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In- Vitro Testing The Bio –Efficacy of Different Virulent *Trichoderma* Isolates

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Abstract: The various management strategies has been developed for the management of early blight of tomato but no one has been proved quite effective as due to the development of new fungal pathotypes. The pathogen *Alternaria solani* has developed resistant against various systemic and contact fungicides due to their wide usage under field and controlled conditions. Due to their wide consumption there must be need to developed a technology or strategy to manage this disease in an effective manner which could be beneficial for the environment as well. Dual culture results showed that *Trichoderma* isolates substantially inhibited the growth of tested fungi In case of *Alternaria solani* a maximum inhibition of 54.16% was recorded in T-6 and followed by 43.85% in T-5, where in T-4 is 41.22 % and in T-1 is 40.22 % followed by T-2 and T-3 is 37.71%, and 37.28%.

Keywords: Antagonistic, *Trichoderma*, Isolates, *Alternaria solani.*

1. Introduction: Tomato (*Lycopersicum esculentum*), which is also called as Nightshades that include more than 300 species belongs to the family *Solanaceae*. The other examples of different crops within the Nightshade family includes potato, tobacco, pepper & eggplants etc. Although tomato plants have

ISSN:0971-1260

Vol-22-Issue-16-August-2019

been attacked by a wide range of pathogens which are in soil and air borne in nature and causes huge economic loss every year in terms of crop production and monetary loss to farmers. Out of various diseases early blight of tomato is most devastating disease among all other diseases. It causes approximately tonnes of loss in crop production throughout the year and about 79% of total production loss throughout the world. In India it causes nearly about 72% of total production loss every year and about 1.36% of yield loss every year (Gomes *et al*., 2010). This pathogen causes severe symptoms on leaves, stem, fruits and stalks as well. It produces concentric ring spots and later on fruits become macerated and shows severe infestation of *Alternaria* spp. in plants (Martin and Hepperly, 1987). The reason behind its devastating nature is that fungi also produces some mycotoxins which are the types of pathotoxin. Hence also produces the same symptoms when applied on plants as same symptoms produced by pathogen itself. They directly influence the pathogenicity of pathogen (Singh, V. 2015).

2.Materials and Methodology

2.1 Collection of specimens of infected diseases and pathogen isolation

From the field of Lovely Professional University, India, tomato leaves with typical early blight symptoms were identified. *Alternariasolani*pathogen are isolated by the standard tissue isolation method and sterilized surface are of infected parts of leaves with typical symptoms in a 0.1 % mercury chloride, or 1% sodium hypochlorite solution for one minute. The bits have been washed and transferred three times repeatedly and incubated in a sterile plate containing PDA (Potato Dextrose Agar) at room temperature (27±1 ° C). for a period 3 to 7 days ,and observed the fungal growth and sporulation under aseptic conditions. Colonies that develop from the bits were identified by the use of mycelial and

ISSN:0971-1260

Vol-22-Issue-16-August-2019

spore characters by microscopic observation. They have been transferred to PDA slants and incubated for the further use at (27 ±1 ° C)after identification.

2.2 Collection of soil samples, isolation and purification of *Trichoderma* from soil sample

Soil samples from the different states have been collected and isolated on PDA for proper identification, with the help of serial plate dilution technique (Johnson and Curl, 1972).In a 1-10 dilution flask of 90 mal of sterile water,10 g well pulverized air dried soil as added to a sample and vigidly shaken on a magnetic shaker between 20-30 min. for homogeneous suspension. One ml of suspension was transferred from the flask to a 9ml sterile water test tube to make 1:100 dilutions in aseptic conditions.in addition , the dilution was made to 103 by pipetting 1 ml of suspension into additional water as described above. One ml of the suspension was transferred from 103 dilutions to 10 sterile Petri plates that had already been poured with 15 mal sterile PDA medium and spread consistently.The inoculated plate Petri has been incubated for seven days at 23±2˚C. After I ncubation, the method described in the soil manual was identified..Jha (2004).

