Biological Forum – An International Journal **13(2): 62-69(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Biocontrol of Fusarium Wilt in Tomato: An Eco-friendly and Cost Effective Approach

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(Received 17 March 2021, Accepted 29 May, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Tomato is the second most widely produced vegetable in the world. All through growth time or even after harvesting, tomatoes are prone to several diseases brought about through viruses, fungal pathogen, bacteria, and nematodes. Tomato wilt is a fungal disease, caused by *Fusarium oxysporum* f.sp. *lycopersici*, that limiting tomato output severely around the world. Several measures were offered to restrict the spread of *Fusarium oxysporum* f.sp. *lycopersici* but were difficult because of the ability of the fungus to stay long in the soil. A wide range of chemical pesticides currently available and the continuous use of these pesticides affect the food substance of tomatoes as well as their texture and the performance of soil in order to control all these vulnerabilities. Thus, this review focuses on the possible use of microbial pesticide potential along with mechanisms for the management of wilt disease in tomato. Additionally, different constraints in tomato production, symptoms produced by wilt disease, nature of causal organism, epidemiology and losses, infection stages and disease cycle are also discussed. The use of a microbial pesticide is an environmentally friendly and effective way to prevent tomatoes from wilt disease and its devastating consequences. Different microorganisms have been used in tomato wilt treatment and are now being discussed. The beneficial inoculum not only suppresses the disease, but it also contributes in the healthy growth of crops.

Keywords: Microbial pesticides, Bioagents, PGPR, ISR, *Trichoderma, Pseudomonas, Glomus, Baccillus*

INTRODUCTION

Tomato is a vital solanaceous crop grown worldwide significantly contributing in human nutrition. In tomato cultivation, the major concern is regarding Wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Loganathan *et al.,* 2009). An eco-friendly approach towards management of the disease will abolish the harsh outcome of chemicals like residual toxicity, pollution of environment, and development of resistance in pathogens against continuously used fungicide for their control. Enormous communities of bacterial residing in the rhizosphere and actively participating in growth promotion activity of plant are referred as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.,* 1980). The PGPR creates competition for ecological niche/substrate by forming antibiotics, hydrogen cyanide, releasing siderophores and secretion of fungal cell wall lysis enzymes thus behave as biocontrol agents (Glick and Bashan 1997; Wang *et al.,* 2000; Saravanakumar *et al.,* 2007). PGPR additionally lead to activation of induced systemic resistance (ISR) in varied crops against several diseases (Kloepper and Beauchamp 1992; Liu *et al.,* 1995; Chen *et al.,* 2000; Sangeetha *et al.,* 2010). For a wide range of crops, use of PGPR to control different pest and diseases and to improve production turned out be studied in depth, but knowledge regarding its effect on quality of fruit, especially about fruit texture and amount of lycopene available stays quite inadequate. Among different carotenoid, lycopene is one available in tomato which is a key composite required in supplement for humankind because it helps to reduce chances of prostate cancer diseases and lower down the cardiovascular and prostate cancer diseases (Giovannucci 1999; Giovannucci *et al.,* 2002).

In the soil, other than PGPR, there are other microbes known as biocontrol agents like *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Trichoderma* spp. have been demonstrated to have a substantial effect against soil-borne pathogens that are extremely race specific (Upadhyay *et al.,* 2021) (Table 1).

Table 1: List of some biocontrol agents reported to act against Fusarium wilt disease of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*.

