Bio-Efficacy of 'SUDO™' (Pseudomonas fluorescens) as a Bio-Control Agent against Early Blight (Alternaria solani) Disease of Tomato

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ABSTRACT
Tomato (Lycopersicon esculentum Mill) is the most popular vegetable crop grown in India as well as in the world which is attacked by several diseases. Among the various diseases, early blight caused by Alternaria solani has become the constraint resulting in huge yield losses. Continuous usage of chemical methods leads to environment, soil and water pollution. However, biological control of diseases is long lasting, inexpensive, eco-friendly and harmless to target organisms. A field bio-efficacy study was conducted for evacuation of SUDO (Pseudomonas fluorescens) for control of tomato early blight disease. The results compiled suggest that the two sprays with 'SUDO' @ 2.0 ml/L followed or preceded by chemical fungicides was found effective in the management of early blight disease of tomato. Minimum PDI of 9.88 was recorded in T-1 treatment (chemical), followed by combination of treatments of chemicals + SUDO with 11.88 to 28.34 PDI which are significantly lower than control treatment (42.40 PDI). Even SUDO @ 2.0 ml/L alone recorded significantly more plant height (17.42%), and fruit yield (58.10%) with reducing early blight disease by 33.16% over control treatment. Similarly spraying of SUDO did not show any phyto-toxic effect on tomato plants.

Keywords- Tomato, Lycopersicon esculentum, SUDO, Pseudomonas fluorescens, early blight, Alternaria solani, PDI, Yield.

I. INTRODUCTION
Tomato (Lycopersicon esculentum Mill) is the second most important vegetable crop next to potato. It is native of Andean region of South America and cultivated throughout the world. It is mostly used in preparation of soup, pickles, ketchup, purees, sauces and also used to add variety of colours and flavours to the food materials by various ways. Therefore, it is one of the most important vegetable crops in the world as well as in India due to its importance as protective food and nutritive value. According to Saini (2006) the nutritive value of tomato per 100 gm of edible portion are as moisture 93.19%, protein 1.90 gm, potassium 144 mg, copper 0.19 mg, sulphur 24 mg, chlorine 38.00 mg, Vitamin 31.00 mg, thiamine 0.07 mg, riboflavin 0.01 mg, nicotinic acid 0.40 mg, magnesium 15.00 mg, oxalic acid 2.00 mg, phosphorus 36.00 mg, Iron 1.80 mg and Vitamin-A 320 mg. As tomato contains sufficient amount of antioxidant lycopene pigment which helps to protect the body against cancer and heart disease (Bohm et. al. 2012). Due to its wide use and nutritive value, there is a high demand for both fresh and processed tomato varieties.

Tomato crop is found to suffer from a variety of diseases caused by fungi, bacteria, viruses and nematodes. The most important diseases are early blight, damping off, late blight, fusarium wilt, verticillium wilt, bacterial wilt and tomato mosaic virus. Among these diseases, early blight caused by Alternaria solani is one of the most yield limiting factors in India. The survival of the pathogen mainly in the soil and penetrates into the plant through the root system. The early blight or fruit rot disease caused by Alternaria solani (Ellis and Martin) is one of the most destructive diseases of tomato in most of the tomato growing regions. The causal organism is air borne and soil inhibiting and is responsible for early blight, seedling collar rot and fruit rot of tomato (Datar and Mayee 1986). This early blight disease was the most destructive disease causing heavy loss at pre and post-harvest stages causing 35 to 78% reduction in yield. Since, hybrids are more susceptible to early blight and constitute more than 70% of total cultivation losses can be enormous.

