AgriCos e-Newsletter
ISSN: 2582-7049
Volume: 02 Issue: 03 March 2021 Article No: 27

In-Vitro Efficacy of PGPR *Bacillus Subtilis* against *Fusarium* Wilt in Tomato

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SUMMARY

Tomato (Solanum lycopersicum L.) is one of the most important, commercial and widely grown vegetable crop 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 production losses between 30 % to 40 %. In the present investigation an attempt has been made to study the in vitro efficacy of Bacillus subtilis against Fusarium oxysporum f.sp. lycopersici. The antagonistic effect of Bacillus subtilis were observed by the Dual culture technique under the in vitro conditions.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated and popular vegetable crop across the world (Pastor et al. 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 amount of Tomatoes produced worldwide stood at 188 million tonnes, rising by 3.5% against the previous year. India occupies second position in the world with respect to area, but occupies only fifth place in terms of production. Total area under tomato cultivation in India is 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, *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 production losses between 30 to 40% and may even raise up to 80% if so, climatic conditions favour the growth of the fungus. The PGPR having ideal potential to combact various pathogen, has been used in different forms of application. PGPR playing a vital role and capable of colonizing the plants 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 the pathogens and reduce the disease severity (Mahendra Prasad et al. 2019). Mechanisms used by PGPR are involved in biocontrol such as direct antagonism via 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. *B. subtilis* is also having highest antagonistic activity against *F. oxysporum* in both *in vitro* and *in vivo* conditions. The *B. subtillis* strain EU07 reduced the incidence of disease caused by *F. oxysporum* f.sp. *lycopersici* by 75% (Francine et al. 2017) consortium. These bacteria have been broadly described for a wide range antagonistic activities to combat phytopathogens.

Yield Losses

Fusarium wilt is one of the most important constraint 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 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) repoted 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 favor 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 favored 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 invade the plants mostly through the wounds in the cortical tissues of roots and also through the wounds on adventitious roots produced on stem. The symptoms of Fusariosis begin with a 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 with in 48 h after the entry of the pathogens. In the infected plants the leaves becomes yellow followed by dropping of leaves which occurs may be on one side of the plant or on both the sides of shoot.The fungus blocks the xylem vessels by invading the vascular tissues and reduces the movement of water and causes severe wilting. A 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.

Vascular discoloration

Yellowing of leaves

Antagonistic Effect of *Bacillus Subtilis*

Bacillus subtilis is an on-pathogenic bacteria that lives in soil, often in association with roots of higher plants. *B. subtilis* cells are capable of forming dormant spores that are resistant to extreme conditions and thus can be easily formulated and stored. *B. subtilis* also produces a variety of biologically active compounds with a broad spectrum of activities towards phytopathogens and that are able to induce host systemic resistance. Various strains of *B. subtilis* have also been shown to be capable of forming multicellular structures or biofilms. The colony morphology of the six isolates showed an irregular form, rough surface and cream color on NA. A macroscopic variability was noted between the isolates in terms of margin which was undulate, curly and lobed or irregular (Ben Abdallah *et al*. 2015). Rhizo-bacterial strains namely *Brevibacillus brevis*, *B. agri*, *Stenotrophomonas* *maltophilia* and *B. formosus* found to be highly effective in suppression of *Salvia afficinalis* wilt and root rot diseases caused by different isolates of *F. oxysporum* and *F. solani* when applied as seedling treatment.

Efficacy of *Bacillus Subtilis* 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 15 ml of sterilized and solidified PDA medium. The bacterial antagonist *B. subtilis* was streaked 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 to 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

Ahmadi et al. (2019) reported that the 303 *Bacillus* isolates were screened by dual culture and volatile metabolite tests. Among them, 20 isolates were identified as highly effective *Bacillus* isolates, because they could limit the radial growth of *Fol* considerably. This antagonistic effect may be attributed to the production of diffusable and antifungal compounds such as cyclic peptides (e.g. iturin) and macrolactones including plipastatins, fengycins and surfactins. Similarly, Gong et al. (2015) also mentioned that antagonistic effect of *B. amyloliquefaciens* against *F. graminearum* and it will shows the 93.26 percent inhition in dual culture studies. Monda (2002) reported that bacterial biocontrol agents with promising biocontrol activities against *F. oxysporum* f.sp. *lycopersici* including *B. subtilis*. Singh et al. (2008) reported that *B. subtilis* isolates exhibited strong antagonistic activity *F. solani*.

CONCLUSION

PGPR (Bacillus subtilis) are the encapsulated members of Rhizosphere and considered to be an 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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