



## Effect of Bioagents and their Consortia in the Management of Early Blight Disease of Potato

Suleiman Abdullahi

Suleiman<sup>1\*</sup>,

<sup>1</sup>Department of Crop Production, Federal University Dutse, P.M.B. 7156, Jigawa State, Nigeria

Sobita Simon<sup>2</sup>

<sup>2</sup>Department of Plant Pathology and Entomology, Sam Higginbottom Institute of Agriculture, Allahabad 211 007, Uttar Pradesh, India

Midhun Babychan<sup>2</sup>

<sup>2</sup>Department of Plant Pathology and Entomology, Sam Higginbottom Institute of Agriculture, Allahabad 211 007, Uttar Pradesh, India

\* Corresponding author: [suleiman.ab@fud.edu.ng](mailto:suleiman.ab@fud.edu.ng)

**Abstract** – Early blight incited by *Alternaria solani* is a destructive disease of potato worldwide. The plant growth-promoting antagonists, which elicit induced systemic resistance (ISR) and enhance plant growth, are being used as safe alternatives to synthetic fungicides for the management of plant diseases. In this study, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus thuringiensis* were tested individually and in combinations, as slurry seed treatment, for their efficacy against *Alternaria solani*, the causative agent of early blight disease of potato, under *in vitro* and field conditions. Consortium treatment of *T. viride* and *B. thuringiensis* showed highest early blight disease suppression (30.76%, disease incidence) and highest yield of potato at harvest (2.78  $\text{tha}^{-1}$ ). The mixture of *T. viride* and *P. fluorescens* revealed highest growth parameters (25.5cm, shoot length) and highest inhibition of *A. solani* under *in vitro* condition (97.48%). The study indicates that seed treatment with combination of *T. viride* and *P. fluorescens* and that between *T. viride* and *B. thuringiensis* can become part of integrated management of early blight disease of potato and plant growth promoting.

**Keywords** – Biological Control, Consortia, Early blight, Potato.

### 1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of mankind's most valuable food crops [1]. It is the most important vegetable crop in terms of quantities produced and consumed worldwide [2]. Potato is prone to more than a hundred diseases caused either by bacteria, fungi, viruses or mycoplasmas. However, early blight or *Alternaria* blight is one of the most important foliage diseases in areas with favourable weather conditions [3]. The fungus can cause disease on foliage (leaf blight), stem (collar rot) and tuber, and can result in severe damage during all stages of plant development [4]. The leaf blight phase, commonly referred to as early blight, is the most important phase of the disease and can result in complete loss of the crop when incidence is severe [5].

Early blight is difficult to control because of its capacity to produce huge amounts of secondary inoculum [6], [7]. In order to suppress early blight and to prevent the losses it causes, potato fields are intensively sprayed with fungicides. Several effective fungicides have been recommended for use against this pathogen, but they are not considered to be long term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. In an attempt to modify this condition, some alternative methods of control have

been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases.

The use of *Trichoderma spp.* in the biological control of many plant diseases has been a subject of many studies. Antibiosis was potentially a principal component of mechanism of the biocontrol by *Trichoderma spp.*, which produced an array of metabolites identified as antifungal and antibacterial compounds [8], [9]. Antagonistic bacteria have been extensively studied as biocontrol agents effective against various soil-borne pathogens. Several *Bacillus spp.* are antagonistic to plant pathogenic fungi and bacteria. However, investigation on mechanisms of biological control by *Pseudomonas spp.* revealed that *P. fluorescens* produced an antibiotic highly effective which protect plants from various pathogens through inducing systemic resistance [10], [11] and [12]. Several reports however showed that disease suppression can be increased by utilizing combinations of biological control agents and plant growth promoting rhizobacteria (PGPR) and their combined effects are pronounced in improving crop yields and enhancing nutrient uptake by plants over single organism inoculation [13].