Although satisfactory control of the disease by using various chemicals has been reported by Choulwar and Datar (1998), Maheshwari et. al. (1991) and Abdul-Mallek et. al. (1995) in tomato. However, continuous use of agro-chemicals for controlling the disease may pose several problems like toxicity to non-target organisms, development of resistance in the populations of the pathogen and environmental pollution. Biological agents and plant extracts are considered to be a new way because they are eco-friendly and can be used as an effective alternative measure to control plant diseases (Verma et. al. 2008). The use of plant growth promoting rhizobacteria, Pseudomonas fluorescens is one of the promising biological control modules and its commercial formulations have been tested against several crop disease caused by pathogens (Kaur et. al. 2016).
Bioagents possess antagonistic effects against pathogen of plants (Muriungi et. al. 2013). Bio-control treatments of plant diseases with *Pseudomonas fluorescens* have proved to be eco-friendly and effective against many plant pathogens and considered as a long-term solution to management of plant diseases (Kaur et. al. 2016; Verma et. al. 2008). Kaur et. al. (2016) reported 39 to 46% reduction of tomato early blight disease with talc based formulation of *P. fluorescens*.

Certain strains of *P. fluorescens*, when applied to soil or soil or aerial spray, provide biological control of soil borne pathogens in tomato (Ngoc et. al. 2013, Vanitha et. al. 2009). Farm yard manure (FYM) has been reported as the best substrate for multiplication and an ideal source for *P. fluorescens* (Nazam and Singh 2004) and Jayraj et. al. (2007) in the management of early blight disease of tomato.

Hence the present study was conducted to evaluate the bio-efficacy of ‘SUDO’ liquid formulation of *Pseudomonas fluorescens* for the management of tomato early blight disease in field conditions.

**II. MATERIALS AND METHODS**

Field experiment was conducted to evaluate ‘SUDO’ (*Pseudomonas fluorescens*) a bio-fungicide against tomato early blight disease caused by *Alternaria solani* and yield of tomato. The trial was conducted during 2016-17 at the Main Agricultural Research Station, Institute of Organic Farming, University of Agricultural Sciences, Dharwad Karnataka State. The present experiment was laid out in a Randomised Block Design (RBD) with three replications and eight treatments. A promising and adaptive tomato variety ‘DMT-2’ was used in the present study. A fertilizer dose of 60:50:30 NPK/ha was used by using all the other agronomical practices for raising a good crop. A plot size 3.6 m X 3.0 m with 60 cm X 60 cm distance between two rows and two plants was kept. The treatment details are given in the following table :-

| Sr. No. | Treatments                        | Dosage  g/ml/l | Application Time & Method |
|---------|-----------------------------------|----------------|--------------------------|
| 1       | POP (Sectin 60WG@3 g/l)           | 3              | Spray started soon after the onset of Leaf spot disease/ Early blight disease of tomato & two sprays were given at 15 days interval with Knapsack sprayer. |
| 2       | SUDO @ 2ml/l                      | 2              |                          |
| 3       | POP+SUDO (1 ml/l)                 | 3+1            |                          |
| 4       | POP+SUDO (2 ml/l)                 | 3+2            |                          |
| 5       | SUDO(1ml) + Chemical (Mancozeb 2.0g/l) | 1+2        |                          |
| 6       | SUDO(2ml) + Chemical (Mancozeb 2.0g/l) | 2+2        |                          |
| 7       | Chemical (Difencomazole-Score) +SUDO (2ml/l) | 1+2    |                          |
| 8       | Control                           |                |                          |

Total 500 L/ha water was used for spraying with Knapsack sprayer. In each plot 5 plants were randomly selected and graded for leaf blight disease incidence using 0-5 scale as described by Dattar and Mayee (1986) as given below:

| Sr. No. | Grades | Description                        |
|---------|--------|------------------------------------|
| 1       | 0      | No symptoms of early leaf spot     |
| 2       | 1      | 0-5% leaf area affected and covered by spot, no spot on petiole and branches |
| 3       | 2      | 6-20% leaf area affected and covered by spot, no spot on petiole and branches |
| 4       | 3      | 21-40% leaf area and branches affected are covered by spot |
| 5       | 4      | 41-70% leaf area and branches affected are covered by spot |
| 6       | 3      | >71% spots appear on leaf, branches and fruits |

The percent disease index (PDI) was further calculated using the formula of Wheeler, 1969 or Mc Kinny (1923) as given below:-

\[
\text{Percent Disease Index} = \left( \frac{\text{Sum of Numerical ratings}}{\text{Total No. of fruits/leaves observed} \times \text{Maximum grade}} \right) \times 100
\]
Similarly, observations on plant height and fruit yield (T/ha) were recorded and statistically analysed according to the method suggested by Panse and Sukhatme (1985).

'SUDO' (Pseudomonas fluorescens) is a biological fungicide which is eco-friendly and effective against a wide variety of seed and soil borne plant pathogenic fungi. Pseudomonas fluorescens is a fast growing competitive microbe which secretes enzymes and binds iron, making it unavailable for pathogenic fungi. As a result the pathogenic fungi do not get Fe which is required for growth. It is highly active on roots, stem rots, foliar fungal diseases caused by Alternaria, Ascoytha, Cercospora, Myrothecium, Rumularia, downy mildew and powdery mildews. It is also acts as a plant growth promoter to stimulate seed germination and early flowering and fruiting. It is also functional over a broad temperature range of 10 to 35°C and therefore it is advisable in case of Cercospora/Downy mildew when temperature is below 15°C on leaf surface/atmosphere. The treatments were also observed for the visual phytotoxic symptoms like injury on leaf tips and leaf surface, wilting, vein clearing, necrosis, epinasty and hypomasty and are recorded on 1, 3, 5, 7 and 10 days after spray by following 0-10 rating scale as below:

| Sl. No. | Grades | % Phytotoxicity injury on foliage |
|---------|--------|----------------------------------|
| 1       | 0      | No adverse effect                |
| 2       | 1      | 1-10%                           |
| 3       | 2      | 11-20%                          |
| 4       | 3      | 21-30%                          |
| 5       | 4      | 31-40%                          |
| 6       | 5      | 41-50%                          |
| 7       | 6      | 51-60%                          |
| 8       | 7      | 61-70%                          |
| 9       | 8      | 71-80%                          |
| 10      | 9      | 81-90%                          |
| 11      | 10     | 91-100%                         |

III. RESULTS AND DISCUSSION

The data on bio-efficacy of 'SUDO' against tomato early leaf blight disease (PDI), plant height and yield are presented in Table-1 and Figure-1.

i) Effect on tomato early leaf blight

The first symptom of the early blight disease caused by Alternaria solani on tomato appeared as small brown water soaked lesions on the older leaf. The symptoms were oval or angular in shape from one to four mm diameter and there was a narrow chlorotic zone around the spot. These spots enlarged and covered the entire stem and petioles leading to withering of the plants. Symptoms also developed on calyx and flower buds in the form of minute brown to dark brown spots which enlarged later and spread to sepalns and fruits resulting in pre-mature dropping of fruits.

Incidence of tomato early leaf blight was recorded on leaves and fruits and the PDI values are calculated and presented in Table-1. On leaves and fruits a minimum PDI of 9.88 was recorded in T-1 treatment followed by T-7 (11.88), T-6 (13.09), T-4 (14.82), T-3 (19.30), T-5 (21.29) and T-2 (28.34) which are significantly superior over control for reduction of leaf blight disease as compared to control (42.40). It is also significant to note that the highest % reduction in disease was recorded in T-1 (76.69%), followed by T-7 (71.98%), T-6 (79.13%), T-4 (65.05%), T-3 (54.48%), T-5 (49.78%) and T-2 (33.16%) treatment over control treatment. Similarly, 'SUDO' @ 2.0 ml/L, individually recorded 33.16% reduction in disease control.

