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Potentiality of Formulated Bioagents from Lab to Field: A Sustainable Alternative for Minimizing the Use of Chemical Fungicide in Controlling Potato Late Blight

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Abstract: Late blight of potato caused by an oomycete, *Phytophthora infestans* (Mont.) De Bary limits the production of potato worldwide. Late blight management has been based on chemical fungicide application, and the repeated use of these fungicides introduces new and more aggressive genotypes, which can rapidly overcome host resistance. Therefore, innovative and effective control measures are needed if fungicide use is to be reduced or eliminated. Some potential formulated bacterial bioagents viz. *Pseudomonas putida* (BDISO64RanP) and *Bacillus subtilis* (BDISO36ThaR), and fungal bioagents viz. *Trichoderma paraviridicens* (BDISOF67R) and *T. erinaceum* (BDISOF91R), were evaluated for their performance in controlling late blight of potato under growth chamber and field conditions. Both artificial inoculation and field experiments revealed that eight sprays of these bacterial (*P. putida* and *B. subtilis*) and fungal (*T. erinaceum*) bioagents were found to be most effective at reducing late blight severity by 99% up until 60 days after planting (DAP), whereas these bioagents were found to be partially effective until 70 DAP, reducing late blight severity by 46 to 60% and 58 to 60% in the field and growth chamber conditions, respectively. However, these bioagents can reduce the spray frequencies of Curzate M8 by 50% (four sprays instead of eight) when applied together with this fungicide. Economic analysis revealed that T_6 (eight sprays of formulated *P. putida* + *B. subtilis* + four sprays of Curzate M8) and T_{16} (eight sprays of formulated *P. putida*, *B. subtilis*, and *T. erinaceum* + four sprays of Curzate M8) performed better in consecutive two years, applying less fungicidal spray compared to T_1 (eight sprays of Curzate M8 (Positive control)), which indicated that the return ranged, by Bangladeshi Currency (Taka), from 0.85 to 0.90 over the investment of Bangladeshi Currency (Taka) 1.00 in these treatments, and these results together highlight the possibility of using bioagents in reducing late blight of potato under a proper warning system to reduce the application frequency of chemical fungicide.

Keywords: bioagents; chemical fungicide; complementary approach; late blight management; potato

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1. Introduction

Late blight caused by *P. infestans* (Mont.) De Bary restricts the yield of potato notably in cool temperature regions globally. *P. infestans* (Mont.) De Bary is an oomycete that is well recognized for its explosive expansion when environmental circumstances are adequate and host plants are vulnerable to infection [1]. In 2019, Bangladesh produced 9.7 million tonnes of potato on 0.5 million ha, which represents 2.6% of the world production [2]. The yield of potatoes in Bangladesh in 2019 as calculated by the FAO [2] was 20.6 t/ha, which was

lower compared to the potential yield and to the yield of other potato growing countries of the world. Recently, Bangladesh exported 45,000 tonnes of fresh potato in the world market in 2019–2020, as the production exceeded the demand [3]. The annual consumption of potato per capita also increased and reached 25.66 kg in 2016 from 23.65 kg in 2010, bringing the growth rate to 8.5% during only a six-year period [4]. The estimated losses in the world's economy vary from 3 to 5 billion dollars annually due to the investment cost for the production of potatoes destroyed by late blight [5,6]. In Bangladesh, the late blight disease of potatoes has caused a big drop in yields, which has been estimated to be between 25% and 57% [7]. The genome structure of *P. infestans* allows itself to adapt by fostering genetic diversity [8,9].

As potato late blight may quickly cause large economic losses, potato growers must apply synthetic fungicides to plant surfaces almost weekly before sporangia appear [10]. However, the heavy use of synthetic pesticides causes serious concerns for human health and also affects the environment, as well as favoring the development of fungicide resistant *P. infestans* genotypes [11,12], due to the fast development in the number of physiological races that can overcome a set of resistance genes (R1–R11) [13]. At the same time, two counter-balancing factors have also developed: societal pressure for reducing pesticide use on crops and acreage of organically-grown food crops—potato and tomato included [14–16]. For many years, copper-based fungicides (e.g., Bordeaux combination, fixed-copper hydroxide, copper oxide, and copper oxychloride) have been used to suppress late blight in organic potato and tomato cultivation. Organic fields in Brazil [15], USA [17] and Japan (Maff Notification no. 59, 2000) may employ these chemicals (www.maff.go.jp/soshiki/syokuhin/hinshitu/organic/engyukihow.pd accessed on 22 January 2022). Currently, in the European Union, only 6 kg of elemental copper per ha per year is allowed in organic production [16]. As soon as reliable alternatives to manage late blight are available, a complete ban of copper compounds should take place [18]. On the other hand, the misuse of pesticides has resulted in a severe danger to food safety and to the natural environment [19]. Fungicides have been widely used to treat late blight and for the emergence of novel pathogen genotypes [20]. Randall et al. [21] reported field isolates insensitive to phenylamide-based chemicals, including metalaxyl and insensitive strains exhibiting cross-resistance to multiple phenylamide compounds. In the meanwhile, in 2020, EU countries decided to ban mancozeb, the last cheap contact fungicide of the dithiocarbamates family, because of its reproductive toxicity and endocrine disruptive action (regulation (EU) 2020/2087). It was largely used in potato late blight control, and is one of the two or three most common pesticides in use worldwide, with a history of 60 years since its introduction in 1962.

Several biopesticides and biofungicides products have already been registered for the treatment of late blight or have been pending registrations [22,23]. However, these products have elicited mixed results and, as of yet, have not demonstrated sufficient and consistent levels of late blight suppression in order to significantly curb the heavy use of synthetic and copper-based fungicides [22]. A tremendous increase in the application of pesticides, especially fungicides, has led to a number of health problems, including reproductive problems [24,25], genetic damage [26], neurological disorders [27], increases in bladder cancer [28], and even breast cancer [29], because farmers are directly and indirectly exposed to pesticides. So, to minimize or eliminate fungicide usage, such as in organic potato cultivation, creative and effective control strategies are required. To safeguard potato crops from the most dangerous foliar disease, researchers are searching for non-chemical alternatives.

