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**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/3725
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/3725>



RESEARCH ARTICLE

IN VITRO EFFICACY OF *TRICHODERMA* ISOLATES AGAINST SOME FUNGI CAUSING FUNGAL ROT DISEASE OF TOMATO.

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Manuscript Info

Manuscript History

Received: 01 January 2017
 Final Accepted: 11 February 2017
 Published: March 2017

Key words:-

Tomato, *Trichoderma* isolates, *Alternaria alternata* (Fr.) Keissl, *Penicillium* sp., *Aspergillus niger* Van Tiegh,

Abstract

Biological control has been found efficient eco-friendly approach for the management of several fungal diseases caused by pathogenic fungi. Therefore, present study was carried out to evaluate the *in vitro* efficacy of some *Trichoderma* isolates against the pathogenic fungi, *Alternaria alternata* (Fr.) Keissl, *Penicillium* sp and *Aspergillus niger* Van Tiegh known to infect tomato fruits in Kashmir. It was observed from the results that all the *Trichoderma* isolates brought about significant inhibition in the mycelial growth. The inhibition in mycelial growth of *Alternaria alternata*, by PPT3 isolate of *Trichoderma* was 26.79%, followed by isolate PPT1 (19.47%) and isolate PPT2 (14.64%) of *Trichoderma* respectively. Likewise, the highest inhibition in mycelial growth of *Penicillium* sp. was shown by isolate PPT3 (34.36%) followed by isolate PPT2 (23.44%) and by isolate PPT1 (9.37%) respectively. In case of *Aspergillus niger*, the inhibition in mycelial growth was highest by isolate PPT3 (42.5%) followed by isolate PPT1 (36.67%) and by isolate PPT2 (31.67) respectively. The results indicate that different local isolates of *Trichoderma* proved effective against the tested pathogenic fungi and can be evaluated further for their antifungal activity against other fungi.

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Introduction:-

Tomato (*Lycopersicon esculentum* Mill.) is a member of the family Solanaceae which comprises short-lived perennial herbaceous plants. It is one of the most popular vegetable crops widely grown for its edible fruits, high nutritive values and also for its diversified uses (Afroz *et al.*, 2008; Ewulo *et al.*, 2008). They are important source of vitamins and important cash crop for both small holders and medium scale commercial farmers (Ana *et al.*, 2003). The fruit also contains plenty of antioxidant carotenoid lycopene that has recently attracted interest because of its role in preventing cancer heart disease and muscular degeneration (Wener, 2008). Several pathogenic fungi are known to cause damage to tomato especially under storage conditions and also on the aerial parts of the plants. The main postharvest disease of tomato caused by various pathogenic fungi are *Rhizopus stolonifer* (Stevens *et al.*, 1997; Schena *et al.*, 1999), *Botrytis cinerea* (Lee *et al.*, 2006) and *Alternaria alternata* (Feng and Zheng, 2007). *A. alternata* is a saprophytic pathogen of tomato causing postharvest black rot at high frequency (Akhtar *et al.* 1994). Harvested fruits and vegetables decay due to fungal infestation is the main cause of major losses in food production. Postharvest decay of fruits and vegetables can be traced to infections, occur either between flowering and fruit maturity or during harvesting and subsequent handling and storage (Eckert and Ogawa 1988). The loss resulting

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from these infections have been reduced primarily by applying fungicides in the field or after harvest (Eckert and Ogawa 1988).

Trichoderma spp. is the most widely studied biocontrol agents (BCAs) against plant pathogens because of their ability to reduce the population of soil borne plant pathogens (Papavizas, 1985). They are soil borne fungi and show significant activity against a wide range of plant pathogenic fungi (Elad *et al.*, 1982). Mechanism used by *Trichoderma* spp. for control of plant pathogen includes competition, mycoparasitism, antibiosis and induced resistance of the plant host (Chet, 1987; Schirmbock *et al.*, 1994).

Trichoderma is one of the common fungal biocontrol agent is being used worldwide for suitable management of various foliar and soil borne plant pathogens. (Dominguesa *et al.*, 2000). Therefore, the objective of the present investigation was to assess the efficacy of local isolates of *Trichoderma* under in vitro condition against some fungal pathogens isolated from tomato fruits.

Materials and Methods:-

Tomato (*Lycopersicon esculentum* Mill.) fruits were obtained from different vegetable local markets of Kashmir Valley. Samples of infected fruits were brought to the laboratory in clean polythene bags and these samples were either used immediately or stored at 10°C in the laboratory for different pathological studies. Small portions of rotted tissues were isolated aseptically from the diseased tomato fruits and transferred to Potato Dextrose Agar (PDA) medium. Pure colony cultures were obtained by sub-culturing the isolated fungi such as *Alternaria alternata* (Fr.) Keissl., *Penicillium sp* and *Aspergillus niger* Van Tiegh, in separate Petri plates containing the same medium. The pathogen was identified by their morphological, reproductive and cultural characteristics (Ellis, 1971; Barnett and Hunter, 1972; Watanabe, 2002; Gilman, 2008). For pathogenicity, pathogens were re-inoculated after isolation onto the healthy pear fruits (Tomkin and Trout, 1931). Then all the fruits were kept in clean polythene bags and incubated at 25±2°C for ten days. These pathogenicity tests were used for the identification of plant pathogens and to confirm the detection of a particular disease. Identification of the disease and the pathogen was done following Koch's postulates.