Sr. No.	Biocontrol Agents	Mechanisms	References
1	*Bacillus subtilis*	Induced the activities of defense related enzymes	Loganathan *et al.,* 2014
2	*Pseudomonas fluorescens*	Increased resistance	M'piga *et al.,* 1997
3	*Bacillus subtilis*	Antagonistic activity	Mohammed and Toama 2019
4	*Pseudomonas fluorescens*	Antagonistic activity	Mohammed and Toama 2019
5	*Trichoderma harzianum*	Antagonistic activity	Arenas *et al.,* 2018
6	*T. spirale*	Antagonistic activity	Vargas-Inciarte *et al.,* 2019
7	*Pseudomonas fluorescens*	ISR and antagonistic activities	Boukerma *et al.,* 2017
8	*Pseudomonas putida*	ISR and antagonistic activities	Boukerma *et al.,* 2017
9	*Trichoderma viride*	Increase growth and chlorophyll content	Wani and Mir 2009
10	*T. harzianum*	Increase growth and chlorophyll content	Wani and Mir 2009
11	*Glomus mossae*	Increase growth and chlorophyll content	Wani and Mir 2009
12	*G. fasciculatium*	Increase growth and chlorophyll content	Wani and Mir 2009
13	*Bacillus sphaericus*	Antagonistic activities	Wani and Mir 2009
14	*Pseudomonas putida*	Antagonistic activities	Kouki *et al.,* 2012
15	*Burkholderia gladioli*	Antagonistic activities	Kouki *et al.,* 2012
16	*Stenotrophomonas maltophilia*	Inhibition of the systemic fungus progress	Aydi-Ben-Abdallah *et al.,* 2020
17	*Azotobacter chroococcum*	Inhibition of the systemic fungus progress	Aydi-Ben-Abdallah *et al.,* 2020
18	*Serratia marcescens*	Inhibition of the systemic fungus progress	Aydi-Ben-Abdallah *et al.,* 2020
19	*Trichoderma virens*	Induce JA and SA signalling cascades for the elicitation of *Fusarium oxysporum* resistance	Jogaiah *et al.,* 2018
20	*Alcaligenes faecalis*	Antifungal potential	Abdallah *et al.,* 2016
21	*Bacillus cereus*	Antifungal potential	Abdallah *et al.,* 2016
22	*Bacillus amyloliquefaciens*	Anti-fungal secondary metabolites production	Gowtham *et al.,* 2016
23	*Bacillus pumilis*	Induction of resistance	Benhamou *et al.,* 1998

Among them, in the worldwide the most explored are *Trichoderma* sp. (Howell and Stipanovic 1995); this is due to its ubiquity nature, its easy isolation property and its capacity to quickly grow on a huge number of substrates (Candela *et al.,* 1995; Verma *et al.,* 2007). Due to ongoing research for the past 10 years, isolation, selection and evaluation of native species of *Trichoderma* spp. have been done. The aim behind was to establish biological control against diverse diseases by anticipated numerous mechanisms and for the employment of this fungus providing satisfactory outcomes (Cook and Baker 1989; Chet *et al.,* 1998; Sandoval 2011; Awad *et al.,* 2014; Hamed *et al.,* 2015). *Trichoderma's* mechanism of action with different plant pathognes may turn collaborative *Septoria triticii, Sclerotium rolfsii, Sclerotiniasclerotiorum, Rhizoctonia solani, Fusarium oxysporum* and *Pythium splendens* in wheat, cucumber, soybean and lettuce, soybean, tomato and beans, respectively (Ghisalbertí *et al.,* 1991; Harman *et al.,* 2004). There are several mechanisms of genus *Trichoderma*, through which they compete with phytopathogen. Among them, the vital ones are grounded into triocategories: (i) Straight struggle for niche or supplements (Elad and Baker 1985; Elad and Chet 1987; Chet and Ibar 1994; Belanger *et al.,* 1995) (ii) Production of volatile or non-volatile antibiotic metabolites (Sid 2000; SIAP 2016) and (iii) mechanism of direct parasitism by few species of *Trichoderma* spp. (Yedidia *et al.,* 1999; Ezziyyani 2004). There are many reports which explained the use of different fungal biocontrol agents and VAM fungi to control of numerous root-borne diseases caused by *Fusarium* species (Dehne and Shoenbeck 1979).

