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# Management of *Alternaria solani* Causing Early Blight in Tomato through Fluorescent Pseudomonads in Central Himalayas

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**ABSTRACT**

*Plant growth-promoting rhizo-bacteria play a vital role in the suppression of plant diseases. Pseudomonads are well known to show the growth promotion activity in plants and simultaneously activating the disease incidence delay. Promising strains of Pseudomonas isolated from different soil samples collected from various geographical regions of central Himalayas were applied as seed bio priming (SB), SB+ Root Dip (RD), SB+RD+ Drenching (DR) and SB+RD+DR+ Spraying (SPR) individually and in combination with different strains and evaluated for their effect on management of early blight caused by Alternaria solani an important disease of tomato. Disease incidence was reduced by the application of bio-inoculants individually as well as in combinations. The minimum disease incidence was recorded when different strains as consortia were applied as SB+RD+DR+ SPR.*

***Key words:*** *Alternaria solani, Rhizo-bacteria, Pseudomonads, Plant growth promotion*

## INTRODUCTION

Vegetables an essential to human diet are being frequently infected by the plant pathogens. Among vegetables, tomato is known to be of wide use and nutritional values, leading to its high demand in both fresh and processed market tomato varieties. The yield of tomato is restricted to a great extent due to an array of diseases associated with the crop[1]. Early blight is one of the most destructive diseases of tomato limiting the yield of glasshouse, field and soilless grown tomato crops in tomato producing countries around the world in the tropical and subtropical regions. The causal organism, *Alternaria solani* is air borne and soil inhabiting and is responsible for leaf blight, seedling collar rot and fruit rot of tomato[2]. The disease is most common in tropical and sub-tropical regions of the world and is favoured by high temperature and humidity (crowded plantation, high rainfall and extended period of leaf wetness from dew) and the plants are more susceptible to the blight infection during fruiting period[3]. Yield losses of up to 80 per cent have been reported due to disease in the field[4].

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Every one per cent increase in intensity can reduce yield by 1.36 per cent, and complete crop failure can occur when the disease is most severe[5].Use of potentially hazardous fungicides for the management of *A. solani* has been the subject of growing concern for plant pathologist as it may lead to the development of fungicidal resistance in the pathogen[6]. Thereby, novel approach requires low amount of chemicals to reduce pollution hazards as well as the cost of management.

Plant growth-promoting rhizo-bacteria play a vital role in the suppression of plant diseases. *Pseudomonads* are well known to show the growth promotion activity in plants and simultaneously activating the disease incidence delay. Bacterial inoculants, widely used as bio-control agents, are applied to soil, seed or roots of agriculture crops for the suppression of soil born plant diseases. Rhizosphere bacteria with the ability to provide biological control appear to comprise less than 10% of the total population of bacteria in the rhizosphere[7]. Specific rhizobacteria associated with plant roots and stimulate plant growth promoting rhizobacteria (PGPR)[8]. PGPRs such as *P. fluorescens* induce resistance in plants and suppress plant pathogens causing fungal, bacterial and viral diseases [9]. Keeping the above consideration in view the present study was conducted to assess the potentialities of *Pseudomonas fluorescens* for management of early blight disease of tomato.

## MATERIAL AND METHODS

### Collection of rhizospheric soil samples

Tomato Plants were grown in plastic pots filled with soil samples collected from different agro climatic locations of central Himalaya *viz* Auli (9000') Raiwala (942') and Pithoragarh (5500'). The plants were gently uprooted with approximately 10cm diameter core of surrounding soil with least possible injury to the roots. Root system and surrounding soil placed in plastic bags was subjected to vigorous shaking of excited roots to remove all but tightly adhering soil. 1gm. of soil was kept in 9ml. sterilized distilled water under aseptic conditions and after shaking for 5 min it was mixed well to prepare the dilution $10^{-1}$. The process was repeated to make sterile dilutions up to $10^{-7}$.

### Isolation and identification of *Pseudomonads*

The rhizospheric soil suspensions were plated (1ml) on Kings' B medium (KB)[10] streaking method. After 48 h. of growth at 28±1ºC, dominant fluorescent colonies were viewed under UV light and selected on the basis of colony characters and morphology of the bacteria and subjected to single colony isolation. The bacterial strains then selected were sent to Institute of Microbial Technology (I M Tech) Chandigarh for identification as well as transferred on the Kings'B slants and kept at 4ºC for further studies.