2.3 Screening of *Trichoderma* isolates for their antagonistic potential

2.3.1Trichoderma's Dual Cultural Assay isolates against *Alternaria solani*:

The relative viability of *Trichoderma* isolates with *Alternaria solani* was assessed by dual culture techniques.Nine mm of the width circle of testing growth and the hostile organisms are reversed at a distance of 5mm from the edge of a 5 day culture on the petri plate fringe. On the same circle of the test organism was fixed to another Petri plate on another side of the PDA plate, which was taken as control and each treatment was repeated 3 times and kept at 25 ± 1 °C.After 7 days, the plates were analysed for the development of restrain zones between *Trichoderma* and pathogen.. Outspread development lessening

ISSN:0971-1260

Vol-22-Issue-16-August-2019

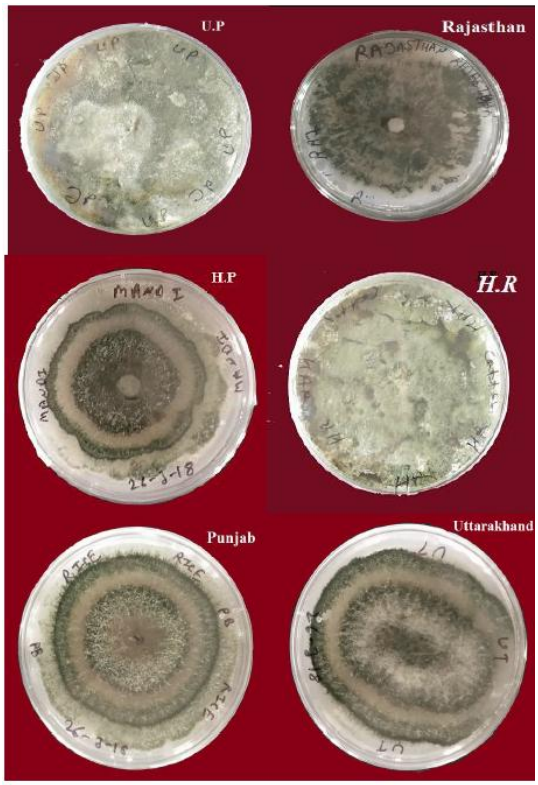
was computed in connection to development of the control (Edington et al. 1971)as given underneath.

$$\text{Inhibition \%} = \frac{C - T}{C} 100 \text{ \% Inhibition of radial mycelial growth}$$

Where, C = Radial growth of the pathogen measured in control plates (mm)