A. The Host Crop

Tomato (*Solanum lycopersicum*) is a flowering plant which belongs to nightshade family (Solanaceae). It is extensively grown for its palatable fruits. Tomatoes serve as rich source of vitamin C and lycopene and for its high nutritional values, they are labelled as a vegetable. The fruits are consumed in different ways like raw form as salads. Cooked vegetable is made and also utilised as an ingredient to make pickle and cook different dishes. Moreover, by-products of huge part of world's tomato crop are prepared like ketchup, puree, products, tomato juice, paste and dehydrated pulp. Canned tomatoes and "sun-dried" tomatoes are also available. Requirement of this plant requires generally warm weather and considerable sunlight. During cool climates, it is cultivated essentially in hothouses. To retain tomato stems and fruits off the ground, they are generally staked, tied, or caged and to escape blossom-end rot and fruits cracking, constant irrigating is required (Adam *et al.,* 2019).

B. Constraints in Tomato Production

As it is a central crop cultivated worldwide (Fig. 1 and 2), there are numerous limitations which leads to huge loss in yield and seed production. The main reason of crop losses in worldwide is diseases of plants caused by fungi, bacteria, viruses or nematodes. Different pest and disease attacking tomato plants like Fusarium wilt, early blight, bacterial wilt, mosaicvirus, tomato hornworms and nematodes etc. In addition to pest's and pathogen's losses, post-harvest conditions also restrict the overall production of the crop. In the international markets, the food security norms have been strengthened and mycotoxins causing contamination of food have been acknowledged unfit for intake of human (WHO, 2002).

C. Wilt Disease of Tomato

Symptoms

Wilt of tomato is caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) which is considered a major tomato disease (Borisade *et al.,* 2017). Epidermis of root is starting point, where the pathogen comes with contact of host. Pathogen penetrates epidermis and spreads to vascular tissue gradually. It occupies plant's xylem vessels and causes clogging of vessels resulting in acute stress of water, thus wilt like symptoms is ultimately observed (Singh *et al.,* 2017). Visually and morphologically, this disease is recognized by yellow coloured leaves on partial or complete wilted plants. Fusarium movement in host is a very compound phenomenon, and the successive advances associated with its infection progression (Di *et al.,* 2016).

Causal Organism

FOL has three identified races (Races 1, 2 and 3) which are distinct by their principle resistance genes. Availability of races 1 and 2 has been reported from all the parts of world cultivating tomato, however presence of race 3 is stated in nations like Georgia, Mexico and California etc. In the whole, world maximum cultivated commercial varieties of tomato are found to be resistant against races 1 and 2, but against race 3 only few varieties show resistant (Biju *et al.,* 2017). Small range transmission of FOL occurs primarily through water system and ranch hardware's which are contaminated by pathogen, whereas infected transplants, soils etc. play a major role in long distances transmission (Agrios, 2005). It has been reported that the fungus generally sustains indefinitely in an area, if once gets contaminated with FOL (Animashaun *et al.,* 2017; Prihatna *et al.,* 2018).

Epidemiology and Losses

The global dispersal of FOL is identified as cosmopolitan and surviving as soil saprophyte. This pathogen is extensively known among varied fungi and in agricultural soil, it is regarded as a prevalent fungus. This pathogen has wide dispersal area due to presence of the diverse formae speciales. Both in greenhouse and field conditions the influence of disease in tomato plant is at 28°C (Bawa 2016; Debbi *et al.,* 2018). Thus, this ailment causes huge loss in production of fruit i.e. nearly 60-70 percent along with dry wilted crops (Singh *et al.,* 2015).

Infection Stages and Disease Cycle

Being a soil-born pathogen, FOL own characteristic to easily and broadly survive as dormant structure is known as chlamydospores in the soil and its germination is triggered by presence of host root. After the germination, infection hyphae are form which stick to root surface and gradually penetrate.