Several genera of microorganisms show an anti-oomycete activity, such as *Bacillus* [30,31], *Penicillium* [32], *Pseudomonas* [33,34], *Fusarium* [35,36], and *Trichoderma* [37–40]. The use of bacteria as bio-control agents for the treatment of potato late blight has recently gained popularity in recent years, with numerous research finding promising outcomes [41–46]. Among the bacterial antagonists, many belong to the genus *Bacillus* and there are several other major genera of smaller practical value than *Bacillus* [47]. En-

dosporic and enzymatic components of *B. subtulis* have been found to be very potent against numerous fungal infections. Potato associated cyanogenic *Pseudomonas* spp. displays a volatile-mediated high potential against *P. infestans* [48,49]. In addition to supplying biofungicides as effective alternatives to synthetic fungicides, bacteria have an enormous potential for agricultural advantages such as secreting plant growth regulating hormones, fixing atmospheric nitrogen, and enhancing phosphorus nutrition [34]. Unlike synthetic fungicides, numerous microorganisms may also have the capacity to increase their hostile activity against plant pathogens over time by effectively colonizing plant surfaces [50]. Plant growth reduction is caused by drought stress [51], heavy metals [52], weed infestation [53], salt stress [54], and several adverse environmental states. PGPM may alter plant performance directly by producing chemicals that enhance plant growth, boost nutrient availability, and absorption under biotic stress, and trigger plant defense responses, or indirectly by suppressing plant infections [55]. Surprisingly, biocontrol agents (BCAs), including microorganisms and their secondary metabolites, were shown to be promising as efficient and environmentally friendly alternatives to chemicals [19,56]. Because disease symptoms occur early in the growth stage, chemical control programs should use prediction models and eco-friendly plant protection methods to minimize the fungicide dose and lengthen the treatment intervals [57].

Two new native fungal isolates identified from the rice rhizosphere and bacterial isolates identified from potato phylloplane and rhizosphere have been used in this study. We assessed their efficacy for controlling the late blight of potato, but *P. infestans* is a polycyclic pathogen that can hardly be completely controlled with bioagents only. The effects of fungicide application have numerous hazards to mankind and the environment, and apart from that, many fungicides are banned in developed countries due to their toxic effects to human beings and animals. Thus, in this study, we focused on the use of both fungicides and native formulated bioagents, considered as a novel approach in reducing the application frequency of chemical fungicide, to minimize the impact of late blight severity on potato yield.

2. Materials and Methods

2.1. Culture and Growth Condition for Bacterial and Fungal Bioagents

The cultures of bacterial bioagents were maintained in Luria−Bartani (LB) medium [58] and fungal bioagents were maintained in potato dextrose agar (PDA) medium. Two bacterial isolates viz. *P. putida* (BDISO64RanP) and *B. subtilis* (BDISO36ThaR) were isolated from potato phylloplane and rhizosphere identified previously by sequencing 16SrDNA [59] and were grown on the LB agar medium during the experimental period. Two fungal strains viz. *T. paraviridicens* (BDISOF67R) and *T. erinaceum* (BDISOF91R) (Islam et al., unpublished data) isolated from the rice rhizosphere were identified with ITS primer and were cultured on the PDA medium.

2.2. In Vitro Antagonist Test in the Laboratory

In order to test the efficacy of different bioagents against *P. infestans* in vitro, the growth inhibition of *P. infestans* by different bio-agents was compared with the controls (positive and negative) (Figure 1). For the bacteria, the bioagents were sub cultured for one week after being removed from −80 °C and then, overnight, the culture of *B. subtilis*/*P. putida* was inoculated in a triangle on pea agar plates. Then, 5 mm disc of *P. infestans* (9 days old) were placed at the center of the triangularly inoculated bacterial plates. In the control plates, only a 5 mm disc of *P. infestans* (9 days old) was inoculated. The radial growth inhibition of *P. infestans* was assessed at two to three weeks after inoculation by measuring the radial growth of *P. infestans* in the dual and control plates. The percent radial mycelial growth inhibition was calculated as follows:

$$\% \text{ Radial growth inhibition } = \frac{(R1 - R2) \times 100}{R1} \quad (1)$$

where R1 = radial growth of *P. infestans* in the control plates and R2 = radial growth of *P. infestans* in the dual culture plates.

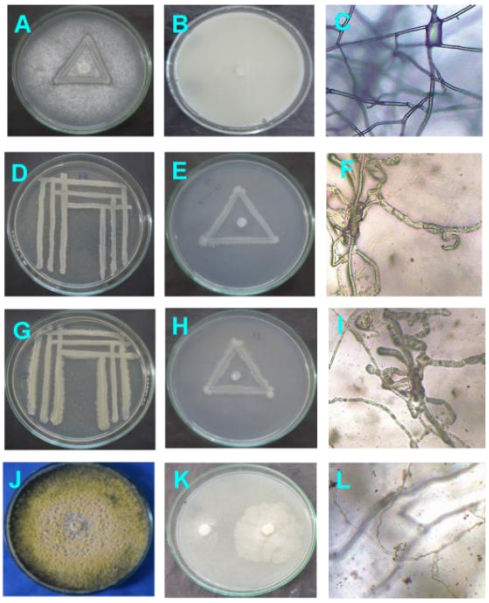

Figure 1. Growth inhibition of *P. infestans* by bioagents with the control. (**A**) Negative control, (**B**) positive control (Curzate M8), (**C**) mycelia of *P. infestans*, (**D**) pure culture of *P. putida*, (**E**) growth inhibition of *P. infestans* by *P. putida*, (**F**) deformation of mycelial structure of *P. infestans* by *P. putida*under stereo binocular microscope, (**G**) pure culture of *B. subtilis*, (**H**) growth inhibition of *P. infestans* by *B. subtulis*, (**I**) deformation of mycelial structure of *P. infestans* by *Bacillus subtilis* under stereo binocular microscope, (**J**) pure culture of *T. erinacium*, (**K**) growth inhibition of *P. infestans* by *T. erinacium*, and (**L**) deformation of mycelial structure of *P. infestans* by *T. erinacium* under stereo binocular microscope.