The main objective of this study was to evaluate the individual and combined inhibitory effect of some selected bioagents against *A. solani* and to study their solo and consortial effects on growth and development of potato under field conditions.

### 2. MATERIALS AND METHODS

#### 2.1. Experimental site

The experiment was conducted in the laboratory and research field, Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Allahabad, India during the spring season of 2015-2016. There was net cultivated area of 84m<sup>2</sup> consisting of seven treatments replicated three times.

#### 2.2. Isolation and purification of the fungal pathogen

Fungal pathogen was isolated from the infected potato leaves. The infected sections were cut-off from the margins of leaf lesions together with some portions of the healthy plant and surface sterilized in 1% sodium hypochlorite followed by rinsing in three series of distilled water. Inside laminar flow chamber, the sections were carefully and quickly transferred onto the sterilized PDA media, and incubated at 20-25°C for 5-7days, and obtained

the culture. The culture of *Alternaria solani* was purified and maintained by periodic sub-culturing on PDA petri plates and slants, and incubated at  $25 \pm 2^{\circ}\text{C}$  temperature [14].

### 2.3. Source of bioagents and potato seed treatment

Commercial based Powder formulations of *Trichoderma viride* ( $2 \times 10^7$  c.f.u./g), *Pseudomonas fluorescens* ( $2 \times 10^8$  c.f.u./g) and *Bacillus thuringiensis* ( $2 \times 10^8$  c.f.u./g) obtained from Yash Green Land Pvt.Limited, Allahabad, was used as slurry seed treatment for this experiment. All the formulations contained 0.5% w/w Carboxymethyl cellulose (CMC) as an adhesive material. In single bioagent treatments, 10g of formulation was used to treat 1kg of potato tubers. However, in mixtures of bioagents, combinations of the two powders were formed in ratio 1:1 to make the same quantity as recommended by the manufacturer. The seeds were allowed to dry under shade for 2 hours, before sowing.

### 2.4. Artificial inoculation of the pathogen

The pure cultures of *A. solani* obtained during isolation were transferred to fresh plates of acidified PDA for multiplication. Full grown cultures were macerated and strained through a layer of muslin cloth and made up to three-four litres of suspension. This stock suspension was taken to the field and diluted in a compressed air sprayer. Spray was done into the whorls of the plants where it will be retained for longer period to permit the conidial germination. Inoculation was made when plants were 15-20 cm high with the spore suspension load ( $5 \times 10^6$  spores/ml) of *A. solani*.

### 2.5. Isolation of bioagents from talc formulations

Serial dilutions and spread plate techniques were adopted on the talc formulations to obtain the pure cultures of the bioagents. For *T. viride*, PDA was used as medium and incubated at  $25^{\circ}\text{C}$  for 3 days while *Pseudomonas fluorescens* and *Bacillus thuringiensis* KB and NA were used respectively at  $37^{\circ}\text{C}$  for 48 hours.

### 2.6. Dual culture technique

Dual culture method was applied to evaluate the inhibition (%) of the pathogen by the bioagents *in vitro*. A mycelia plug from an actively growing *A. solani* on PDA was taken with a cork borer (7mm diameter) and kept at 1cm from one side of the agar medium contained in 90mm diameter petri dish. Another mycelia disc of *T. viride* was kept at the other side. The bacterial isolates were streaked on the other side of the pathogen. In consortial treatments, however, both mycelial plug and bacterial streaks were used. The experimental design was complete randomized design (CRD) with three replications. PDA medium was used in the experiments in order to favor the growth of *A. solani* and the potential antagonists.

