auto Dock is most applicable tools in virtual screening, identify to ligand that bind to a specific receptor as predict to binding site of biomolecule, energetically most favorable binding pose and approaches try to calculate the absolute free binding energy in kcal/mol., KJ/mol., respectively (Raschka,2014).

*In-silico*, we selected wilt causing protein, enzyme of *Fusarium oxysporum* spp. and secondary metabolites of *Trichoderma* spp. and predicted docking score by Autodockvina. The best ligand was 3, 3, 4, 4, 5, 5-hexamethyl, 6-(5-

hydroxy) pentyl-24-Pyran-2-one of *Trichoderma* spp. and best receptor was TOM of *Fusarium oxysporum* spp.. Interaction of ligand and receptor was scoring as -19.6. TOM was represented as best receptor that could be inhibited by 1010849747 (3, 3, 4, 4, 5, 5-hexamethyl, 6-(5-hydroxy) pentyl-24-Pyran-2-one) as such ligand. Vinale *et al.*, 2008 had been reported about 6pp which was volatile, secondary metabolite, played a crucial role in cell wall degradation of fungi and Rabinal and Bhat (2017) was also reported this metabolite play role in inhibition of cell wall biosynthesis and aid in mycoparasitic behavior for Plantpathogen. During docking, compound prescribed as binding site of receptor and ligand, thus estimate to binding affinity of the complex, as an important part of the structure based drug design process (Seeliger D. and Groot de L. bert; 2010).*In-silico*, 379 metabolites of *Trichoderma* strain was tested against wilt protein and enzyme as SIX, TOM, *pel D*, Sg1 by molecular docking *viz.* Autovina dock. Morever, among all metabolites, 3, 3, 4, 4, 5, 5- hexamethyl, 6-(5-hydroxy) pentyl-24-pyran-2-one (CID-101849747) showed excellent result based on binding score.

From this study, it concluded that the nine isolates of *Trichoderma* from rhizosphere zone and none of them was from rhizoplane zone. Total no. of isolate *Trichoderma* spp. represented antagonistic potential against *Fusarium oxysporum* spp., examined *in-vitro, in silico*. In-vitro, result T2 (*Trichoderma virens*) was best species in comparison to other. However, 3, 3, 4, 4, 5, 5-hexamethyl, 6-(5-hydroxy) pentyl-24-Pyran-2-one (101849747) was best for four *Fusarium* wilt causing agent (TOM> Sg1> SIX>pel D) in comparison to 376 metabolite molecules based on binding energy.

**Author statement**

PS performed the experiments and anaiyzed the results. MP involved in study design.

**Funding**

This study was self-finance.

**Declaration of competing interest**

Author declare that they have no conficts of interest.

**Acknowledgments**

Authors would like to acknowledge department of biotechnology, NFCC, Agharkar Research Institute, Pune and NBAIM, Kushmaur, MAU (U.P.) for laboratory workplace.

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