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IN-VITRO EFFICACY OF PGPR *Pseudomonas flourescens* AGAINST FUSARIUM WILT IN TOMATO

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Tomato (*Solanum lycopersicum* L.) is one of the most important, commercial and widely grown vegetable crops in the world. Tomato plays a critical role in nutritional food requirements, income and employment opportunities for the people. However, its production is threatened by the *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* and productionlossesbetween30 %to40 %. In the present investigation, an attempt has been made to study the *in vitro* efficacy of *Pseudomonas flourescens* against *Fusariumoxysporum* f.sp. *lycopersici*. The antagonistic effect of *Pseudomonas flourescens* was observed by the Dual culture technique under the *in vitro* conditions.

Tomato(*Solanum lycopersicum* L.) is one of the most cultivated and popular vegetable crops across the world (Pastoretal. 2012). It belongs to the *Solanaceae* family, and it is the most important vegetable after Potato. It is used as a fresh vegetable and can also be processed and canned as a paste, juice, sauce, powder or as a whole. Tomato grows well in a relatively cool and dry climate, it is well adapted to all climatic zones around the globe. Tomato is used for consumption due to its high nutritive values, antioxidant and curative properties and it contains Vitamin A, Vitamin C and Vitamin E with 95.3% of Water, 0.07% Calcium and Niacin, which have great importance in metabolic activities of humans (Sahu et al. 2013). In 2018 the number of Tomatoes produced worldwide stood at 188 million tonnes, rising by 3.5% against the previous year. India occupies the second position in the world with respect to area, but occupiesonly fifth place in terms of production. Total area under tomato cultivation in Indiais 7.97 lakh ha with a production of 207.08 lakh tonnes (Anonymous 2018). Tomato plants are susceptible to various diseases caused by different agents such as Bacteria, Viruses, Nematode, Fungi and Abiotic factors. Among the fungal diseases,

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Fusarium wilt is caused by *Fusarium oxysporum* f.sp. *lycopersici*, and it causes economic loss of tomato production in worldwide. *F. oxysporum* f.sp. *lycopersici* is a soil-borne pathogen, persists in soil for about 8-10 years in the form of chlamydospores as resting structure. The fungus *F. oxysporum* f.sp. *lycopersici* is exerting pressure on productionlossesbetween30to40%andmay even raise upto80%if so; climatic conditions favour the growth of the fungus. The PGPR having the ideal potential to combat various pathogen has been used in different forms of application. PGPR playing a vital role and capable of colonizing the plant's root system and improve the growth and yield. Plant growth-promoting rhizobacteria with biocontrol traits can be considered as an alternative to the high doses of pesticides applied on crops to deter pathogens and reduce the disease severity (Mahendra Prasad et al. 2019). Mechanisms used by PGPR are involved in biocontrol, such as direct antagonism via the production of Antibiotics, Siderophores, HCN, Hydrolytic enzymes or indirect mechanisms in which the biocontrol organisms act as a probiotic by competing with the pathogen for a niche. *P. fluorescens* are the most exploited bacteria for biological control of soil-borne and foliar plant pathogens. In the past three decades, numerous strains of *P. fluorescens* have been isolated from the rhizosphere soil, and plant roots by several workers and their biocontrol activity against soil-borne and foliar pathogens are reported.

Yield Losses

Fusarium wilt is one of the most important constraints to tomato (*Solanum lycopersicum* L.*) production in major tomato growing areas in the world. That results were* reported 10-90% losses in yield of tomato in the temperate region. *Fusarium* wilt causes 90% of crop losses with repeated infections, especially in the same growing season in greenhouses. Sustainable losses in the yield of tomato is due to vascular wilt and early blight caused by the fungi *F.oxysporum* f.sp. *lycopersici* and *A.solani*, respectively. Nirmaladevi (2016) reported that among the diseases of tomato, the Fusariosis caused by the fungus *F. oxysporum* f.sp. *lycopersici*, bringing up production losses between 30 and 40 per cent and may even reach up to 80 per cent if climatic conditions favour the growth of the fungus.

Epidemiology

F. oxysporum occurs, survives and grown in all the type of soil, but sandy soils are most favourable for growth and development. Infection and disease development in *Fusarium* wilt is favoured by warm soil temperature and low soil moisture. The disease tends to be most severe in sandy soil and generally less in heavier clay soil. This disease affects the tomato grown at warm temperature (28°C) in both greenhouse and field condition (Debbi et al. 2018). Disease development is favoured by warm temperatures (27–28°C), dry weather, and acidic soil (pH 5–5.6). Rapidly growing, highly succulent tomato plants exposed to fertilization with Ammonium nitrate are especially susceptible to the disease. The fungus can be disseminated by infected seeds or by transplants grown in infested soil. The fungus can be introduced into a field on contaminated equipment, training stakes, packing crates or shoes. Soil particles from infested fields may be blown into disease-free fields.