For fungal bioagents, a dual culture method was used to analyze whether *T. paraviridescens*/*T. erinaceum* inhibits the growth of *P. infestans* [60]. *Trichoderma* isolates were maintained in PDA strains at 4–8 °C for a short period of time. Briefly, a 5 mm diameter mycelial plug of *P. infestans* (9 days old) was placed on one side of a petri dish (9 cm diameter) containing pea agar and was pre-incubated at 18 °C for 2 days to initiate growth. Later, a 5 mm diameter disc of *T. paraviridescens*/*T. erinaceum* (7 days old) was placed 6 cm away from the pathogen on the dual plates, whereas a sterile PDA disc was placed in the control plates. The assay was done twice with five replications and the radial growth of the pathogen was measured 4 days after incubation at 18 °C. The percent radial mycelial growth inhibition (I) was calculated as follows [59]:

$$I = [(C - T)/C] \times 100 \quad (2)$$

where C is the radial growth measurement of the pathogen in the control plates and T is radial growth of the pathogen in the dual plates.

2.3. Experimental Location and Design

The efficacy of some selected formulated bio-agents was evaluated in both the plant growth chamber (18 °C and RH 90%) and field conditions. Plant growth chamber experiments were conducted at the Professor Golam Ali Fakir Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh. The growth chamber was equipped with an air cooler and sprinkling watering system, and sensors to maintain temperature (18–20 °C and adjust humidity (85–90%) two to three times in a day. Field experiments were conducted in the same farmer's field, Sutia Khali, Mymensingh Sadar, Mymensingh, from 2018–2021. Pot experiments were conducted in a plant growth chamber with completely randomized design (CRD) and field experiments were with randomized complete block design (RCBD)

by maintaining three replications. The plot size for field experiments was 3 × 2 m^2. The row to row distance was 60 cm, while the plant to plant distance was 20 cm.

2.4. Treatment Design and Combination

We assessed the efficacy of two bacterial (viz; *P. putida* and *B. subtilis)* and two fungal (*T. paraviridescens* and *T. erinaceum*) bioagents compared to the chemical fungicide (Curzate M8) in a different combination. Treatment combinations were T_0 (water (negative control), T_1 (foliar spray of formulation of *T. paraviridescens*), T_2 (foliar spray of formulation of *T. erinaceum*), T_3 (foliar spray of formulation of *P. putida*), T_4 (foliar spray of formulation of *B. subtilis)*, T_5 (foliar spray of formulation of *T. paraviridescens* and *P. putida*), T_6 (foliar spray of formulation of *T. erinaceum* and *P. putida)*, T_7 (foliar spray of formulation of *T. paraviridescens* and *B. subtilis*), T_8 (foliar spray of formulation of *T. erinaceum* and *B. subtilis*), T_9 (foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida,* and *B. subtilis*), T_{10} (foliar spray of Curzate M8 (Cymoxanil + Mancozeb), and T_{11} (foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida,* and *B. subtilis* with T_{10}).

According to the results of the previous experiments on the efficacy of the two formulated bacterial and two fungal bioagents in reducing late blight severity of potato under growth chamber conditions and field conditions during 2018–2019, we selected two bacterial (viz; *P. putida* and *B. subtilis)* and one fungal (viz. *T. erinaceum*) bioagents that were found to be effective for the total growth inhibition of late blight pathogen. The next step was to compare treatments (A) exclusively based on the current number of sprays of chemical fungicide; (B) based on the same number of sprays, but applying single or mixed bioagents; and (C) the same as (B), but reinforced by one to four additional sprays with chemical fungicide. Thus, the efficacy of these bioagents in reducing the application frequency of chemical fungicides for controlling late blight of potato was evaluated in the following treatments: T_0 = water (negative control), T_1 = eight sprays of Curzate M8 (positive control), T_2 = eight sprays of formulated *P. putida* + *B. subtilis*, T_3 = T_2 + one spray of Curzate M8, T_4 = T_2 + two sprays of Curzate M8, T_5 = T_2 + three sprays of Curzate M8, T_6 = T_2 + four sprays of Curzate M8, T_7 = Eight sprays of formulated *T. erinaceum*, T_8 = T_7 + one spray of Curzate M8, T_9 = T_7 + two sprays of Curzate M8, T_{10} = T_7 + three sprays of Curzate M8, T_{11} = T_7 + four sprays of Curzate M8, T_{12} = Eight sprays of formulated *P. putida*, *B. subtilis* and *T. erinaceum*, T_{13} = T_{12} + one spray of Curzate M8, T_{14} = T_{12} + two sprays of Curzate M8, T_{15} = T_{12} + three sprays of Curzate M8, and T_{16} = T_{12} + four sprays of Curzate M8.

2.5. Growing Potato for Field Experiments

Land was fertilized with cow dung (7.5 t/ha), DAP (260 kg/ha), MOP (260 kg/ha), Gypsum (120 kg/ha), zinc (7.5 kg/ha), boron (7.5 kg/ha), magnesium (45 kg/ha), furadan (7.5 kg/ha), and urea (120 kg/ha) just before the final land preparation. Apparently disease free and uniform tubers of a popular potato cultivar (Diamant, a variety showing susceptibility under severe outbreak) were cut into pieces with at least one bud and were left for 24 h for suberization. Then, the suberized tuber pieces were treated by drenching with the formulated bioagents (0.4% *w/v*) and the treated tubers were left for at least 1 h for adherence. Treated and non-treated tuber pieces were planted in the pots filled with prepared soils, which were then kept in the net house until two days before the inoculation. For field experiments, the treated and non-treated tuber pieces were planted in respective experimental plots. Two top dressings of urea (120 kg/ha) were applied at 33 and 60 DAP along with two irrigations at 27 and 60 DAP. Weeding was performed at 25 DAP followed by earthen up at 33 and 43 DAP.