### Screening of *Fluorescent Pseudomonas* against *A. solani* through *in vitro* antagonism

Pure culture of pathogenic fungi *A. solani* was brought from IARI Delhi and maintained on PDA slants. The antagonistic activity of *Pseudomonas* strains against the pathogenic fungi was assessed on agar plates as described by [7] with slight modifications. A combination of two media (KB+PDA 1:1) was used for this purpose. Pure culture of bacterial strains was grown on Kings'B broth and fungal pathogen maintained on PDA slants was transferred to Petri dishes containing fresh PDA to produce fungal mycelium plugs. Antibiosis inoculation plates were inoculated with the help of sterilized glass bangle and a 5 mm mycelial disc (from actively growing colony of the test pathogen) was cut with the help of sterilized cork borer and placed in the centre of inoculation Petri dish. These inoculated plates were incubated at 28±1ºC for 48 hours. Maximum and minimum inhibition zones were measured after 48, 72, 96 hours and strains which do not allowed the growth of pathogenic fungi *in vitro* were taken as consideration as potential one. The plates without bacteria served as control. Three replications were taken for each treatment.

**Assessment of disease suppression potential of *Pseudomonads***

To study the potentialities of *Pseudomonas fluorescens* for management of early blight disease talc based formulations of effective strains of *Pseudomonas* were prepared by mixing autoclaved Carboxy methyl cellulose (CMC) and talc powder @ 1% w/w with bacterial strains grown on KB broth for 48 hours in incubator shaker at 150 rpm and 27±1°C for 24 hours on each of two consecutive days. 500ml of the bacterial suspension containing $2x10^9$ colony forming units (cfu) per ml was added to one kg of the carrier, mixed thoroughly and left overnight for complete drying. For the preparation of mixed formulation 100ml broth of each bacterial suspension was mixed in one kg carrier. Seeds of tomato var. Punjab Chhuhara were surface sterilized with 2 per cent sodium hypochloride for 30 seconds and rinsed in sterilized distilled water and dried overnight. Sterilized seeds were treated with 10 per cent solution of bio formulations for 30 minutes and then dried in sterile airflow overnight. Bioprimed seeds were sown into the pots (60X45cmX10cm Size), and field under glass house conditions. Three replications were made for each treatment and observations with respect to disease incidence were recorded for two successive cropping seasons. To record the performance of various treatments, during crop growth stage, transplanting was done in pots as well as in field under glass house and open field. Bio formulation treatments were given as (a) Seed bio-priming + root dip (SB+RD) (b) SB+RD+Dreanching (SB+RD+DR) (c) SB+RD+DR+ foliar spray (SB+RD+DR+SPR). Drenching and root dip @ 10 per cent where as foliar spray @ 5 per cent bio formulation was done. Experiments were set in triplicate, in randomized arrangement. Observations with respect to disease incidence at seedling and crop stage were recorded for two crop seasons.

The data was analysis statistically using simple ANOVA on Completely Randomized Block Design (CRBD) or/and Randomized Block Design (RBD). Critical difference was calculated at probability level of 0.05 to identify the significant effect of treatment means.

## RESULTS AND DISCUSSION

**Isolation and identification *Fluorescent pseudomonads***

Eighty six strains of *Pseudomonads* were isolated from the soil samples collected from different regions of central Himalayas. The isolated cultures were sent to Institute of Microbial Technology (IMTech) Chandigarh for identification, and were identified as different strains of *Fluorescent pseudomonas*, on the basis of morphological and chemical characters. Pure cultures of pathogenic fungi, *A. solani* causing wilt disease of tomato in central Himalayan region collected from IARI Delhi were used for antagonistic activity assessment as well as for inoculation of tomato plants.