Symptoms

Fusarium oxysporum f.sp. *lycopersici* is a soil-borne pathogen that invades the plants mostly through the wounds in the cortical tissues of roots and also through the wounds on adventitious roots produced on the stem. The symptoms of Fusariosis begin with foliar chlorosis in a region of the plant, and as the disease is established, the yellowing is observed in the majority of the plant, causing the wilt and later the death of the plant, without producing fruit or the fruit production is scarce. The earliest symptoms appear within 48 h after the entry of the pathogens. In the infected plants, the leaves become yellow, followed by dropping of leaves which occurs may be on one side of the plant or on both the sides of the shoot.

The fungus blocks the xylem vessels by invading the vascular tissues and reduces the movement of water, and causes severe wilting. Lengthwise brown streaks or vascular discolouration may be seen when the infected stem is cut open. This is the characteristic symptom and used for the identification of disease (Mui-Yun 2003). This discolouration often extends far up the stem and is especially noticeable in a petiole scar. *Fusarium* disease occurs in two forms, also called syndromes.

Antagonistic Effect of *Pseudomonas Flourescens*

The use of plant growth-promoting rhizobacteria (PGPR) in agriculture is effective in integrated pest management due to improved nutrient cycling to the crop and protection of crop from phytopathogens. *P. fluorescens* has been extensively used against different soil-borne pathogens in several crops (Gopi et al. 2016). The PGPR colonize the plant rhizosphere

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and influence plant growth by secreting essential phytohormones for growth and development, increasing nutrient uptake due to production of siderophores, increasing mineral and nitrogen availability in the soil. The PGPR are antagonistic against phytopathogenic microorganisms by producing siderophores, antibiotics, chitinase, β-1, 3 glucanase and Hydrogen cyanide (HCN). These bacteria are involved in the solubilization of phosphate, inducing systematic resistance in the host system to fight against a wide range of phytopathogens, and withstanding abiotic stresses.

Vascular discoloration

Yellowing of leaves

Fluorescent Pseudomonads are non-pathogenic rhizobacteria that suppress the soil-borne pathogens through rhizosphere colonization, antibiosis, iron chelation by siderophore production and ISR. The studies on the mechanism of growth promotion indicate that PGPR promotes plant growth directly by production of plant growth regulators or indirectly by stimulating nutrient uptake by producing siderophores or antibiotics to protect plants from soil-borne pathogens or deleterious rhizosphere organisms. *Pseudomonas* sp. may increase plant growth by producing Gibberellins like substances, mineralizing phosphates or by other mechanisms which are not clearly understood.

Efficacy of *Pseudomonas Fluorescens* Against *Fusarium Oxysporum* F.Sp. *Lycopersici* (Dual Culture)

A nine mm culture disc obtained from the periphery of the seven days old culture of *F. oxysporum* f.sp. *lycopersici* was inoculated at 75mm approximately away from the edge of the Petri dish containing 15ml of sterilized and solidified PDA medium. The bacterial antagonist *P. fluorescens* and *B. subtilis* were streamed gently made onto the medium using two days old culture just opposite to the pathogenic culture at equidistance. The zone of inhibition (mm) and the mycelial growth of *F. Oxysporum* f.sp. *lycopersici* were recorded. The effective antagonists were selected based on the inhibition of the growth of the pathogen. The per cent inhibition of mycelial growth was calculated, according to Vincent(1927).

$$I = \frac{C - T}{C} X 100$$

Where, I = Percent inhibition over control

C = Radial growth (mm) in Control

T = Radial growth (mm) in Treatment

In a recent study, Shanmuga Priya *et al.* (2019) reported that the *in vitro* antagonism of ten isolates of *Pseudomonas* sp. against the mycelial growth of *Fusarium* sp. and indicated that the isolate *Ps* Ap was most effective in inhibiting the mycelial growth of virulent isolate of *Fusarium* spp (FI3) to the maximum of 57.77 per cent. Shahzaman *et al.* (2016) reported the bioefficacy of thirty isolates of *P. fluorescens* against the *Fusarium* sp. The antagonist Pf 3 was found most effective, with an inhibition percentage of 93.33 per cent.

Conclusion

PGPR (*Pseudomonas flourescens*) are the encapsulated members of the Rhizosphere and considered to be effective symbionts by protecting the plants from root rot pathogens simantaneously increasing plant growth and imparting plant tolerance to various stress factors.

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