T = Radial growth of the pathogen in the presence of *Trichoderma*

Photograph:1 *Trichoderma* isolates from different locations of India

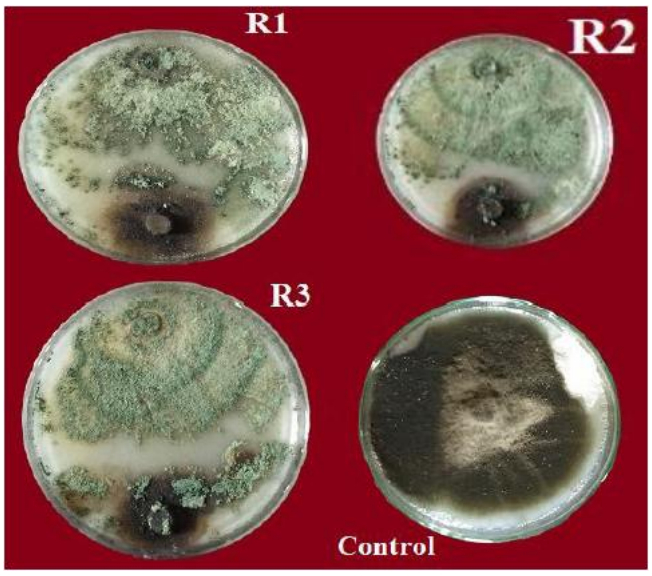


Photograph:2 *Trichoderma* isolates slants from different locations of country

ISSN:0971-1260

Vol-22-Issue-16-August-2019

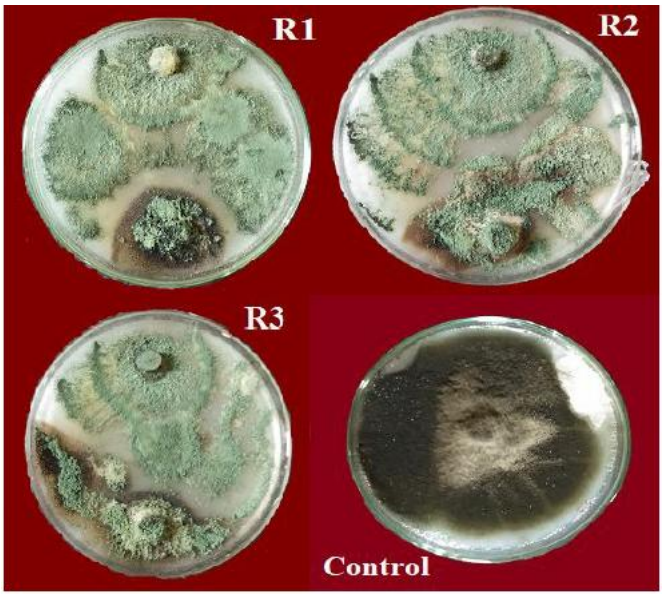
Photograph:3 Antagonistic effect of *Trichoderma's* Dual Cultural Assay isolates against *Alternaria solani*:



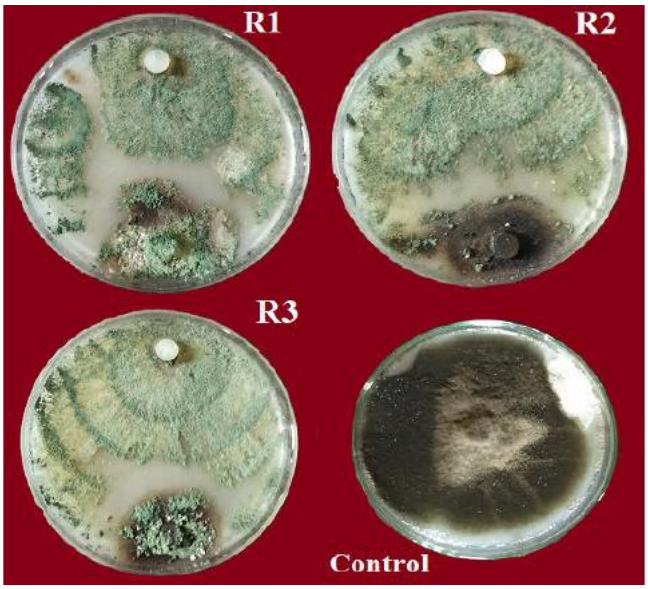
Photograph:4 *Trichoderma* Isolates from Haryana against *Alternaria solani*

ISSN:0971-1260

Vol-22-Issue-16-August-2019



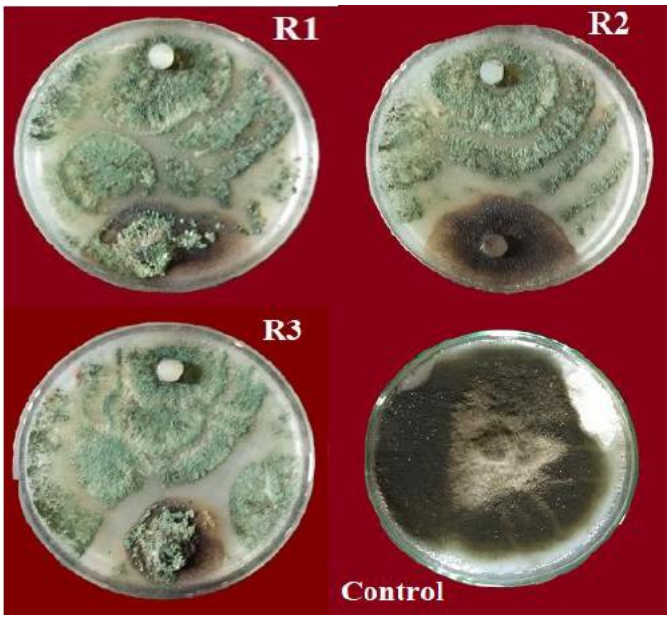
Photograph:5 *Trichoderma* Isolates from Punjab against *Alternaria solani*