## 2.7. Assessment of disease symptoms and potato growth promotion in the field

Plants were observed weekly for the symptoms of early blight, and plant growth promoting activity. The disease incidence was recorded six days after inoculation and rated by estimating the affected percentage leaf area of all leaves [15]. Percentage of disease severity incidence was calculated using the formula Amin [16].

$$DI (\%) = \frac{\text{Sum of Individual numerical rating}}{\text{Total No. of assessed} \times \text{Max. score in scale}} \times 100$$

Also, disease severity was estimated by scoring individual plants on a 0-5 visual scale described by Latunde-Dada [17] of increasing severity: 1-no symptom, 2-moderate lesion development on the leaf, 3-heavy lesion development on and below the leaf, 4-severe lesion development on all parts of the leaf, 5-plant dead. Yield was also recorded in respect to challenged control. The experiment was designed in Randomized Block Design (RBD). Data were analyzed statistically using analysis of variance according to SAS procedure for a completely randomized design. The differences of means were identified by Duncan's Multiple Range Test (DMRT).

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of bioagents on early blight incidence under field conditions

The result of this study shows that all the three bioagents have significant benefit in suppressing the early blight incidence in comparison to control (74.48%). However, the consortium of *T. viride*+*P. fluorescens* (31.67%) and that of *T. viride* + *B. thuringiensis* (30.76%) recorded the least disease incidence and disease rating. Our results are in agreement with Kumar [18] who reported that combination of *Trichoderma* spp. and *Pseudomonas* spp. is a potential biocontrol mean for management of various soil-borne phytopathogens.

### 3.2 In vitro evaluation of antagonistic potential of bioagents

Following the dual culture technique between the *A. solani* and the biological control agents, *in vitro* analysis revealed that the six treatments, except control, inhibited the growth of *A. solani* significantly. The results obtained (Table i) showed that *T. viride*+*P. fluorescens* inhibited the growth of *A. solani* most by 97.48% with only 2.00 mm growth in diameter of the pathogen. This is followed by *T. viride* + *B. thuringiensis* with inhibition of 92.43% and 6.00 mm diameter of pathogen growth, and then *P. fluorescens* + *B. thuringiensis* with 79.58% inhibition and pathogen diameter of 17.00mm.

**Table i: Effect of bioagents and their consortium on disease severity, inhibition of *A. solani* and yield of potato.**

Treatment	Disease Incidence (%)	Disease Rating	Inhibition (%)	Yield (t/ha)
<i>Trichoderma viride</i>	38.92 <sup>b</sup>	2.17 <sup>b</sup>	74.77 <sup>d</sup>	2.19 <sup>c</sup>
<i>Pseudomonas fluorescens</i>	40.90 <sup>b</sup>	2.27 <sup>b</sup>	68.51 <sup>c</sup>	2.11 <sup>c</sup>
<i>Bacillus thuringiensis</i>	54.05 <sup>c</sup>	2.60 <sup>b</sup>	58.40 <sup>f</sup>	1.56 <sup>d</sup>
<i>T. viride</i> + <i>P. fluorescens</i>	31.67 <sup>a</sup>	1.63 <sup>a</sup>	97.48 <sup>a</sup>	2.39 <sup>b</sup>
<i>P. fluorescens</i> + <i>B. thuringiensis</i>	51.48 <sup>c</sup>	2.13 <sup>b</sup>	79.58 <sup>c</sup>	2.13 <sup>c</sup>
<i>T. viride</i> + <i>B. thuringiensis</i>	30.76 <sup>a</sup>	1.67 <sup>a</sup>	92.43 <sup>b</sup>	2.78 <sup>a</sup>
Control	74.48 <sup>d</sup>	3.63 <sup>c</sup>	---	1.30 <sup>e</sup>

Means in the same column followed by different letters indicate highly significances according to DMRT (P< 0.05).