**Symptomatology**

The symptoms exhibited by *A. solani* as small, isolated, scattered, pale brown spot on the leaflets. These spots were covered with a greenish blue growth of the fungus. The disease first appeared on lower leaves and then progressed to upper leaves. As the spots mature, concentric rings develop to produce a target board effect. Narrow chlorotic zone around the spots fades and increase with increase in size of the spots. Heavily infected leaves shriveled and fall off. The symptoms on the stems appeared as water soaked spots which soon turn light to dark brown to black necrotic lesions. Stem infections on the plant girdle the stem and caused premature death. The symptoms from the leaves and stem progressed to the fruits.[11] described the symptoms of early blight of tomato as minute, oval or angular, dark brown to black, necrotic, leathery spots on leaf lets with concentric rings, producing a characteristic shooting target board effect. Each spot surrounded by narrow chlorotic zone. The spots on the fruit were also similar to that on the leaves showing brown leasions with dark concentric rings. Both green and ripe fruits showed infection

(Plate-1). A mass of black spores was evident on lesions on fruits. The lesions gave velvety appearance on the surface of the fruits due to fungal growth and spore development.

***In vitro* screening of Fluorescent *Pseudomonads* against *F. oxysporum***

*Pseudomonads* when screened against *A. solani*, the strains isolated from the soil samples collected from Auli (AULI-200, AULI-178, AULI-185, AULI-188, AULI-187) were most antagonistic followed by those from the soil samples of Pithoragarh (P GGu-667, P JB-669, P JB-531, P GI-446, P JB-662) and Raiwala (R RWL-128, R RWL-308, R GRW-329, R GRW-118, R RWL-330) where maximum zone of inhibition was recorded. The Auli strains isolated from the higher hills were comparatively more effective than that of lower hills.[12] also reported reduction in the mycelial growth, spore germination, spore production and germ tube formation of *A. solani* and *A. alternata* by *P. fluorescens*.

***In vivo* screening through seed bio-priming**

The effect of formulated products on disease incidence was studied in tomato variety Punjab Chhuhara. Seeds were bio- primed with bio-formulated products RS-01 (strain R RWL-128), PS-01 (strain P GGu-667), AS-01 (strain AULI-185) and consortia of different strains RC-05 (consortia of R RWL-128, R RWL-308, R GRW-329, R GRW-118, R RWL-330), AC-05 (consortia of AULI-200, AULI-178, AULI-185 , AULI-188, AULI-187), and PC-05 (consortia of P GGu-667, P JB-669, P JB-531, P GI-446, P JB-662) before sowing. Formulated products which were prepared by mixing five effective strains showed higher efficacy towards disease suppression as compared to those prepared using single bacterial strain. The pooled data for two cropping seasons revealed that amongst the bio- products used AC-05 was found most effective in reducing the disease incidence as minimum 2.0 per cent, 3.0 per cent, 8.0 per cent and 10.33 per cent blight incidence was recorded after 10, 15, 20 and 25 days after germination respectively in pot conditions. Bio-inoculant AC-05 was also found highly significant in reducing the disease under field conditions in nursery stage (Table 1). All other formulations when bio-primed in seeds showed significantly effective outcome in reducing the blight as compare to control. Disease incidence was reduced in all the treatments as compared to control where the seeds were shown without any treatment. Suppression of plant pathogenic fungi and production of antifungal compounds by *Pseudomonas* spp. is also documented[13,14,15]. *Pseudomonas fluorescens* could act as strong elicitors of plant defense reactions[16]. All the products when bio-primed in seeds, shows significantly effective results in reducing the blight disease of seedlings as compared to control. Several PGPR strains have been reported to induce systemic resistance in plants against pathogens that eventually leads to reduction in disease incidence in many crops[17,18,19].