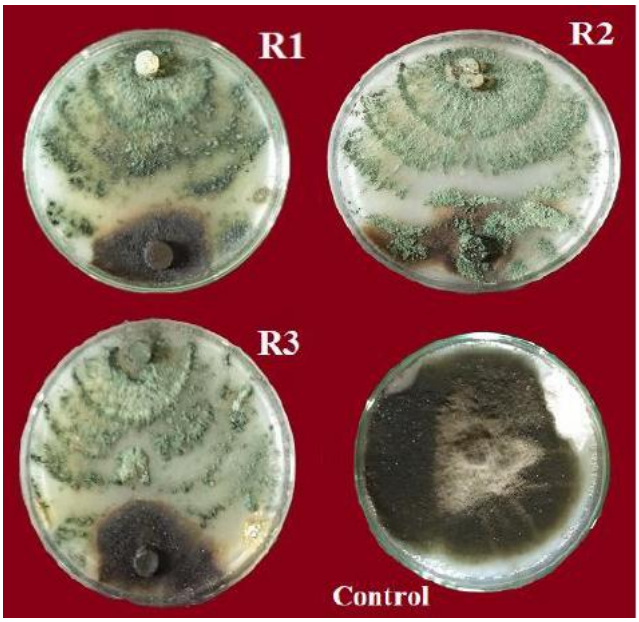
Photograph:6 *Trichoderma* Isolates from Himacah Pradesh against *Alternaria solani*

ISSN:0971-1260

Vol-22-Issue-16-August-2019



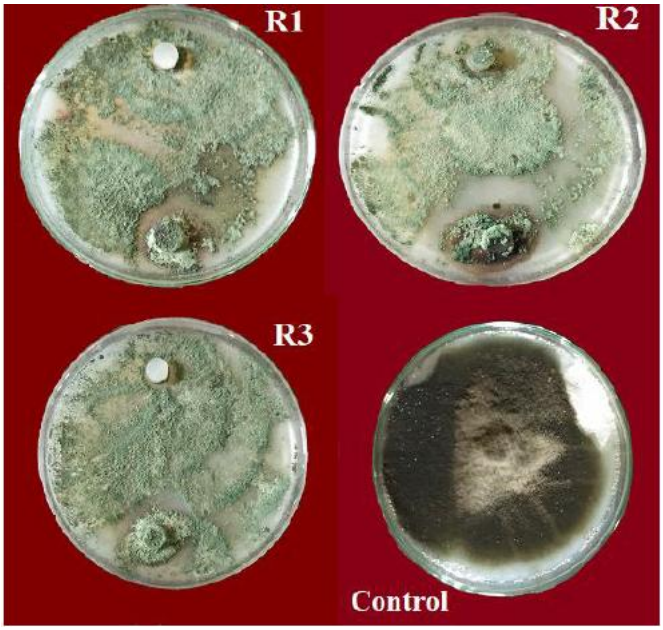
Photograph:7 ***Trichoderma*** **Isolates from Rajasthan against** ***Alternaria solani***



Photograph:8*Trichoderma* Isolates from Uttar Pradesh against *Alternaria solani*