**Table ii: Effect of bioagents and their consortium on growth attributes of potato at 30 days after germination.**

Treatment	Shoot length (cm)	Root Length (cm)	No. of Branches/ Plant	No. of Leaves/ Plant
<i>Trichoderma viride</i>	19.83 <sup>b</sup>	7.33 <sup>a</sup>	10.00 <sup>a</sup>	99.33 <sup>c</sup>
<i>Pseudomonas fluorescens</i>	18.43 <sup>b</sup>	6.97 <sup>a</sup>	9.67 <sup>a</sup>	93.33 <sup>c</sup>
<i>Bacillus thuringiensis</i>	23.80 <sup>a</sup>	6.70 <sup>a</sup>	10.33 <sup>a</sup>	99.33 <sup>c</sup>
<i>T. viride</i> + <i>P.fluorescens</i>	25.50 <sup>a</sup>	7.10 <sup>a</sup>	9.33 <sup>a</sup>	122.67 <sup>a</sup>
<i>P. fluorescens</i> + <i>B. thuringiensis</i>	16.97 <sup>c</sup>	6.93 <sup>a</sup>	9.00 <sup>a</sup>	77.67 <sup>d</sup>
<i>T. viride</i> + <i>B. thuringiensis</i>	24.37 <sup>a</sup>	7.07 <sup>a</sup>	9.00 <sup>a</sup>	105.00 <sup>b</sup>
Control	15.77 <sup>c</sup>	7.30 <sup>a</sup>	8.93 <sup>a</sup>	68.00 <sup>e</sup>

Means in the same column followed by different letters indicate highly significances according to DMRT (P< 0.05).

**Table iii: Effect of bioagents and their consortium on growth attributes of potato at 30 days after germination.**

Treatment	Fresh Shoot Weight(g)	Fresh Root Weight (g)	Dry Shoot Weight (g)	Dry Root Weight(g)
<i>Trichoderma viride</i>	9.20 <sup>b</sup>	1.70 <sup>c</sup>	1.33 <sup>c</sup>	0.71 <sup>a</sup>
<i>Pseudomonas fluorescens</i>	7.33 <sup>c</sup>	1.83 <sup>c</sup>	1.23 <sup>c</sup>	0.20 <sup>b</sup>
<i>Bacillus thuringiensis</i>	8.67 <sup>b</sup>	1.90 <sup>c</sup>	1.73 <sup>b</sup>	0.71 <sup>a</sup>
<i>T. viride</i> + <i>P.fluorescens</i>	14.97 <sup>a</sup>	3.50 <sup>a</sup>	2.20 <sup>a</sup>	0.83 <sup>a</sup>
<i>P. fluorescens</i> + <i>B. thuringiensis</i>	8.10 <sup>b</sup>	1.97 <sup>c</sup>	1.20 <sup>c</sup>	0.17 <sup>b</sup>
<i>T. viride</i> + <i>B. thuringiensis</i>	8.97 <sup>b</sup>	2.63 <sup>b</sup>	1.60 <sup>b</sup>	0.31 <sup>b</sup>
Control	5.67 <sup>d</sup>	1.07 <sup>d</sup>	1.00 <sup>c</sup>	0.10 <sup>b</sup>

Means in the same column followed by different letters indicate highly significances according to DMRT (P< 0.05).

Based on this *in vitro* study, the mixture between the three bioagents gave higher inhibition (%) of *A. solani* in comparison with single bioagents. This is in agreement with the work of Kumar<sup>[19]</sup> who reported highest zone of inhibition in consortium between *T. harzianum*(OTPB3) and *Bacillus subtilis*(OTPB1) over single treatments and control. Similar results were reported on mixture of *Trichoderma* spp. and *Pseudomonas* spp. by Singh<sup>[20]</sup>. Sarkar<sup>[21]</sup> reported the same trend between *Pseudomonas aeruginosa* (KUCd1) and *Bacillus subtilis*.