2.6. Talc-Based Formulation of Selected Bacterial and Fungal Bioagents

First, 500 g talc powder, 5 g CMC (Carboxy methyl cellulose), and 7.5 g $CaCO_3$ were mixed at 121 °C for 30 min. To formulate the bioagents, the bacteria were cultured for 24 h on LB media. The bacteria were then cultured in LB broth for 6 h. They were then centrifuged and resuspended in 200 mL peptone broth with bactopeptone. This broth

culture was shaken for 2 h more. Then, 5 mL of sterile 100% glycerol was added in a 200 mL culture. These cultures (5×10^8 CFU/mL) were added to 500 g powdered talc in the tray. The formulations were then air dried overnight in a laminar flow hood and later the formulations were powdered with hand wearing gloves and mask. The formulated bacterial antagonists were packed in plastic bags. For fungal bioagents, a mycelial disc (5 mm diameter) for each isolate was inoculated in 100 mL PDB broth. Conidia production was counted after 7 days and the mycelial mat along with conidia from PDB was mixed thoroughly with previously autoclaved talcum powder pretreated with 0.5% CMC (5 g CMC dissolved in 100 mL water mixed with 1 kg talcum powder). The mixture was then air-dried in a laminar flow hood and was kept in plastic bags, accordingly.

2.7. Artificial Inoculation of P. infestans

Inoculum was prepared from Petri plate cultures of the *P. infestans* isolates on pea agar with β-sitosterol (50 mg/L) grown until the maximum vegetative growth stage; on the day before inoculation, the mycelia were smashed with a sterile test tube and the plates were left at 18 °C in an incubator (VELP SCIENTIFICA) overnight for the production of sporangia. The sporangia were harvested by washing them off the plates with Sato's solution [61] and the concentration was determined by counting with a hemocytometer and was adjusted to 10^4 sporangia/mL of Sato's solution. The viability of the formulated bioagents was more than four months.

2.8. Application of Formulated Bacterial and Fungal Bioagents

In case of net house experiments in the growth chamber, formulated biagents and Curzate M8 were sprayed four times on plants before inoculation at 34, 41, 48, and 53 DAP and 2, 4, 7, and 9 days after inoculation, i.e., 57, 59, 62, and 64 DAP, whereas the inoculation was done at 55 DAP. In the case of field experiments, the chemical fungicide (s) and formulated bioagents were sprayed at 34, 41, 48, 53, 57, 62, 69, and 75 DAP over the potato plant surface when applied alone. However, in case of combined application with chemical fungicides, one chemical spray at 53 DAP; two chemical sprays at 53 and 57 DAP; three chemical sprays at 53, 57, and 62 DAP; and finally, four chemical sprays at 48, 53, 57, and 62 DAP were applied together. The formulated bacterial and fungal bioagents were sprayed (0.4% *w/v*) two days after fungicide application to avoid the interactions effects with chemical fungicide. The application concentration of each bioagent was reduced to half in case of the combined application of two bioagents, and one third when three bioagents were applied together.

2.9. Assessment of Late Blight Incidence and Severity

Ten potato plants were randomly selected and tagged for data collection. Late blight incidence and severity were recorded for Net house experiments at 61 and 65 DAP for field experiments at 48, 59, and 71 DAP, following the formula and the scales mentioned bellow. Parameters for field experiments were (i) plant height at 34, 52, and 71 DAP; (ii) number of plants per hill at the time of harvest; (iii) number of tubers per plant; and (iv) yield.

$$\text{Late blight incidence } (\%) = \frac{\text{Number of late blight infected plants}}{\text{Total number of plants examined}} \times 100 \quad (3)$$

The late blight severity scale followed was by James [62]. Briefly, 1 = 0% blight (no disease observed), 2 = 0.1% blight (a few scattered plants blighted; no more than 1 or 2 spots in 12-yard radius), 3 = 1% blight (up to 10 spots per plant; or general light infection), 4 = 5% blight (about 50 spots per plant; up to 1 in 10 leaflets infected), 5 = 25% blight (nearly every leaflet infected, but plants retain normal form; plants may smell of blight; field looks green although every plant is affected), 6 = 50% blight (every plant affected and about 50% of leaf area destroyed), 7 = 75% blight (about 75% of leaf area destroyed; field appears neither predominantly brown or green), 8 = 95% blight (only a few leaves on plants, but stems green), and 9 = 100% blight (all leaves dead, stems dead or dying).

2.10. Economic Analyses of Formulated Bioagents

The benefit−cost ratio (BCR) was calculated for each treatment according to the method of Mondal et al. [63]. The cost−benefit analysis compared the profitability of each treatment based on the gross returns and costs. Each treatment's gross and net returns were computed as follows. Gross return (TK/ha) = tuber Yield (kg/ha) × price (TK/kg); net return (TK/ha) = gross return (TK/ha) − cost of production plus treatment cost (TK/ha); the BCR was calculated as shown below:

$$\text{BCR} = \frac{\text{A} \times \text{C} - \text{B}}{\text{B}} \quad (4)$$

where A = selling price (Tk./kg), B = cost of cultivation + treatment cost (Tk./ha), and C = yield (kg/ha).

2.11. Statistical Analysis

Data were analyzed using the MStatC statistical program. Means were compared using Duncan's multiple range test (DMRT).

Experimental procedures are presented in Chart 1.

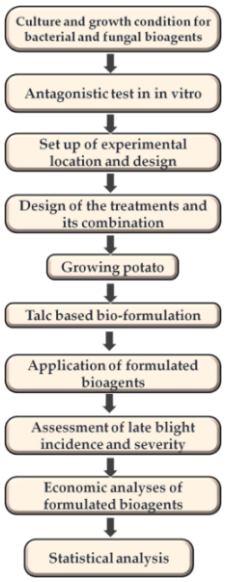

Chart 1. A flow chart depicting the entire experimental procedures.

3. Results

3.1. Development of an Eco-Friendly Sustainable Management Alternative against Late Blight of Potato Using Potential Formulated Bio-Agents under Field Conditions

The present study was designed to develop an eco-friendly sustainable management alternative against potato late blight using some potential formulated bio-agents under both growth chamber and field condition. Experiments were conducted in both a net house with artificial inoculation and in the field with natural infection conditions to compare the efficacy of the selected formulated bacterial and fungal bio-agents for controlling late blight of potato. Before using those with chemical fungicide (Curzate M8), the interactions effect of different bacterial and fungal bioagents were studied. The results showed that no interactions effect was observed among the bioagents. However, the growth of both bacterial bioagents (*P. putida* and *B. subtilis*) and fungal bioagent (*T. paraviridescens* and *T. erinaceum*) were slightly delayed due to CurzateM8 (Supplementary Figure S1). Thus, the bioagents were applied after two days of Curzate M8 application.

3.2. In Vitro Growth Inhibition and Morphological Changes of P. infestans by Bacterial and Fungal Bioagents

The in vitro antagonistic assay of *B. subtilis* and *P. putida* with *P. infestans* revealed that the growth of *P. infestans* was inhibited by 93.99% over the control (Figure 2). On the other hand, *T. paraviridicens* and *T. erinaceum* inhibited the growth of *P. infestans* by 46 and 51.5%, respectively, over the control (Figure 3). Considering the morphological changes, we observed the deformation of mycelial structures when bioagents were applied against *P. infestans* in a duel culture method in the laboratory (Figure 1).

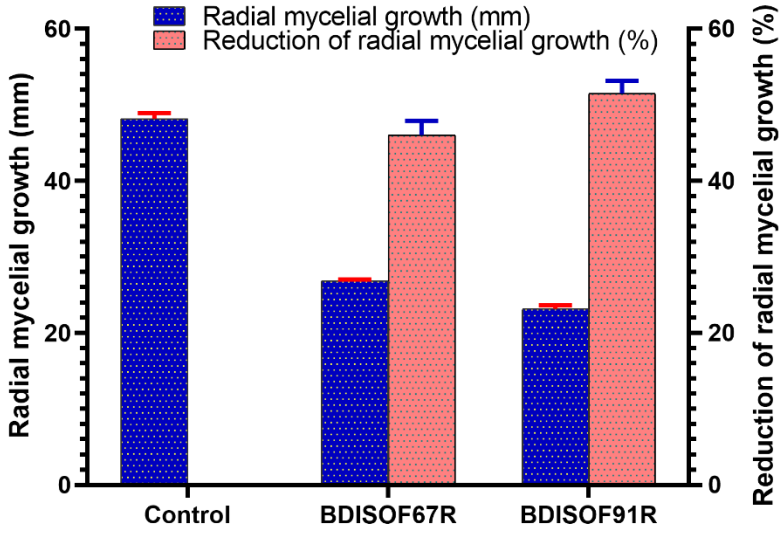

Figure 2. In vitro growth inhibition (mm) and percent reduction of mycelia growth of *P. infestans* by two antagonistic fungal isolates (BDISOF67R and BDISOF91R).

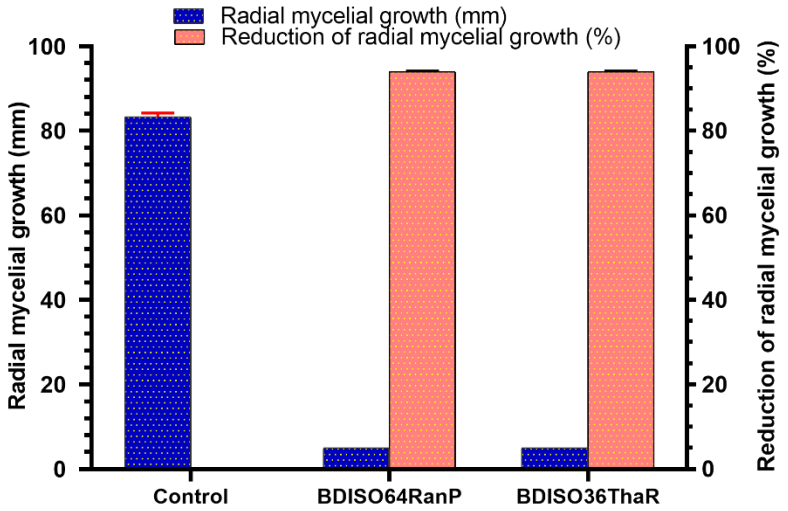

Figure 3. In vitro growth inhibition (mm) and percent reduction of mycelia growth of *P. Infestans* by two antagonistic bacterial isolates (BDISOF64RanP and BDISO36ThaR).

3.3. Efficacy of Formulated Two Bacterial and Two Fungal Bioagents in Reducing Late Blight Severity of Potato under Artificial Inoculation Conditions

The minimum severity (3.67% and 5.00%) was recorded in T_{11} at 61 and 65 DAP, respectively, in 2018–2019 compared to the control and treatments, as T_0 showed maximum severity at both 61 and 65 DAP. However, for T_1 to T_{10}, all exhibited statistically similar data in both 61 and 65 DAP, except T_5 in the 65 DAP. Considering the percent reduction of severity at 65 DAP, T_{11} showed the best (92.69%) result, followed by T_9 (72.44%), T_{10} (71.87%), and T_2 (70.47%) compared to the other treatments (Table 1).

Table 1. Efficacy of formulated two bacterial and two fungal bioagents at reducing late blight severity of potato under growth chamber conditions during 2018–2019.

Treatments	% Severity (0.1–100)		% Reduction of Late blight Severity over Control at 65 DAP
	Days after Planting (DAP)		
	61	65	
T_0	41.67 ± 8.33 ^{a}	73.33 ± 13.02 ^{a}	0.00
T_1	18.33 ± 6.67 ^{ab}	33.33 ± 8.33 ^{bc}	52.34
T_2	11.67 ± 6.67 ^{ab}	18.33 ± 6.67 ^{bc}	70.47
T_3	11.67 ± 6.67 ^{ab}	26.67 ± 13.02 ^{bc}	60.78
T_4	26.67 ± 13.02 ^{ab}	33.33 ± 8.33 ^{bc}	52.34
T_5	33.67 ± 16.33 ^{ab}	41.67 ± 8.33 ^{b}	43.57
T_6	25.33 ± 14.15 ^{ab}	33.33 ± 8.33 ^{bc}	54.68
T_7	18.33 ± 6.67 ^{ab}	33.33 ± 8.33 ^{bc}	46.78
T_8	11.67 ± 6.67 ^{ab}	25.00 ± 0.00 ^{bc}	63.45
T_9	14.67 ± 2.91 ^{ab}	17.83 ± 3.93 ^{bc}	72.44
T_{10}	10.33 ± 7.42 ^{ab}	17.00 ± 8.00 ^{bc}	71.87
T_{11}	3.67 ± 1.33 ^{b}	5.00 ± 0.00 ^{c}	92.69
Level of significance	*	*	-
CV (%)	85.15	49.40	-

Data are the averages of three replications. Values with same letters in the same column are statistically similar. NS = non-significant and * indicates the means were significant at 5% level of probability. T_0 = water (Negative control); T_1 = foliar spray of formulation of *T. paraviridescens*; T_2 = foliar spray of formulation of *T. erinaceum*; T_3 = foliar spray of formulation of *P. putida*; T_4 = foliar spray of formulation of *B. subtilis*; T_5 = foliar spray of formulation of *T. paraviridescens* and *P. putida*; T_6 = foliar spray of formulation of *T. erinaceum* and *P. putida*; T_7 = foliar spray of formulation of *T. paraviridescens* and *B. subtilis*; T_8 = foliar spray of formulation of *T. erinaceum* and *B. subtilis*; T_9 = foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P.putida,* and *B. subtilis*; T_{10} = foliar spray of Curzate M8 (Cymoxonil + Mancozeb); and T_{11} = foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida,* and *B. subtilis* with T_{10}.

3.4. Assessment of Field Potential of Formulated Two Bacterial and Two Fungal Bioagents in Reducing Late Blight Infection and Severity under Field Conditions

The performance of the treatments on the percent of infected plants and late blight severity was recorded at three different time point viz. 48, 59, and 71 DAP in 2018–2019. Maximum (74.36%) and no plant infection were found in T_8 and $T_{11,}$ respectively, while at 59 and 71 DAP, 100% infection was calculated, with almost all treatments possessing statistically identical data except T_9 (95.00), T_{10} (64.96%), and T_{11} (29.91%). Regarding the percentage of late blight severity at 48 DAP, no infected plant was found in T_{10} and T_{11} and maximum severity was recorded in T_5 (2.84%), and the others were calculated as a moderate rate of severity. At 59 DAP, minimal severity was recorded in T_{11} (2.43%), followed by T_{10} (3.37%) showing statistically identical data. These treatments performed better compared to all other treatments. In the case of 71 DAP, the minimum (10.33%) severity was recorded in T_{11} followed by T_{10} (21.30%), which was statistically similar and performed better among all of the other treatments. Considering the percent reduction of late blight severity over the control, the highest reduction was found when applied with T_{11} (89.16%) followed by T_{10} (77.50%), T_9 (27.07%), and T_2 (16.71%) (Table 2).

Table 2. Efficacy of formulated two bacterial and two fungal bioagents in controlling late blight infection and late blight severity of potato under field condition during 2018–2019.

Treatment	% Plant Infection			% Late Blight Severity			% Reduction of Late Blight Severity over Control at 71 DAP
	Days after Planting			Days after Planting			
	48	59	71	48	59	71	
T_0	66.67 ± 5.34 ^{ab}	100 ± 0.0 ^{a}	100	1.50 ± 0.38 ^{abc}	69.33 ± 2.92 ^{a}	95.50 ± 2.08 ^{a}	0.00
T_1	70.09 ± 9.86 ^{ab}	100 ± 0.0 ^{a}	100	2.14 ± 0.67 ^{ab}	50.83 ± 5.92 ^{b}	90.50 ± 1.80 ^{ab}	5.07
T_2	58.12 ± 9.86 ^{ab}	100 ± 0.0 ^{a}	100	1.34 ± 0.38 ^{abc}	47.50 ± 3.69 ^{b}	79.50 ± 3.75 ^{bc}	16.71
T_3	67.52 ± 9.40 ^{ab}	100 ± 0.0 ^{a}	100	1.46 ± 0.68 ^{abc}	59.50 ± 6.43 ^{ab}	91.00 ± 3.21 ^{ab}	4.74
T_4	71.12 ± 7.31 ^{ab}	100 ± 0.0 ^{a}	100	1.60 ± 0.38 ^{ab}	53.50 ± 4.25 ^{a}	85.00 ± 1.04 ^{ab}	10.93
T_5	69.86 ± 7.40 ^{ab}	100 ± 0.0 ^{a}	100	2.84 ± 0.12 ^{a}	60.17 ± 5.83 ^{ab}	83.33 ± 3.18 ^{ab}	12.75
T_6	49.47 ± 3.63 ^{b}	100 ± 0.0 ^{a}	100	1.32 ± 0.31 ^{abc}	46.67 ± 4.34 ^{b}	86.00 ± 1.50 ^{ab}	9.93
T_7	67.52 ± 6.16 ^{ab}	100 ± 0.0 ^{a}	100	2.37 ± 0.87 ^{ab}	56.83 ± 2.92 ^{ab}	93.67 ± 0.44 ^{a}	1.83
T_8	74.36 ± 6.78 ^{a}	100 ± 0.0 ^{a}	100	1.71 ± 0.65 ^{ab}	57.50 ± 8.40 ^{ab}	84.17 ± 8.35 ^{ab}	12.15
T_9	56.00 ± 3.80 ^{ab}	95.00 ± 1.48 ^{a}	100	1.60 ± 0.15 ^{bc}	47.50 ± 0.88 ^{b}	76.17 ± 6.17 ^{c}	27.07
T_{10}	1.71 ± 1.71 ^{c}	64.96 ± 13.27 ^{b}	100	0.00 ± 0.00 ^{c}	3.37 ± 1.07 ^{c}	21.30 ± 6.33 ^{d}	77.50
T_{11}	0.00 ± 0.00 ^{c}	29.91 ± 2.26 ^{c}	100	0.00 ± 0.00 ^{c}	2.43 ± 0.62 ^{c}	10.33 ± 3.53 ^{d}	89.16
Level of significance	*	*	NS	*	*	*	-
CV (%)	21.57	7.53	0.00	56.06	17.08	9.07	-

Data are the averages of three replications. Values with same letters in the same column are statistically similar. NS = non-significant and * indicates the means were significant at 5% level of probability. Data are the averages of three replications. Values with same letters in the same column are statistically similar. T_0 = water (Negative control); T_1 = foliar spray of formulation of *T. paraviridescens*; T_2 = foliar spray of formulation of *T. erinaceum*; T_3 = foliar spray of formulation of *P. putida*; T_4 = foliar spray of formulation of *B. subtilis*; T_5 = foliar spray of formulation of *T. paraviridescens* and *P. putida*; T_6 = foliar spray of formulation of *T. erinaceum* and *P. putida*; T_7 = foliar spray of formulation of *T. paraviridescens* and *B. subtilis*; T_8 = foliar spray of formulation of *T. erinaceum* and *B. subtilis*; T_9 = foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida*, and *B. subtilis*; T_{10} = foliar spray of Curzate M8 (Cymoxanil + Mancozeb); and T_{11} = foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida*, and *B. subtilis* with T_{10}.

3.5. Economic Analysis of Formulated Two Bacterial and Two Fungal Bioagents Used for Reducing Late Blight Infection and Severity under Field Conditions

The benefit−cost ratio (BCR) was calculated based on the data obtained from formulated bacterial and fungal bioagents during 2018–2019 for each of the treatments, and is tabulated in Table 3. The results from the table of the cost−benefit analysis revealed that all treatments provided BCR lower than 1, except T_{10} (0.45) and T_{11} (0.50), which previously recorded significant results in the reduction of severity over the control. The maximum gross return (Tk. 321,760.00/ha) and the net return (106,660.00 Tk./ha) were obtained from the treatment T_{11}. Thus, the highest BCR was calculated from treatment T_{11} (0.50) followed by T_{10} (0.45). The results indicated that a return of Tk. of 0.45 and 0.50 was obtained over the investment of Tk. 1.00 in case of T_{10} (0.45) and T_{11} (0.50) (Table 3), respectively.

3.6. Field Potential of Formulated Two Bacterial and One Fungal Bioagents in Reducing the Application of Chemical Fungicides for Controlling Potato Late Blight under Growth Chamber Conditions

Based on the findings obtained from 2019–2020, the minimum severity (0.40%) was recorded in T_{16} at 61 DAP, followed by T_{15} (0.70%), T_{11} (1.70%), T_{10} (1.73%), T_{14} (2.03%), T_9 (2.03%), and T_1 (2.33%), which performed better than the control. According to 65 DAP, the same treatment with T_{16} showed the lowest severity (10.00%), followed by T_{15} (10.33%), T_{14} (10.33%), T_1 (10.33%), and T_6 (12.67%) exhibiting statistically significant data, while other treatments showed insignificant outcomes including the control. With regards to the percent reduction of late blight severity over the control, at 65 DAP, T_{16} (90.00%) resulted in

the highest reduction, followed by T_{15} (89.67%), T_{14} (89.67%), and T_{11} (85.00%) compared to all of the other treatments applied, including T_1 (88.33%) (Table 4).

Table 3. Cost−benefit analyses of selected two bacterial and two fungal bioagents used for controlling late blight of potato during 2018–2019.

Treatment	Yield (t/ha)	Gross Return (Tk./ha)	Production Cost (Tk./ha)	Total Cost of the Treatment (Tk/ha)	Total Cost with Treatment (Tk/ha)	Net Return (Tk./ha)	BCR
T_0	7.22	115,555.56	192,500	0	192,500	−76,944.444	−0.40
T_1	6.44	103,111.11	192,500	9600	202,100	−98,988.89	−0.49
T_2	6.56	104,888.89	192,500	9600	202,100	−97,211.11	−0.48
T_3	6.28	100,444.44	192,500	9600	202,100	−101,655.56	−0.50
T_4	6.67	106,666.67	192,500	9600	202,100	−95,433.33	−0.47
T_5	7.22	115,555.56	192,500	9600	202,100	−86,544.44	−0.43
T_6	6.33	101,333.33	192,500	9600	202,100	−100,766.67	−0.50
T_7	6.89	110,222.22	192,500	9600	202,100	−91,877.78	−0.45
T_8	6.89	110,222.22	192,500	9600	202,100	−91,877.78	−0.45
T_9	10.06	160,888.89	192,500	9600	202,100	−41,211.11	−0.20
T_{10}	18.67	298,720.00	192,500	13,000	205,500	93,220.00	0.45
T_{11}	20.11	321,760.00	192,500	22,600	215,100	106,660.00	0.50

Price: Potato Tk. 16.00/kg, Fungicide Tk. 1625/kg, bioagents Tk. 600/kg, fungicide 8 kg/ha, bioagents 16 kg/ha. T_0 = water (negative control); T_1 = foliar spray of formulation of *T. paraviridescens*; T_2 = foliar spray of formulation of *T. erinaceum*; T_3 = foliar spray of formulation of *P. putida*; T_4 = foliar spray of formulation of *B. subtilis;* T_5 = foliar spray of formulation of *T. paraviridescens* and *P. putida*; T_6 = foliar spray of formulation of *T. erinaceum* and *P. putida*; T_7 = foliar spray of formulation of *T. paraviridescens* and *B. subtilis*; T_8 = foliar spray of formulation of *T. erinaceum* and *B. subtilis*; T_9 = foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida,* and *B. subtilis*; T_{10} = foliar spray of Curzate M8 (Cymoxonil + Mancozeb); and T_{11} = foliar spray of formulation of *T. paraviridescens*, *T. erinaceum*, *P. putida,* and *B. subtilis* with T_{10}.