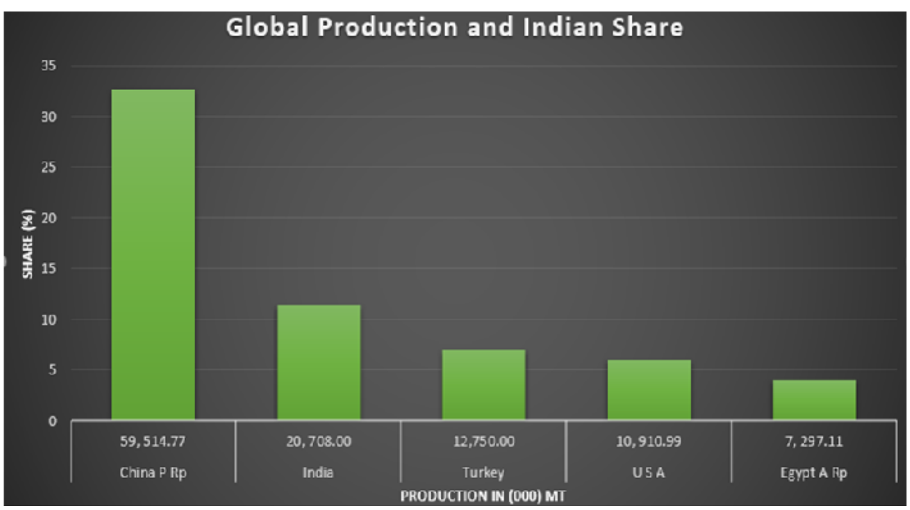

Fig. 1. Tomato Production in top five chilli producing countries in the world (FAO, 2017).

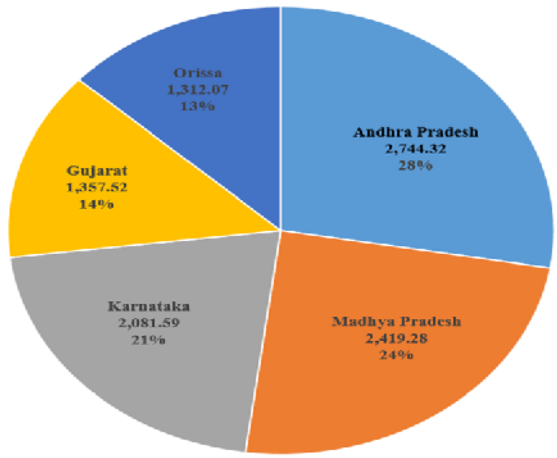

Fig. 2. Production (T) and percent share of tomato in top five chilli producing states in the India (NHB, 2017-18).

Invasion of mycelium occurs intercellularly in the cortical cells of root and eventually enters vascular framework via xylem pits. In this way, this pathogen exhibits an exclusive infection pathway, where initially it colonizes entire xylem vessels and then, further quickly colonizes the host completely. Microconidia are produced by fungus itself in the xylem vessels and after detachment, these are transported through sap stream to up part of plants. This causes mycelial penetration of the upper vessels as germination of microconidia occurs in that part. Vessel blockage due to accumulation of fungal hyphae, secretion of toxins and formation of gum, gel and tylose lead to the characteristic wilt symptoms appearance. Throughout this time, wilt pathogen restrictes in the xylem vessels, spread through parenchymatous tissue and reproduction occurs forming spores on plant surfaces like leaf, steam etc. (McGovern 2015; Joshi 2018).

D. Disease Management

Microbial pesticides

Pseudomonas fluorescens PF27 and *P. putida* PP15 both have proved their capability to defend plant against wilt disease.PF15 showed antagonistic activity and reduced of pathogen growth by 47% i*n vitro* condition, mean while in PP27 showed comparatively less inhibition of mycelium i.e. 10 percent. A trial was performed in *in-situ* condition for understanding induced systemic resistance (ISR) for which they took split-root design and to gauge antagonistic activity and ISR both, split-root design was not used. Commencement of symptoms was deferred and lowering of kinetics in comparison to pathogen control was observed due to Fluorescent *Pseudomonas*. The severity of the disease was reduced by 37–72% based on McKinney's index, and incidence level by 7–36% (Lamia *et al.,* 2017).