### 3.3. Effects of bioagents on plant growth promotion of potato

Although *Bacillus subtilis*(23.80cm) performed significantly the same with *T. viride*+*P.fluorescens* (25.50cm) and *T. viride* + *B. thuringiensis*(24.37cm), the consortial treatments gave higher shoot lengths than other singles and control treatments(Table ii). These findings are in agreement with Srivastava<sup>[22]</sup> as they also demonstrated that by the use of consortium of *T. viride*+*P.fluorescens* in the management of Fusarium wilt in tomato highest shoot length was obtained. Similar results were obtained by Yobo<sup>[23]</sup> who demonstrated that *Trichoderma* and *Bacillus* combinations were better than the *Trichoderma* isolated and *Bacillus* isolates used alone. They reported that there was potential in using mixtures of *Trichoderma* and *Bacillus* for improving plant growth. But in terms of root length and number of branches per plant, the results signified absence of significant difference among the treatments. Nevertheless, the results on table ii indicated highest number of leaves in *T. viride*+*P.fluorescens* (122.67), so also fresh and dry weights of leaves, fresh and dry weights of stems (Tableiii). These tallied with Schneider and Ullrich<sup>[24]</sup>, Raupach and Kloepper,<sup>[25]</sup> who reported that tomato seeds coated with fresh suspensions of microbial mixture

containing *T. harzianum* and *B. subtilis* resulted in significant increase in number of leaves in comparison with *P. putida* and untreated control. Srivastava<sup>[20]</sup> demonstrated that combination of *Pseudomonas* spp. and *Trichoderma* spp. displayed a vital role in increasing tomato biomass compare to solo treatments and control.

### 3.4. Effect of bioagents and their consortia on potato yield at harvest

The maximum yield is reported in *T. viride* + *B. thuringiensis* (2.78ton<sup>-1</sup>), followed by *T. viride*+*P.fluorescens* (2.39 ton<sup>-1</sup>). All the remaining treatments performed better than the control which recorded only (1.30ton<sup>-1</sup>). The present results are in harmony with the work of Radjcommare<sup>[26]</sup> who reported that seeds and seedlings treatment with consortium of these bioagents(*Trichoderma* spp. & *Pseudomonas* spp.) results in yield promotion in contrast to application of individual bioagents. Alagawadi and Gaur<sup>[27]</sup> reported similar result on mixture of *Trichoderma* spp. and *Bacillus* spp. Ganeshmoorthi<sup>[28]</sup> and Latha<sup>[29]</sup> all illustrated that combinations of biological control agents and plant growth promoting rhizobacteria (PGPR) and their combined effects are pronounced in improving crop yields and enhancing nutrient uptake by plants.

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**AUTHORS' PROFILE**


**S.A. Suleiman**, born at Bichi Local Government of Kano State, Federal Republic of Nigeria, on May 3, 1985. Attended Hagagawa Primary School (1991-1996). Went to Senior Secondary School Bichi (1999-2005). Studied Bsc. Agriculture (Crop Science) at Kano University of Science and Technology, Nigeria in 2011. Moved to Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh State of India and obtained M.sc in Plant Pathology in 2016. He worked as an Assistant Lecturer at Audu Bako College of Agriculture Kano, Nigeria in 2014. Currently, he is an ASSISTANT LECTURER at the Department of Crop Production, Federal University Dutse, Nigeria.



**S. Simon** was born on January 1, 1960. Acquired her first B.sc degree in Agriculture from Allahabad University, India in 1981. She obtained M.sc degree in Plant Pathology from Allahabad University in 1984. From the same university, she completed her P.hd in 1990. Her area of specialization is "Endoparasitic Nematodes Associated Soil borne Fungal Diseases and their Management". She is currently the HEAD of the department of plant pathology and entomology of Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad. She works as a teacher and researcher and guided 78 M.sc and 17 P.hd students. She has 81 national and 50 international published journals and participated in 20 national and international conferences. Professor, (Dr.) Mrs. Simon has 3 books published, 10 projects carried out, and 131 research publications. She has 5 years industrial and 18 years teaching experiences.



**M. Babychan** was born in Erumad, Panthalur, Tamil Nadu India. He attended Shimhas High school in Erumad. He went to Adhiparasakti Agriculture and Horticulture College in Tamil Nadu where he obtained his B.sc degree in Agriculture in the year 2013. He obtained his M.sc degree in Plant Pathology in 2016 from Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad.