International Journal of Research in Engineering and Applied Sciences(IJREAS)
Available online at http://euroasiapub.org
Vol. 9 Issue 6, June-2019,
ISSN (O): 2249-3905, ISSN(P): 2349-6525 | Impact Factor: 7.196 |

ASSESS THE MORPHOLOGICAL AND MOLECULAR VARIABILITY AMONG THE PYTHIUM SPECIES IN TOMATOS

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Abstract

Tomato (Solanumlycopersicum L) is a vegetable crop found all over the world that belongs to the solanaceae family. Tomatoes are a prominent vegetable crop for low-income farmers in tropical nations. It is a popular vegetable due to its use as a basic ingredient in a wide range of raw, processed, and cooked dishes. The tomato fruit, which is commercially important, can vary in colour, size, and form, and contains a pigment that produces red colour and has antioxidant characteristics. Fruits, which are high in carotenoids, are the primary source of lycopene in the human diet. Polyphenolic chemicals and carotenoids increase the nutritional value of tomatoes while also improving its functional and sensory properties, such as scent, texture, and taste. Tomato fruits produced a high concentration of metabolites such as citrate, hexoses, malate, sucrose, and ascorbic acid.

1. INTRODUCTION

Tomatoes are grown on an area of 814 million hectares in India, with an annual yield of 20515 million tonnes of tomato fruits. Gujarat, Andhra Pradesh, Karnataka, Maharashtra, Orissa, Rajasthan, and West Bengal are the main tomato-growing states in India. Tomatoes account for 25% of the acreage and output in Orissa, accounting for 9361.80 tonnes of India's total production [1].

Tomato crop is subjected to be infected by various soil inhibiting plant pathogens viz., Rhizoctoniasolani, Verticillium, Fusariumsolani, Fusariumoxysporum and Sclerotiumrolfsii,

causing damping-off or root rot diseases [2]. Among these, P. aphanidermatum (Edson) Fitzpatrick is a major diseases of damping-off, wilt and root rot of tomato all over the worldwide. It is reported that the Pythium spp. major cause of seedling rot is caused by dampingoff which are responsible for seed rot in addition to pre and post-emergence damping-off of tomato seedlings. It is reported that more than 60 per cent mortality of seedlings both in nursery and main field caused by Pythium species inciting chilli damping-off. Damping-off caused by Pythium spp. leads to seed decay and the death of developing and premature weakening seedlings [3]. Higher cooler soil temperature and soil moisture content induces damping-off infection.

The most common way for controlling the plant infection caused by this fungus-like organism is to employ fungicides, however this strategy is costly and has damaging consequences on the environment and humans. As public knowledge of the hazards of fungicides has grown, more people are turning to ecologically benign biological approaches as a disease management option.Bio-control is an alternative approach to the fungicides and it may be an out of harmful, effective and eco-friendly method for the plant disease management [4].

Majority of the existing bio agents were isolated from the rhizosphere soil for the management of soil-borne diseases. Soil has vast unutilized potential antagonistic microbes viz., Bacillus spp. fluorescent pseudomonas and Trichoderma spp. which was proven antagonistic effects against numerous soil inhibiting plant pathogens [5]. Various genus of bacteria like Bacillus, Pseudomonas, Alcaligenes, Azotobacter, Arthrobacter and Serratia were called as plant growth promoting rhizobacteria (PGPB). As long as in tomato, several plant growth promoting strains like Azotobacter, Bacillus sp., P. fluorescens, Serratia and Micromonospora are involved in growth enhancement as well as management of diseases [6].

Trichoderma spp. has been shown to be an antagonist to a variety of soil-borne diseases. Several studies have shown that Trichoderma isolates can effectively reduce root rot and damping-off caused by Pythium species in a variety of crops. The ability to use the oil cakes to improve sustainability of growing media such as potting soil by increasing its disease restraining, the replacement of peat and re-use of nutrients by using organic amendments. Addition of

antagonistic microorganisms either combination with organic amendments or alone in might endow with strong disease suppressive effects for soil- borne pathogens. Further, the combination of organic amendments and antagonistic microorganisms provide better disease control [7].