Hence, an experiment was conducted to estimate the impact of biocontrol agents with *Glomus mossae*, *G. fasciculatium*, *Trichoderma viride* and *T. Harzianum* to control wilt disease of tomato. Substantial drop in severity of wilt disease, improvement in chlorophyll content and plant growth parameters were recorded due to use of biocontrol agent's various portion. With increment of biocontrol agent's dosage, there was additionally increase in growth attributes. It was observed that higher dosage of bioagent led to maximum development and increase in content of chlorophyll content in each pot following less dosages of treatments in comparison to control plant where the least plant development was detected (Wani and Mir 2009).

An experiment was performed where they explored capability of *Trichoderma's* ten isolates to protect tomato plant against Fusarium wilt pathogen added to it. They also observed effect of these isolates in the presence and absence of the pathogen on the growth tomato plant. Lincoln Bio-Protection Research Centre Culture Collection was used to get the isolates and seed raising mix (0.5% w/w) was inoculated by them to raise two glasshouse experiments. Tomato Fusarium wilt incidence was reduced significantly (P<0.05) up to 69% by two *Trichoderma* isolates. Some plants only showed vascular discoloration. In the presence of the pathogen, an isolate increased the growth of plant by >50 percent, whereas there was no enhancement in the plant growth parameters by *Trichoderma* isolates, when pathogen was absent. (Ghazalibiglar *et al.,* 2016)

In another research, use of rhizobacteria for the management of Fusarium wilt disease in tomato was studied. They characterized their antagonistic activity against *F. oxysporum*. They took ten rhizobacterial isolates and among them, the highest inhibition of fungal growth (31%) was shown by *Bacillus amyloliquefaciens* CS-1 followed by *B. amyloliquefacie*ns PCfS which inhibited by 28% percent. Characterization for their useful qualities discovered that every isolate showed positive relation towards root colonization and variable results were found for solubilization of phosphate, Indole acetic acid production, production of antifungal secondary metabolite, siderophore formation, secretion of Hydrogen cyanide and disrupting enzymes formation salikechitinase etc. During greenhouse study, Fusarium wilt incidence was significantly suppressed by *Ochrobactrum intermedium* TRB-1, *B. amyloliquefaciens* PCfS *and B. amyloliquefaciens* CS - 1, and treated seedlings also showed enhanced the vigor index (VI) in comparison to untreated one (Gowtham *et al.,* 2016).

Under pot condition, the biocontrol ability contrary to Fusarium wilt by *Funneliformis mosseae*, *Acaulospora laevis* (arbuscular mycorrhizal fungi) and *Trichoderma viride* was evaluated. Results displayed considerable upsurge in growth of plant by entire bioagents. When *F. mosseae, A. laevis* and *T. viride* inoculated collectively, increased the height of plant, fresh weight of shoot, dry weight of root and leaves branches counts of each plant although *F. mosseae* and *T. viride* combined application augmented other growth parameters such as dry weight of shoot, fresh weight of root, root length and leaf area. On single inoculation of AMF, colonisation and spore number of AM was observed maximum, which diminishes on adding *T. viride*. Nevertheless, no effect was observed on the biocontrol efficacy of bioagents of this reduction. Pathogen infection caused considerable reduction in total amount of chlorophyll, photosynthesis, and nutrients. This effect was nullified by application of bioagent and leads to a notable rise in the nitrogen and phosphorus contents of the plant. Between the two AMF, for better tomato strain was *F. mosseae,* as it was more effective in comparison to *A. laevis*. The highest drop in incidence and severity of disease was documented in collective application of *T. viride, F. mosseae and A. laevis*. However, maximum disease incidence was observed in control plants without any bioagent. This study explains that, *F. mosseae* soil inoculation and application of *T. viride* conidial suspension in root former to transplantation causes improved immunity and subsistence capacity in seedlings of tomato against wilt disease (Tanwar *et al.,* 2013).