Mean PDI of Early Blight

![Graph showing Mean PDI of Early Blight](image)

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ii) **Plant height (cm)**

All the treatments gave significantly more plant height than control treatment. Maximum plant height of 150.33 cm was recorded in T-6 (SUDO @ 2.0 ml/L + Mancozeb @ 2.0 g/L) followed by T-7 (Score @ 1.0 ml/L + SUDO @ 2.0 ml/L) with 148.64 cm; T-4 (POP + SUDO @ 2.0 ml/L) with 147.79 cm; and T-5 (SUDO @ 1.0 ml/L + Mancozeb @ 2.0 g/L) with 145.00 cm. which are on par with each other and are significantly superior over control treatment (118.89 cm). Similarly all the above four treatments gave 26.44%, 25.02%, 24.31% and 21.96% more plant height respectively than control treatment. Even, T-6 (SUDO @ 2.0 ml/L + Mancozeb @ 2.0 g/L) treatment gave significantly more plant height than POP (Sectin 60 WG @ 3.0 g/L) also. Likewise, T-2 (SUDO @ 2.0 ml/L) also gave 17.42% more plant height than control treatment.

![Graph of Plant height](image)

### Yield T/ha

iii) **Fruit yield (T/ha)**

All the treatments recorded significantly higher/more fruit yield than control treatment. Fruit yield ranges from 8.33 T/ha (T-1 i.e. POP - Sectin 60 WG @ 3.0 g/L) to 3.58 T/ha (T-8, control). Similarly, T-7 (Score @ 1.0 ml/L + SUDO @ 2.0 ml/L) gave significantly higher yield of 6.76 T/ha followed by T-4 (POP + SUDO @ 2.0 ml/L) with 6.67 T/ha; T-6 (SUDO @ 2.0 ml/L + Mancozeb @ 2.0 g/L) with 6.65 T/ha; T-3 (POP + SUDO @ 1.0 ml/L) with 6.11 T/ha, T-5 (SUDO @ 1.0 ml/L + Mancozeb @ 2.0 ml/L) with 6.02 T/ha and T-2 (SUDO @ 2.0 ml/L) with 5.66 T/ha. Similarly, T-1 treatment gave 132.68% higher yield followed by T-7 (88.83%), T-4 (86.31%), T-6 (85.75%), T-3 (70.67%), T-5 (68.16%) and T-2 (58.10%) over control treatment. Even, the results also indicated that the higher concentrations of 'SUDO' alongwith other treatments were more effective than the lower concentrations.

![Graph of Yield T/ha](image)
iv) Influence of SUDO on phytotoxicity

The test formulation ‘SUDO’ (Pseudomonas fluorescens) was evaluated for toxicity in T-4, T-6, T-7 and T-8 treatments on tomato plants. The visual phytotoxicity of tomato revealed that no phytotoxicity symptoms of the test bio-fungicide ‘SUDO’ was observed in any of the concentrations tested for above treatments.

In the present study tomato plants were sprayed with ‘SUDO’ @ 2.0 ml/L alone and in combination with Pop (Sectin @ 60 WG @ 3.0 g/L) or Mancozeb @ 2.0 g/L or Score @ 1.0 ml/L for evaluating its bio-efficacy against early blight of tomato disease, plant height and yield. Results obtained revealed that application of SUDO at 50 and 65 days after transplanting recorded minimum PDI of 9.88 in T-1 treatment followed by T-7 (11.88) T-6 (13.09), T-4 (14.82), T-3 (19.30), T-5 (21.29) and T-2 (28.34) which are significantly lower than control treatment (42.40). It is also significant to note that the highest % reduction in disease was recorded in T-1 (76.69%), followed by T-7 (71.98%), T-6 (68.13%), T-4 (65.05%), T-3 (54.48%), T-5 (49.78%) and T-2 (33.16%) treatment over control treatment. Similarly, T-4, T-7 and T-2 recorded significantly increase in fruit yield of, 86.31%, 88.83% and 58.10% respectively over control treatment.

