

**Enhancing efficacy of biocontrol of
Fusarium wilt and Early blight of Tomato
(*Lycopersicon esculentum* Mill.) by
Trichoderma and *Pseudomonas***

THESIS

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Agriculture is an oldest and major occupation of the human beings that fulfills the basic needs of the human. In natural or man-made agro-ecosystems, the occurrence of diseases is a common phenomenon. However, the rapid increase in world's population, deterioration of the soil quality and continuous climate change has coupled with the phenomenon of disease and pest incidences that has aggravated the negative impact on plant growth, their yield, and nutritional values. Tomato is one of the most cultivated vegetable crops in the world. Due to its nutritional content and processing values, it is considered as a functional food crop and ranked top in industrial food products.

Tomato crop is sensitive to stresses and its productivity, quality of fruit content is greatly influenced by the biotic and abiotic factors. The occurrence of pathogenic diseases in tomato is common that hampers its yield and reduces market value. Thus the high demand of good quality of tomatoes in industrial sectors as well as for domestic purposes, compel farmers to input excessive amounts of agro-chemicals to obtain high yield. The excessive use of agro-chemicals is no more a sustainable approach due to their intrinsic negative externalities on the environment and living beings. The present study focused on assessing the problems faced by the farmers growing tomato in and around Lucknow city, the trend in use of fertilizers and pesticides followed by them to enhance productivity and to enhance the efficacy to biological approaches in reducing the incidence of fungal diseases on tomato. The major findings of the study were:

A personal interview was conducted with 97 tomato growers based on the pre-structured questionnaire, during different tomato growing seasons (2014-2016). The data regarding farmers age and education was collected that revealed that maximum farmers who indulged in tomato cultivation were between the age of 21-50 years and

about 40% of the farmers surveyed were illiterate. However, most of the farmers below the age of 30 years were found to be educated upto 8th standard. The data show that the low level of literacy was the main reason for the lack of knowledge about the use of modern technologies for sustainable crop cultivation and safety measures while handling agro-chemicals. This was proved by the data of single respondent collected, who was well educated and reported to use sustainable methods for disease management and plant growth and using proper safety measures *i.e.*, use of mask, gloves, studying the direction of wind before spraying agro-chemicals.

The majority of the farmers were marginal and cultivating tomato in small areas, less than a hectare, determining their low socio-economic conditions. The choice of seeds and best timing preferred for tomato cultivation by the farmers was also surveyed and found that maximum numbers of farmers willing to use hybrid seeds over non-hybrid seeds and October-November was the most preferable season for nursery preparation, although tomato can be cultivated all year round as Kharif and Zaid crop. The hybrid seeds were preferred due to their high yield potential, less time required for yield harvesting, better shelf life and quality of fruit content whereas winter or rabi season (October-March) was preferred due to favorable prevailing environmental conditions.

The abiotic, as well as biotic factors, were reported responsible for affecting tomato cultivation during the survey. The fluctuation in temperature, scarcity of water, land fragmentation, low monetary returns were the main abiotic factors whereas infestation of pests like caterpillars, termites, bugs, aphids, and fungal, bacterial, viral pathogens were the biotic agents found responsible for challenging tomato cultivation in the study area. The farmers were heavily reliant on agrochemicals such as organophosphates, synthetic pyrethroids carbamates, and

neonicotinoids to increase reduce plant pests and pathogens and Di-ammonium phosphate (DAP), urea, potash to increase the tomato productivity. The use of biofertilizers and biopesticides were negligible. The reason behind high inputs of the agro-chemicals and the negligible use of sustainable biological approaches was the low educational status of the farmers. This highlights the fact that educational status plays a most influential role in the trend in use of approaches for disease control and plant growth promotion.

Further, the pathogenic fungi responsible for causing biotic stress on tomato cultivation in and around Lucknow city were studied. The infected tomato plant and soil samples were collected from various tomato fields and cultured in laboratory conditions to assess the major fungal pathogens affecting the growth in the study area. The two pathogens *i.e.*, *Fusarium oxysporum* f. sp. *lycopersici* and *A. solani*, responsible for fusarium wilt and early blight, respectively in tomato were found prevalent in and around Lucknow city. The pathogenic nature of isolated strains was established under *in vitro* conditions.

In search of sustainable approach for controlling the growth of these fungal pathogens, a further attempt was made to isolate rhizospheric microbial strains possessing biocontrol ability. The microorganisms such as rhizobacteria, fungi, arbuscular mycorrhiza that colonize rhizosphere and plant roots are known as rhizospheric microbes. The association of several rhizospheric microorganisms with plants is reported to be a beneficial association. These microbes have demonstrated a significant role in the management of plant diseases and tailoring abiotic stress, ultimately leading to enhanced plant productivity. The seven fungal and fifteen rhizobacterial strains were isolated from roots and soil from the vicinity of healthy plants. The isolated fungal strains were identified on the basis of phenotypic

characters whereas rhizobacterial strains identified on biochemical tests. Among seven fungal isolates, two strains TvR1 and TbS2 were identified as *Trichoderma*, two strains AcS4 and AbS5 as *Aspergillus*, other two strains PbS3 and PrS7 as *Penicillium* and single isolate RcS6 as *Rhizopus*. The *Trichoderma* strains were selected for further study and rest were discarded owing to their ability to produce mycotoxins. The phenotypic characters of the isolates of *Trichoderma* were further studied in details for confirmation. Among isolated Rhizobacteria, 47% of the isolates were found to be *Bacillus* species (TS1, LS3, BS5, BS6, ES4, CS13, and Ts23) and only single strain of *Pseudomonas* (Tr20) was isolated.

The isolated strains observed for their antagonistic/biocontrol potential under *in vitro* condition. Total five isolates including two isolates of *Trichoderma* (TvR1 and TbS2) and three rhizobacteria (BS6, CS13, and Tr20) displayed positive biocontrol activity. The isolates of *Trichoderma* displayed mechanism of competition, antibiosis and mycoparasitism against pathogens. Similarly, the rhizobacterial isolates involved mechanism of antibiosis and competition. The strains BS6, CS13, Tr20, TvR1 and TbS2 with positive biocontrol attributes were further identified at a genomic level on the basis of 16s rRNA and ITS region sequencing as *Bacillus subtilis* BS6, *B. subtilis* CS13, *Pseudomonas aeruginosa* Tr20, *Trichoderma lixii* TvR1, and *T. brevicompactum* TbS2, respectively. The sequences were submitted to NCBI for obtaining their accession numbers. The accession numbers obtained were *Bacillus subtilis* BS6 (MF780728), *B. subtilis* CS13 (MF678835), *Pseudomonas aeruginosa* Tr20 (MF797805), *Trichoderma lixii* TvR1 (MF780730) and *T. brevicompactum* TbS2 (MF780729).

The isolated strains were characterized for their ability to produce secondary metabolites, phytohormones, and solubilization of inorganic minerals involved in

biocontrol and plant growth promoting activity. Among antagonistic isolates, production of hydrolytic enzymes chitinase and cellulase; siderophores; phytohormones Indole acetic acid and solubilization of Zn was reported by *Trichoderma lixii* TvR1, *T. brevicompactum* TbS2, and *Pseudomonas aeruginosa* Tr20. The production of Hydrogen cyanide and solubilization of inorganic phosphate was displayed only by *P. aeruginosa* Tr20. All the three rhizobacterial antagonistic isolates found producing protease and the ammonia production was documented for *T. lixii* TvR1, *T. brevicompactum* TbS2, *B. subtilis* CS13, and *Pseudomonas aeruginosa* Tr20. The production of exopolysaccharides (EPS) and biofilm forming ability that participates in root colonization was assessed for antagonistic isolates. The *Trichoderma* isolate TvR1 and TbS2 and rhizobacterial isolates Tr20 were found positive for the production of EPS and biofilm formation. The volatile and non-volatile metabolites of antagonistic isolates were also found instrumental in their biocontrol activities.

The seed germination and seedling vigor index was promoted by all the antagonistic isolates except *T. brevicompactum* TbS2, over control. However, *T. brevicompactum* TbS2 displayed plant growth promoting (PGP) activities *i.e.*, ability to produce IAA, ammonia, siderophores, EPS, solubilization of Zn and formation of biofilm under *in vitro* condition. Similar results were reported in pot experiment where *T. brevicompactum* TbS2 reduced the incidence of fusarium wilt and early blight but does not produce a significant effect on plant growth promotion. The strain *T. brevicompactum* is documented to produce a phytotoxic metabolite that assists in biocontrol activity but hinders plant growth. The overall results of the *in vitro* study displayed that the two isolates *Trichoderma* TvR1 and *Pseudomonas* Tr20 were the most effective strains that possessed biocontrol as well as PGP activities.

Further, the pot experiment was undertaken to screen the biocontrol as well as PGP activity of isolated antagonistic isolates under *in vivo* conditions. The results obtained were in line with the result obtained under *in vitro* conditions. The maximum percentage of disease reduction and plant growth promotion was displayed by *T. lixii* TvR1 and *P. aeruginosa* Tr20. *T. lixii* TvR1 and *P. aeruginosa* Tr20 reduced the incidence of fusarium wilt and early blight by 31.58 and 26.32; and 30.50 and 27.87%, respectively, and increased plant height by 47.3 and 41.4; 74.0 and 69.1; and 50.9 and 49% over positive control; *Fol* infected control and *A. solani* infected control, respectively. The use of consortia is known to have broader activity spectra due to the synergistic effects of the compatible members. The isolates *Trichoderma lixii* TvR1 and *Pseudomonas aeruginosa* Tr20 were found compatible with each other and formulated in a consortium for effective management of fusarium wilt and early blight.

On the basis of the result obtained through screening experiment and compatibility between antagonistic isolates, *T. lixii* TvR1, *P. aeruginosa* Tr20 and their consortium were investigated for management of fusarium wilt and early blight and promotion of growth of tomato plants. During the investigation, all the three treatments were found effective in reducing disease incidences and promoting growth. However, the consortium of two increased the biocontrol and PGP activity of the individual microbial isolates. These results confirmed that the application of consortium of compatible isolates has synergistic effects that promote the efficacy of individual isolate. The consortium also improved the photosynthetic pigments of tomato plants over positive and negative control. The results confirmed the biocontrol attributes of isolated antagonistic strains and further examination of roots sections under SEM revealed the effective root colonization ability of *T. lixii* TvR1 and *P.*

aeruginosa Tr20 suggesting root colonization as one of the mechanisms for biocontrol and PGP.

The antagonistic isolate *Pseudomonas aeruginosa* Tr20 was reported as effective biocontrol and PGP strain, however, the *P. aeruginosa* is an opportunistic human pathogen. Therefore to minimize the risk associated with the use of living cells of *P. aeruginosa*, an attempt was made to replace it with its biosurfactant. The biosurfactant was extracted from *P. aeruginosa* Tr20 and characterized on the basis of some physiochemical parameters and FTIR analysis. Through FTIR studies, the biosurfactant was identified as Rhamnolipid. The biosurfactant obtained was found to be effective in reducing the growth of *Fol* and *A. solani* under *in vitro* condition and its effect was concentration dependent. The compatibility of biosurfactant with *T. lixii* TvR1 was evaluated to develop consortium and their activity was investigated in pot conditions. The biosurfactant was found to be compatible with *T. lixii* TvR1 @ concentration 200 mg/L, a further increase in the concentration of biosurfactant observed to limit the growth of the latter.

The pot experiment was undertaken to assess the effect of biosurfactant and its consortium with *T. lixii* TvR1 @ concentration 200 mg/L on biocontrol of pathogens and PGP. The analysis of result demonstrated that consortium of *T. lixii* TvR1 and biosurfactant @ concentration 200 mg/L enhanced the efficacy of *T. lixii* TvR1, however, the maximum disease control was reported by the inoculation of a consortium of *T. lixii* TvR1 + *P. aeruginosa* Tr20. The consortium *T. lixii* TvR1 + biosurfactant does not displayed significant effect on PGP and fruit yield whereas photosynthetic pigment, carbohydrate and protein content was increased significantly in leaves as compared to the individual treatment of *T. lixii* TvR1.

The inoculation of microbial agents and biosurfactant individually or in the consortium was reported to induce production of pathogenesis-related enzymes. The consortium of *T. lixii* TvR1 + *P. aeruginosa* Tr20 displayed the highest production of polyphenol oxidase (PPO) and peroxidase (PO) enzymes. The PPO production by consortium *T. lixii* TvR1 + biosurfactant was low in comparison to individual inoculation of *T. lixii* TvR1 and *P. aeruginosa* Tr20 in pathogen-challenged plants. The production of PO in plants treated with a consortium of *T. lixii* TvR1 + biosurfactant was significantly same as reported to a consortium of *T. lixii* TvR1 + *P. aeruginosa* Tr20. Investigation of the effect of biocontrol agents and biosurfactant on quality of tomato fruits revealed that microbial agents were effective in improving values for pH, lycopene, and β -carotene than biosurfactant with an exception, where biosurfactant alone found to increase β -carotene content in plants challenged with *Fol*. The treatment of biocontrol agents and biosurfactant did not displayed any effect on the titratable acidity and moisture content of the fruit.

From the findings of the study, it was concluded that the lack of knowledge among farmers in the study area was the major reason for the excessive use of agrochemicals. Therefore there is a need for government and non-governmental organization to organize awareness camps for farmers to educate them about the toxicity and negative impacts of agrochemicals on their health as well as on the health of the consumers and environment and promote the use of sustainable approaches for agricultural activities. Further, the use of native rhizospheric microbial isolates was found as an efficient approach for biocontrol of fusarium wilt and early blight. With this, the study also confirmed that use of consortium in place of individual treatment is more effective in biocontrol of pathogens and plant growth promotion. The use of the microbial metabolites in place of living microbial cells can be another eco-friendly and safer option for sustainable agricultural practices.