2. GENERAL ACCOUNT OF DAMPING-OFF OF TOMATO

The literature available on damping-off caused by Pythium species infecting tomato, chilli, brinjal, ginger, cabbage, cauliflower, pepper, papaya, tobacco and sugar beet etc. Pythium species infecting several vegetable crops in respect of occurrence, distribution and losses caused, taxonomy, morphology, isolation and pathogenicity, symptomatology, disease management and enzymes activity is being reviewed here in this chapter under following subheads.

Several workers reported that P. aphanidermatum causing infection in tomato [7], [8], [9]. Besides P. aphanidermatum, tomato damping-off incited by several other species of Pythium such as P. debaryanum and P. indicum[2], [9].

3. METHODOLOGY

- **Sample Collection**

Soil samples were taken from key tomato-growing areas. The soil sample was 1kg in size. The gathered samples were each seeded with tomato variety PKM-1 in 15 x 30 cm diameter earthen pots. The pathogen linked with diseased samples was isolated using the tissue segment method on Potato dextrose agar (PDA) media after the symptoms were collected individually from each pot on tissue paper.

- **Assessing the virulence of Pythium isolates**

The pot mixture was prepared by thoroughly mixing red soil, farm yard manure and sand at the ratio of 1:1:1. The inoculum from all Pythium isolates expanded on SMM

- **Scoring and statistical analysis**

The aforementioned photograph was used to score SSR products that were clearly apparent. Each isolate's lack or presence of each band was coded as 0 and 1, accordingly. The weighted Pair Group Method with Arithmetic Mean created pairwise similarity (UPGMA). The similarity matrix was used to create a phylogenetic tree.

4. RESULTS & DISCUSSION

4.1EFFECT OF VARIOUS CARBON SOURCES ON THE MYCELIAL GROWTH AND DRY WEIGHT OF*P. APHANIDERMATUM*

The pathogen's development in various carbon sources adjusted solid media and liquid broth showed substantial differences between treatments. Sucrose promoted considerable mycelial growth of 90.00 mm and mycelia dry weight of 620.57 mg in liquid broth, followed by Dextrose, which promoted mycelial growth of 87.00 mm and mycelia dry weight of 615.00 mg among the seven carbon sources studied [10]. The least mycelial growth of 70.45 mm and mycelia dry weight of 385.67 mg were observed in Mannitol (Table 1).

4.2. IN VITRO ANTAGONISM OF NATIVE TRICHODERMA SPECIES AGAINST P. APHANIDERMATUM

Ten native isolates of *Trichoderma* species were tested against the mycelial growth of *P. aphanidermatum* by dual culture techniques [10]. Among the isolates KPTa was significantly superior and recorded the minimum mycelial growth of 15.23 mm which accounted to a maximum growth inhibition of 83.07 per cent over control [10], [11], [12]. This was followed by the ACTa and SCTa in the decreasing order of merit. The least growth inhibition of pathogen (67.42 per cent) was exhibited by KNTh (Table 2).

Table 1: Effect of various carbon and nitrogen sources on the mycelial growth and dry weight of *P. aphanidermatum* (I4) *in vitro*

T.No.	Carbon Sources	Mycelialgrowth (mm)	Mycelial dryweight (mg)	Nitrogen sources	Mycelial growth(mm)	Mycelial dryweight (mg)
T1	Dextrose	87.00^{b}	615.00^{b}	Ammonium nitrate	87.00^{b}	604.00^{b}
T2	Fructose	82.47^{cd}	513.75^{d}	Ammonium oxalate	75.66^{f}	447.78^{g}
T3	Glucose	85.68^{bc}	585.37^{c}	Ammonium sulphate	82.37^{cd}	525.56^{d}
T4	Lactose	77.03^{e}	421.85^{f}	Peptone	85.33^{bc}	514.76^{e}
T5	Maltose	80.35^{de}	476.47^{e}	Sodium nitrate(Czapek's)	90.00a	610.54a
T6	Mannitol	70.45^{f}	385.67^{g}	Sodium nitrite	80.57^{de}	591.78^{c}
T7	Sucrose (Czapek's)	90.00^{a}	620.57^{a}	Urea	78.15^{ef}	456.68^{f}
T8	Control (Withoutcarbon sources)	65.67^{g}	343.67^{h}	Control (Withoutnitrogen sources)	70.45^{g}	299.55^{h}