Isolates of *Trichoderma* were isolated from rhizospheric soil of the tomato plant following serial dilution method (Brown 2004). These isolates were identified on the basis of morphological and cultural characteristics and named as PPT1, PPT2 and PPT3. The antimycotic effect of these pure local isolates of *Trichoderma* against isolated fungi such as *Alternaria alternata* (Fr.) Keissl., *Penicillium sp* and *Aspergillus niger* Van Tiegh was assessed by dual culture method (Bashar and Rai 1994, Prince *et al.* 2011). To assess the effect of *Trichoderma* isolates against fungi, known quantity of mycelia of both was inoculated onto petriplates containing PDA. Then the inhibition in mycelial growth of fungi such as *Alternaria alternata* (Fr.) Keissl., *Penicillium sp* and *Aspergillus niger* Van Tiegh by isolates of *Trichoderma* was observed after incubation. The percent inhibition in mycelial growth of tested fungal pathogens in presence of *Trichoderma* isolates was calculated as per the formula given by Skidmore and Dickinson 1976.

$$\text{Mycelial growth inhibition (\%)} = \frac{dc - dt}{dc} \times 100$$

Where dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group.

Results:-

It was observed from the results (Table 1) that isolate PPT-3 of *Trichoderma* inhibited the mycelial growth of *Alternaria alternata* to an extent of 26.79 per cent. This was followed by *Trichoderma* isolates PPT-1 (19.47%) and PPT-2 (14.64%) respectively. Likewise, growth inhibition of *Penicillium sp* by *Trichoderma* isolates PPT-1 was 9.37%, by PPT-2 was 23.44% and by PPT-3 was 34.36% respectively and inhibition in mycelial growth of *Aspergillus niger* due to *Trichoderma* isolates, PPT1, PPT2 and PPT3 were 36.67%, 31.67% and 42.5% respectively.

The maximum growth inhibition of *Alternaria alternata* and *Aspergillus niger* was due to *Trichoderma* isolate, PPT3 followed by isolate PPT1 and isolate PPT2 respectively. Whereas, isolate PPT3 caused maximum growth inhibition of *Penicillium sp*. However, the least inhibition in mycelial growth of pathogenic fungi was observed by *Trichoderma* isolate, PPT1.

Table 1:- Effect of *Trichoderma* isolates on the mycelial growth of fungi causing rot disease of tomato by dual culture plate method

Treatment	Mycelial growth (mm)		
	<i>Alternaria alternata</i>	<i>Penicillium sp</i>	<i>Aspergillus niger</i>
PPT1	11.00±1.00 (19.47%)	19.33±1.15 (9.37%)	25.33±1.52 (36.67%)
PPT2	11.66±1.52 (14.64%)	16.33±1.52 (23.44%)	27.33±2.08 (31.67%)
PPT3	10.00±1.00 (26.79%)	14.00±1.00 (34.36%)	23.00± 2.00 (42.5%)
Control	13.66 ± 1.52	21.33± 1.52	40.00±1.00

Each value is mean of 3 replicates ± SD

Figures in parenthesis is the mycelial growth inhibition (%)

Discussion:-

It is clear from the above results that different local isolates of *Trichoderma* caused significant inhibition in mycelial growth of *Alternaria alternata* (Fr.) Keissl., *Penicillium* sps and *Aspergillus niger* van Tiegh.

In a similar studies, the antagonistic activities of *Trichoderma harzianum* against several pathogenic fungi have been reported by many workers (Henis and Chet, (1975); Backman and Rodrigues-Kabana, 1974; Hadar *et al.*, 1979 and Elad *et al.*, 1980). Kakde and Chavan (2011) studied the antagonistic activity of *Trichoderma viride* and *Trichoderma harzianum* against storage fungi and found that growth of *Curvularia lunata*, *Rhizopus stolonifer*, *Fusarium oxysporum*, *Macrophomina phaseolina* and *Penicillium chrysogenum* was retarded due to *Trichoderma* species. Dharmaputra *et al.* (1994) also tested two isolates of *T. harzianum* and one isolates of *T. viride* against *Ganoderma* and found that all isolates inhibited the mycelial growth of the pathogen, but *T. harzianum* (isolates B 10-1) showed the best performance. Etabarrian (2006) reported that *T. viridi* (MO) reduced the colony area of *Macrophomina phaseoli* by 19.2 and 34.9% using the dual culture and cellophane methods, respectively. Intana *et al.* (2007) reported the efficacy of three mutant and two wild type strains of *T. harzianum* against *Colletotrichum capsicis*, causal agent of anthracnose of chili. The present study suggests that *Trichoderma* sp. effectively inhibits the growth of fungi causing rot of tomato, hence can be used as a biocontrol agent against rot diseases of vegetables. Bashar and Rai (1994) studied the three isolates of *Trichoderma* viz., *Trichoderma harzianum* Refai, *Trichoderma hamatum* and *T. viride* Pers. were selected to test their antagonistic potential against the pathogens following dual culture technique. However, further investigation is needed for the efficacy of these local isolates of *Trichoderma* against fungal pathogens.

Acknowledgement:-

The authors are highly thankful to the Head Department of Botany, University of Kashmir for providing the necessary facilities for the smooth research.

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