**Table. 1 Progressive disease incidence at seedling stage in pots and field under protected conditions**

| Treatment | Disease incidence (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pot conditions | | | | Field conditions | | | |
| | Days after Germination | | | | Days after Germination | | | |
| | 10 | 15 | 20 | 25 | 10 | 15 | 20 | 25 |
| RS-01 | 10.00 | 12.00 | 22.00 | 24.33 | 14.00 | 19.33 | 26.66 | 28.33 |
| PS-01 | 8.00 | 12.00 | 20.66 | 22.00 | 10.00 | 13.00 | 24.33 | 26.66 |
| AS-01 | 5.00 | 6.00 | 12.00 | 14.33 | 5.33 | 9.33 | 14.00 | 18.66 |
| RC-05 | 8.00 | 11.00 | 16.00 | 18.33 | 9.00 | 11.00 | 16.33 | 21.33 |
| AC-05 | 2.00 | 3.00 | 8.00 | 10.33 | 3.00 | 5.33 | 08.33 | 11.00 |
| PC-05 | 3.00 | 5.00 | 10.00 | 11.66 | 5.33 | 7.66 | 11.66 | 15.00 |
| Control | 18.00 | 20.00 | 35.00 | 37.66 | 15.33 | 17.66 | 31.33 | 47.00 |
| CD at 5 % | 2.515 | 2.603 | 4.356 | 4.589 | 1.426 | 1.511 | 4.293 | 1.868 |
| CV | 18.332 | 14.850 | 13.865 | 13.026 | 9.052 | 7.138 | 12.735 | 4.376 |

**Effect of Fluorescent *Pseudomonads* on disease suppression at different crop stages**

Bio-formulated products RC-05, AC-05 and PC-05 prepared as consortia of different strains which were found effective at seedling stage were further assessed for their affectivity towards disease suppression at different crop stages after transplanting. Different sets of application methods as seed bio-priming (SB), root dip (RD), drenching (DR) and foliar spray (SPR) were followed and all the treatments in either of the application method were found effective as compared to control in both protected and open field conditions.

Under protected conditions, when seedlings were transplanted in pots as well as in field, minimum disease incidence was observed in AC-05 than other formulations in different crop stages up to 90 days of transplanting (DAT). However, all the formulations were found significantly effective than control in reducing the disease incidence (Table 2, 3). Among the methods of application, SB+RD+DR+SPR was found superior as disease incidence was observed least followed by SB+RD+DR and SB+RD. All the formulations were found significantly effective in managing the disease in different methods of applications. Similar trend of observations were also recorded in the open field conditions (Table 4). AC-05 product showed minimum blight in different crop stages. However, during initial 30 DAT there was less difference among the bio-products treatments for disease management but after 90 days of transplanting significant difference was observed. After 90 days of transplanting, minimum (36.22%) blight was recorded in AC-05 product applied as SB+RD+DR and SB+RD as compared to control (94.44%) in pots. In field conditions under protected and open conditions the same results were observed where all the bio-inoculants were found effective when applied in different methods of applications. The use of PGPR such as *P. fluorescens* in recent years has been demonstrated under field conditions for crop plants against different pathogens[20]**.** The studies show that the disease incidence has been delayed with different sets of treatments in different crop stages. Production of elevated levels of defense-related enzymes resulting into increased disease resistance has been reported[21]**.** It confirms that prior application of bio- inoculants in various combinations and different sets of applications, induces the plant's own defense mechanism which enhanced the production of defense related chemicals and enzymes in pants [22, 23, 9, 24].

**Table. 2 Progressive disease incidence at different crop stages in pots under protected conditions**

| Treatments | Disease incidence (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 DAT | | | 30 DAT | | | 45 DAT | | | 60 DAT | | | 90 DAT | | |
| | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD +DR+S PR | SB+RD | SB+RD +DR | SB+RD +DR+S PR | SB+RD | SB+RD +DR | SB+RD +DR+S PR |
| AC-05 | 6.16 | 5.13 | 5.11 | 13.46 | 18.01 | 9.63 | 26.06 | 23.00 | 14.65 | 36.51 | 31.11 | 28.66 | 47.51 | 40.19 | 36.22 |
| RC-05 | 11.43 | 7.83 | 8.93 | 20.13 | 24.23 | 17.30 | 36.25 | 34.39 | 23.32 | 60.65 | 53.21 | 45.32 | 75.63 | 65.37 | 60.16 |
| PC-05 | 8.42 | 5.35 | 6.46 | 17.38 | 20.72 | 11.23 | 33.26 | 25.48 | 17.88 | 41.49 | 35.54 | 30.32 | 58.64 | 50.28 | 46.26 |
| Control | 18.90 | | | 27.76 | | | 47.03 | | | 69.82 | | | 94.44 | | |
| CD at 5 % | (a)=.306 (b)=.354 (a*b)=.613 | | | (a)=.304 (b)=.351 (a*b)=.609 | | | (a)=.160 (b)=.185 (a*b)=.320 | | | (a)=.339 (b)=.392 (a*b)=.679 | | | (a)=.260 (b)=.301 (a*b)=.521 | | |
| CV | 3.579 | | | 1.834 | | | .605 | | | .841 | | | .484 | | |