The mean of three replications was used to get the values. DMRTs do not substantially differ at the 5% level between means followed by a common letter in a column.

Table 2: *In vitro* inhibition of mycelial growth of *P. aphanidermatum* (I4) by native isolates of *Trichoderma* species

S. No.	Isolates	Mycelial growth (mm)	Per cent inhibition over control
1.	JATh	21.97^{de}	75.58
2.	SCTa	17.82^{c}	80.20
3.	PCTh	23.54^{ef}	73.84
4.	TCTa	20.57^{cd}	77.14
5.	ACTa	16.89^{b}	81.23
6.	MMTa	25.67^{fg}	71.47
7.	KPTa	15.23^{a}	83.07
8.	KNTh	29.32^{h}	67.42
9.	IDTh	27.34^{gh}	69.62
10.	BDTh	19.25^{d}	78.61
11.	Control	90.00^{i}	-

The results are the averages of three replications. Means followed by a common letter in a column are not statistically different at the 5% level using DMRTs.

5. FINDINGS

Among the seven carbon sources tested, sucrose promoted significant mycelial growth and mycelia dry weight of P. aphanidermatum in solid media and liquid broth which was similar to the findings of Mandelbaum and Hader (1990); Sundarraj (2000); Chun et al. (2003).

Muthukumar and Eswaran (2008) who explained that sucrose supported the better growth of P. aphanidermatum. The significant variation observed in the efficiency of the different carbon sources tested might be attributed to the quantity of carbon, its form and availability as well as due to variation in the mode of carbon utilization by test fungus. Also reports that sucrose supported the maximum mycelia growth and biomass production of M. phaseolina, S. rolfsii and R. solani. These earlier reports corroborates with the present observations.

The mycelial growth of P. aphanidermatum was inhibited by all natural isolates of Trichoderma species. Among the isolates studied, isolate-KPTa showed the greatest suppression of P. aphanidermatummycelial growth. Similarly, Elshahawy and El-Mohamedy (2019) found that Trichoderma species hindered the mycelial growth of P. aphanidermatum in vitro. Trichoderma isolates were designed to not only stop the disease from spreading, but also to penetrate the colony's surface and sporulate over it. Trichoderma isolate-TR 55 isolated from tomato rhizosphere was found to be the most effective isolate against both Pythium spp. and R. solani Kuhn, with inhibition percentages of 89.26 percent and 87.41 percent, respectively, according to Biam et al. (2019). TR 66, TR 122, and TR 136 were the next most effective isolates. According to Yasser et al. (2020), Trichoderma isolate T-6 inhibited the mycelial growth of P. ultimum by 61.2 percent, followed by isolate T-10 (58.3 percent). Several researchers have found Trichoderma spp. antagonism against Pythium spp. in vitro.

6. CONCLUSION

Tomato (Solanumlycopersicum L) is a major vegetable crop that is susceptible to a variety of fungal, bacterial, and viral illnesses. Damping-off induced by Pythium species is one of the most prevalent and causes significant losses in tomato output. P. aphanidermatum (Edson) Fitzpatrick, a Pythium species, is a prominent cause of tomato damping-off, root rot, and wilt all throughout the world. Management of soil-borne pathogen (Pythium species) with chemical fungicide is effective but its ill effects against other beneficial microbes cannot be ignored. Therefore, bio-control agents and neem cake appear to hold promise in plant disease management.

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