In another experiment, in the tomato plant (*Solanum lycopersicum*) the potential of *T. virens* (TriV_JSB100) using spores for treatment against *F. oxysporum* f. sp. *lycopersici* was studied and found its participation in the regulation and activation of the defence responses. Barley seeds(BGS) used as substrate for growth of *Trichoderma* spore or culture filtrate (CF) free of cell (TriV_JSB100) was used for priming of tomato seeds, these fourteen-day old tomato seedlings were further inoculated with pathogen (*F. oxysporum* f. sp. *lycopersici)*. Priming of bioagent caused considerable reduction in disease incidence in tomato plants, but BGS treated plant showed more reduction in comparison to plants treated with CF. Moreover, for analysing signalling behaviour in this condition, salicylic acid (SA) and jasmonic acid (JA) impaired tomato lines were used as to examine BGS and CF tempted immunity. They found BGS treated mutant which lack JA (def1) plants are prone to pathogen, however, less disease incidence and in BGS-treated wild type (WT) plants higher JA level were found. CF treated SA-deficient mutant NahG plants were also observed to exhibit less SA and also prone to pathogen. CF treated wild type plant showed less disease incidence and significantly increased SA. PDF1 (JA-responsive defensin gene) and PR1a (SA-inducible pathogenesis- related protein 1 gene) were expressed in BGS treated Wild type plants and Wild type plants treated with CF, respectively. These led to a suggestion that induction in plants treated with BGS and CF of TriV_JSB100 causes differentially signalling cascades by jasmonic acid and salicyclic acid for the activation of resistance in tomato plant against Fusarium wilt (Jogaiah *et al.,* 2018).

In vitro bioassay was done in Egypt for the assessment of antagonistic capability of seven isolates of *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici*. Maximum inhibition percentage against the pathogen was shown by *Trichoderma* isolate (T7) followed by T3 isolate. Substantial decline in the disease severity was observed in plants treated with T3 and T7 isolate in comparison to the control treatment under greenhouse trials. In comparison to other isolates, the lowest disease severity was 24.8% which shown by T3 isolate and succeeded by 34.6% via T7 isolate. Real-time RT-PCR was used to quantify the expression of defense-related β-1,3-glucanase gene for assessment of the accumulation kinetics of transcripts encoding PR proteins in the roots of tomato in control plant i.e inoculated with only pathogen, plant treated with T3 isolate and wilt pathogen, and plant treated withT7 isolate and wilt pathogen. Maximum expression of gene was reported in tomato plant treated with T3 and FOL in comparison to control. Using universal primer internal transcribed spacers (ITS1 and ITS4), these two species (T3 and T7) of *Trichoderma* showing antagonistic activity were characterized which evidenced that used isolates were not the same i.e. *T. atroviride* and *T. longibrachiatum* and belonged to different species (Sallam *et al.,* 2019).

Host could acquire resistance against phytopathogens either by direct antagonism or by activating induced resistance via endophytes of root. Induced systemic resistance (ISR) basically relies on signaling pathways of jasmonic acid (JA)/ethylene (ET), but salicylic acid (SA)-dependent signaling pathways could also trigger it. An experiment was conducted to explore the involvement of Jasmonic acid, Saliscylic acid and Ethylene in endophyte-mediated resistance (EMR) conferred by Fusarium endophyte Fo47 against wilt of tomato. The investigations incorporate impaired tomato plants which deduced accumulation of SA (NahG), JA formation (def1) or formation of ET (ACD) and detecting (Nr). Pattern of Fo47colonization in stems was indistinguishable among wild type plants and of hormone mutants. Unexpectedly, JA, ET, or SA impaired lines does not show any compromise in EMR, which showed EMR not dependent on JA, ET, or SA signalling. Thus, it explained difference between classical Induced Systemic Resistance (ISR and induced resistance conferred against tomato wilt disease caused by *F. oxysporum* f.sp. *lycopersici*). This experiment concluded that management of Fusarium wilt is self-regulating and don't rely on basic defense mechanisms (Constantin *et al.,* 2019).