**Table. 3 Progressive disease incidence at different crop stages in fields under protected conditions**

| Treatments | Disease incidence (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 DAT | | | 30 DAT | | | 45 DAT | | | 60 DAT | | | 90 DAT | | |
| | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD+DR | SB+RD+DR +SPR | SB+RD | SB+RD +DR | SB+RD+DR +SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR |
| AC-05 | 4.62 | 2.65 | 4.54 | 11.57 | 16.67 | 7.36 | 23.65 | 20.58 | 12.63 | 34.61 | 28.37 | 25.36 | 45.47 | 39.64 | 34.25 |
| RC-05 | 9.55 | 4.60 | 7.30 | 20.67 | 22.68 | 15.63 | 33.58 | 31.60 | 21.35 | 57.57 | 51.44 | 44.24 | 71.37 | 62.18 | 57.24 |
| PC-05 | 7.34 | 3.25 | 5.23 | 15.29 | 18.47 | 9.26 | 30.54 | 23.42 | 15.26 | 39.36 | 33.56 | 28.40 | 56.57 | 47.56 | 45.58 |
| Control | 22.34 | | | 29.21 | | | 46.03 | | | 68.64 | | | 93.91 | | |
| CD at 5 % | (a)= .288 (b)=.333 (a*b)=.576 | | | (a)=.216 (b)=.249 (a*b)=.432 | | | (a)=.274 (b)=.316 (a*b)=.548 | | | (a)=.290 (b)=.338 (a*b)=.586 | | | (a)=.205 (b)= .237 (a*b)=.417 | | |
| CV | 3.520 | | | 1.360 | | | 1.107 | | | 0.756 | | | 0.393 | | |

**Table. 4 Progressive disease incidence at different crop stages in open field conditions**

| Treatments | Disease incidence (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 DAYS | | | 30 DAYS | | | 45 DAYS | | | 60 DAYS | | | 90 DAYS | | |
| | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR | SB+RD | SB+RD +DR | SB+RD+ DR+SPR |
| AC-05 | 3.22 | 4.39 | 2.79 | 9.65 | 14.59 | 5.52 | 20.37 | 17.26 | 10.55 | 31.53 | 27.49 | 23.68 | 44.20 | 37.47 | 33.70 |
| RC-05 | 8.57 | 7.42 | 3.22 | 17.68 | 20.34 | 13.60 | 31.46 | 27.48 | 18.42 | 55.75 | 48.27 | 42.68 | 70.70 | 61.56 | 55.65 |
| PC-05 | 7.07 | 4.31 | 2.21 | 11.66 | 15.39 | 7.62 | 28.30 | 20.55 | 13.37 | 37.65 | 32.80 | 28.68 | 54.50 | 45.64 | 43.40 |
| Control | 18.09 | | | 25.72 | | | 44.22 | | | 67.10 | | | 92.77 | | |
| CD at 5 % | (a)=.121 (b)=.140 (a*b)=.242 | | | (a)=.246 (b)=.284 (a*b)=.493 | | | (a)=.377 (b)=.436 (a*b)=.755 | | | (a)=.437 (b)=.505 (a*b)=.875 | | | (a)=.346 (b)=.400 (a*b)=.693 | | |
| CV | 1.763 | | | 1.809 | | | 1.670 | | | 1.170 | | | 0.677 | | |

Plate1. Symptoms of early blight in different parts
A. Leaf B. Stem C. Fruit

## CONCLUSION

In conclusion, application of fluorescent *Pseudomondas*, was found to be effective in controlling the tomato early blight disease, out of the five product formulated, product AC-05 showed minimum disease incidence and amongst different methods of applications seed bio priming (SB), SB+ Root Dip (RD), SB+RD+ Drenching (DR) and SB+RD+DR+Spraying (SPR) evaluated for efficient disease management, SB+RD+DR+ SPR was found to be the most promising one.

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