ISSN:0971-1260

Vol-22-Issue-16-August-2019



Photograph:9 *Trichoderma* Isolates from Uttaranchal against *Alternaria solani*

4.Results and discussion

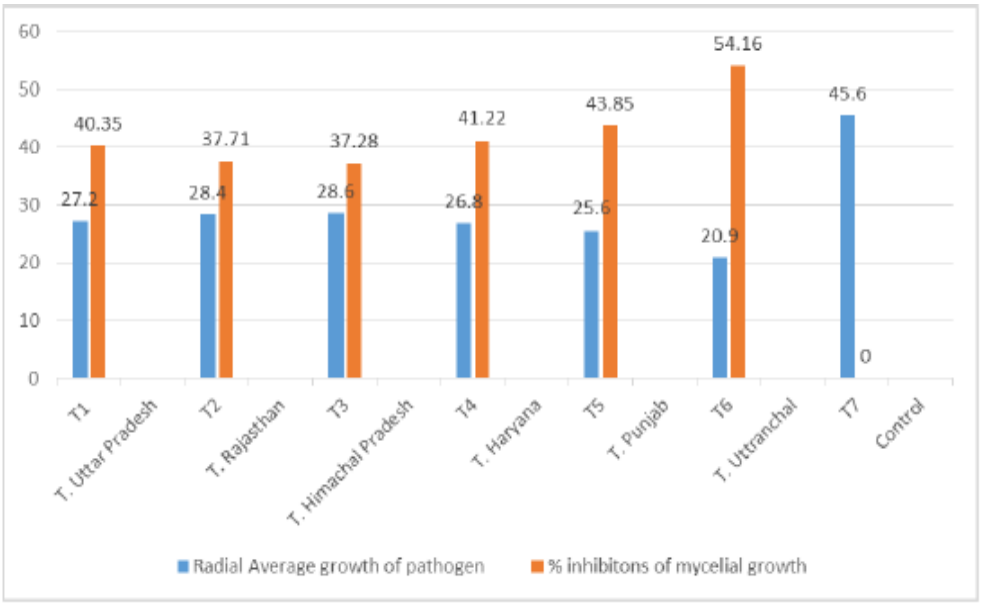
Results from the dual culture technique showed that *Trichoderma* isolates considerably inhibited the growth of tested fungi *Alternaia solani.* .The maximum inhibition of 54.16% was recorded in T-6 and followed by 43.85% in T-5, where in T-4 is 41.22 % and in T-1 is 40.22 % followed by T-2 and T-3 is 37.71%, and 37.28%.

Table&Graph:1Antagonistic effect of different *Trichoderma* isolates against *Alternaria solani*

ISSN:0971-1260

Vol-22-Issue-16-August-2019

Isolates *Trichoderma*	Radial average growth of pathogen	% inhibitons of mycelial growth
T1 *T.* Uttar Pradesh	27.2	40.35
T2 *T.* Rajasthan	28.4	37.71
T3 *T.* Himachal Pradesh	28.6	37.28
T4 *T.* Haryana	26.8	41.22
T5 *T.* Punjab	25.6	43.85
T6 *T.* Uttaranchal	20.9	54.16
T7 Control	45.6	00
CD value	**0.263**	



The continued use of chemical pesticides increases their residual toxicity to crops and raised awareness of contamination. The use of certain eco-friendly alternatives, such as the use of antagonistic microorganisms is possible of non – chemical of the controlling of plant diseases by reducing the pathogen inoculum levels. Attempt were made to isolates and maintain different isolates of *Trichoderma* from several states of India. The isolates confirmed as *Trichoderma* isolates were subjected to testing of antagonistic activity of

ISSN:0971-1260

Vol-22-Issue-16-August-2019

Trichoderma isolates against early blight disease of Tomato pathogen. In general there was increase in the plants height and vigour of the Tomato Plants. The experiments were conducted in order to detect antagonistic potential value of *Alternaria solani* through dual culture technique, (Liu:chienhui *et.al.* (1997). The result was also similar to work of (Leifort *et. al*, 1992; Tomer *et. al*, 2015). *Trichoderma* isolates possess many antagonistic mechanisms like competition, antibiosis and mycoparasitism **(**Dennis and Webster, 1971a, b & c, Transoms, 1986. The results are reported in the above graphs.

5. Conclusion:

To check the anatagonistic effect of different *Trichoderma* spp. isolated from different location such as Punjab, Haryana, Uttaranchal, Uttar Pradesh, Himachal Pradesh and Rajasthan. *In-vitro* condition the isolation and antagonistic effect of different *Trichoderma* isolates *T.* Uttaranchal inhibit the maximum growth of fungal mycelium with 54.16% followed by Punjab (43.85%) *in-vitro* condition.In the current study, *Trichoderma* isolates showed more mycelial path ogen inhibitionof Uttaranchal. This can be obviously be attributed to several possibilities for the existence, such as increased competitiveness, stimulation and antibiotics, of microbial interactions through these *Trichoderma* isolates.

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