To understand induction of phenolic compounds accumulation and defense enzymes in *F. oxysporum* f.

sp. *lycopersici*- infected tomato plants under treatment of salicylic acid (SA) and a biocontrol agent(*T. harzianum*), an experiment was conducted. In each treatment (*F. oxysporum*, *F. oxysporum* + TH, *F. oxysporum* + SA and *F. oxysporum* + TH + SA), accumulation of phenolic compounds was increased in comparison to control healthy plants. In the plants, the highest accumulation was recorded under the combined application of *F. oxysporum* + TH + SA. The accumulation of peroxidase and polyphenol oxidase in plants was raised due to application of salicylic acid at varied concentrations i.e. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM, though at amount,1.5 mM concentration of SA was found the most effective as it showed the highest actions of peroxidase and polyphenol oxidase enzymes on 28th day after the combined application of *F. oxysporum* and salicyclic acid. The same pattern of enhancement in the activities of both enzymes was obtained in Fusarium-infected tomato plants, treated with 1.5 mM of salicyclic acid and *T. harzianum* (Ojha and Chatterjee 2012).

Biological control of wilt of tomato has been also explored where biofortified vermicompost containing biological control agents (*T. harzianum, P. fluorescens* and *B. subtilis*) has been used. Adversarial effect of chosen microorganisms against FOL has been reported in *in-vitro* test. In intended treatments, parameters of plant growth, degrees of various antioxidants and disease occurrence were documented at various time spans which described huge decrease in occurrence of disease, improvement in growth of plant, increase in production along with increased production of antioxidants in vermicompost - applied tomato plants in comparison to untreated plants. Host plant treated with vermicompost biofortified with *T. harzianum* showed utmost improvement (Basco *et al.,* 2017).

CONCLUSION

For the management of Fusarium wilt disease in tomato, many synthetic chemical pesticides are in use. These pesticides are affecting our natural environment, while combating Fusarium wilt disease. To reduce the hazardous effect of pesticide and meet the food demand of increasing world population, researchers investigated and developed different alternative ways for management of diseases. One of them is the use of natural biocontrol agent which has been proved economic and ecological alternative method to manage different plant diseases in effective manner**.** Many investigations showed a potential role of biocontrol agent against plant diseases management. However, research and knowledge gap of plant microbe interactions, especially for the plant-biotic stress-biocontrol agents in rhizosphere region. Improvement in knowledge about rhizosphere ecology interactions and distribution of antagonistic and pathogenic microorganisms can help in enhancing the efficacy of bio-agents against plant diseases.

FUTURE SCOPE

All numerous techniques to control fungal diseases has different mode of action. Thus a comprehensive eco-friendly method including considerate pathogen virulence and genetic diversity, host resistance, and plant-pathogen interactions should be taken into consideration to help a build integrated management solutions. Moreover for advancement of microbial pesticides control method emphasis should be given to determine and improve their efficiency, effectiveness, and long-term viability in the field, not merely in the controlled conditions. Training farmers on the proper application of these microbial pesticides and their incorporation into other tactics for a healthier and nontoxic produce should be given a priority.

Conflict of Interest. The authors declare no conflict of interest.

Abbreviations. PGPR: Plant Growth Promoting Rhizobacteria, ISR: Induced Systemic Resistance, AMF: Arbuscular Mycorrhizal Fungi, SA: Salicylic Acid, JA: Jasmonic Acid, CF: Culture Filtrate, ET: Ethylene, EMR: Endophyte Mediated Resistance, WHO: World Health Organization, FOL: *Fusarium oxysporum* f.sp. *lycopersici.*

Authors' contributions

All authors participated in the development and implementation of the reviewing plan and subsequently written it. The first author RB discussed the different parts of the article with GK and finalized the manuscript. All authors have read and approved the final manuscript.

Acknowledgements. RB, BT, MMR are thankful to Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University for granting fellowship and space for research. SN and GK are thankful to Centurion University of Technology and Management for providing the facilities to complete this review article.

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How to cite this article: Bajpai, R., Teli, B., Rashid, M.M., Nanda, S. Yadav, S.K. and Kumar, G. (2021). Biocontrol of Fusarium Wilt in Tomato: An Eco-friendly and Cost Effective Approach. *Biological Forum – An International Journal*, *13*(2): 62-69.