Though all the bio-control treatments of ‘SUDO’ (P.fluorescens) were found inferior to POP (Chemical treatment), but superior to untreated control with respect to early blight disease and yield as observed in the present study. The use of chemical pesticides cannot be long term solution for the management of disease due to concerns of expenditure, health and environmental hazards. Hence, bio-control treatments of P.fluorescens could be considered for long term solution for the management of plant diseases, as they have proved to be eco-friendly and effective against many plant pathogens. In the present study ‘SUDO’ reduced the early blight disease by 33.16% individually or 71.98% in combination with chemical insecticides as compared to control. This might be due to the antagonistic effect bio-fungicide which could induce systemic resistance against early blight disease in tomato. Many strains of P.fluorescens have been exploited in seed treatment or foliar application to induce systemic resistance in tomato against early blight, leaf blight, root knot disease and bacterial wilt (Babu et. al. 2000, Latha et. al. 2009, Vanitha et. al. 2009, Singh et. al. 2015 and Kaur et. al. 2016).

Pseudomonas spp. are particularly suitable for as agricultural bio-control agent since they can use many exudates compounds as a natural source (Lugtenberget al. 1999) abundantly present in natural soils, particularly on plant root systems, high growth rate, possess diverse mechanisms of actions towards phytopathogens including production of antagonistic metabolites (Rhodes and Powel, 1994) and enable of inducing a systemic resistance to pathogens (Van Loon et al. 1998). The use of biological control agents has been reported as a viable option to synthetic chemicals in plant disease control and is currently being advocated worldwide (Ganeshan and Kumar, 2005). Bio-agents possess antagonistic effects against pathogen of plants (Muriungiet al. 2013). Bio-control treatments of plant diseases with Pseudomonas fluorescens have proved to be eco-friendly and effective against many plant pathogens and considered as a long term solution to management of plant diseases (Kaur et. al. 2016, Verma et. al. 2008).

The use of biological control has also been reported by various workers for the management of early blight disease of tomato. The four species of Pseudomonas viz.; P.fluorescens, P.aeruginosa, P.putida and P.cepacia have resulted in less disease severity and high fruit weight as compared to control in tomato (Joseph et. al. 2017). In addition, the role of fungi such as Trichoderma viride (Sarkar et. al. 2016) and T.harzianum (Chowdappa et. al. 2013) in the management of early blight has been reported. The plant growth promoting rhizobacteria Bacillus subtilis also increases systemic resistance intomato by inducing growth hormones and defense related enzymes such as peroxidase, polyphenol oxidase, and superoxide dismutase (Chowdappa et. al. 2013). Similarly, Pseudomonas species influence plant growth through inhibition of fungal plant pathogens (Moore et. al. 2006). Certain strains of Pseudomonas secrete metabolites that inhibit plant pathogens and stimulate plant growth. Thus, the less disease severities recorded on tomato plants treated with SUDO in the present study might be attributed to the production of metabolites that inhibit the growth of Alternarlasolani. Biological control of crop diseases by antagonistic micro-organisms has become the most effective alternative to synthetic chemical pesticides (Atmayehu, 2014). Therefore, either ‘SUDO’ (P.fluorescens) treatment can be considered along with chemical control for developing environmentally safe, long lasting and effective Integrated Pest Management (IPM) programme for the management of tomato foliar diseases in future.

Table 1: Evaluation of SUDO for bio-efficacy on Tomato early blight disease

| Treat. No. | Treatment details | % Disease Index (PDI) | Plant Height | Yield |
|-----------|-------------------|-----------------------|-------------|-------|
|           | Before Spray      | After II spray        | % reduction in disease | Cm. | % increase in height | T/ha | % increase in yield |

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IV. CONCLUSION

The spray with 'SUDO' @ 2.0 ml/L followed or preceded by chemical fungicide was found effective in the managing the early blight disease and increasing fruit yield of Tomato than SUDO @ 2.0 ml/L alone. Hence, the new bio-fungicide 'SUDO' @ 2.0 ml/L can be integrated with recommended chemical fungicide for effective management of the tomato early blight disease.

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