3.7. Efficacy of Formulated Two Bacterial and One Fungal Bioagents for Reducing the Application Frequency of Chemical Fungicides for Controlling Potato Late Blight Severity under Field Conditions

In 2019–2020, T_1 performed the best, showing the lowest severity (0.007%), followed by T_{13} (0.050%), T_6 (0.683%), T_{16} (0.083%), and T_{15} (0.140%). At 71 DAP, minimum severity was obtained from T_1 (0.45%) followed by T_{16} (1.86%), T_6 (2.76%), T_{13} (0.050%), and T_{15} (6.07%), showing identical statistical interference, whereas at both 59 and 71 DAP, all other treatments showed a moderate to higher level of severity, except T_{16}, T_{13}, T_6, T_{15}, and T_1. However, in case of a reduction of late blight severity over the control at 71 DAP, T_1 (99.54%) showed the highest reduction, followed by T_{16} (98.13%), T_{15} (97.38%), T_6 (97.20%), and T_{11} (94.67%), which were much more fruitful combination than the control and other treatments (Table 5).

Considering 2020–2021, as in the previous year, T_{16} revealed lowest level (6.67%) of severity, followed by T_{15} (8.33%), T_{14} (11.67%), T_{11} (11.67%), and T_1 (11.67%), compared to the control (T_0), which showed 88.33% of severity at 61 DAP. At 65 DAP, similarly, T_{16}, T_{15}, and T_{14} were also effective, revealing only 0.70%, 1.00%, and 2.03% severity, respectively, compared to T_1 (2.33%). As found by percent reduction of late blight severity over control, at 65 DAP, T_{16} (93.33%) performed best followed by T_{15} (91.67%), T_{14} (88.33%), T_1 (88.33%), and T_{11} (86.67%) compared to all of the other treatments, including the control (Table 6). Overall, in these two years, the treatments (T_{16}, T_{15}, T_{14}, T_{11}, and T_1) performed better considering all of the parameters at 61 and 65 DAP.

Table 4. Efficacy of formulated two bacterial and one fungal bioagents in reducing the frequency of application of chemical fungicides for controlling late blight of potato under artificial inoculation condition during 2019–2020.

Treatment	% Severity (0.1–100)		% Reduction of Late Blight Severity over Control at 65 DAP
	Days after Planting (DAP)		
	61	65	
T_0	81.67 ± 6.67 ^{a}	100.00 ± 0.00 ^{a}	0.00
T_1	2.33 ± 1.33 ^{e}	10.33 ± 7.42 ^{f}	89.67
T_2	58.33 ± 8.33 ^{b}	66.67 ± 8.33 ^{b}	33.33
T_3	6.67 ± 1.67 ^{e}	33.33 ± 8.33 ^{de}	66.67
T_4	5.33 ± 2.60 ^{e}	18.33 ± 6.67 ^{ef}	81.67
T_5	5.33 ± 2.60 ^{e}	15.00 ± 5.00 ^{ef}	85.00
T_6	5.00 ± 0.00 ^{e}	12.67 ± 3.71 ^{f}	87.33
T_7	46.67 ± 3.33 ^{c}	58.33 ± 8.33 ^{bc}	41.67
T_8	30.00 ± 5.00 ^{d}	53.33 ± 3.33 ^{bcd}	46.67
T_9	2.03 ± 1.51 ^{e}	20.00 ± 2.89 ^{ef}	80.00
T_{10}	1.73 ± 1.63 ^{e}	17.00 ± 8.00 ^{ef}	83.00
T_{11}	1.70 ± 1.65 ^{e}	15.00 ± 2.89 ^{ef}	85.00
T_{12}	23.33 ± 1.67 ^{d}	41.67 ± 8.33 ^{cd}	58.33
T_{13}	5.03 ± 2.86 ^{e}	33.33 ± 8.33 ^{de}	66.67
T_{14}	2.03 ± 1.51 ^{e}	10.33 ± 7.42 ^{f}	89.67
T_{15}	0.70 ± 0.30 ^{e}	10.33 ± 7.42 ^{f}	89.67
T_{16}	0.40 ± 0.30 ^{e}	10.00 ± 5.00 ^{f}	90.00
Level of significance	**	**	
CV (%)	32.70	33.79	

Data are the averages of three replications. Values with same letters in the same column are statistically similar and ** indicates the means were significant at 1% level of probability. T_0 = water (negative control); T_1 = eight sprays of Curzate M8 (positive control); T_2 = eight sprays of formulation of *P. putida* + *B. subtilis*; T_3 = T_2 + one spray of Curzate M8; T_4 = T_2 + two sprays of Curzate M8; T_5 = T_2 + three sprays of Curzate M8; T_6 = T_2 + four sprays of Curzate M8; T_7 = eight sprays of formulation of *T. erinaceum*; T_8 = T_7 + one spray of Curzate M8; T_9 = T_7 + two sprays of Curzate M8; T_{10} = T_7 + three sprays of Curzate M8; T_{11} = T_7 + four sprays of Curzate M8; T_{12} = Eight sprays of formulation of *P. putida*, *B. subtilis,* and *T. erinaceum*; T_{13} = T_{12} + one spray of Curzate M8; T_{14} = T_{12} + two sprays of Curzate M8; T_{15} = T_{12} + three sprays of Curzate M8; and T_{16} = T_{12} + four sprays of Curzate M8.

Table 5. Efficacy of formulated two bacterial and one fungal bioagents in reducing the frequency of fungicides application for controlling late blight severity of potato under field conditions during 2019–2020.

Treatments	% Severity			% Reduction of Late Blight Severity over Control at 71 DAP
	Days after Planting			
	48	59	71	
T_0	0.020 ± 0.01 ^{a}	44.930 ± 21.44 ^{a}	99.16 ± 0.60 ^{a}	0.00
T_1	0.000 ± 0.00 ^{d}	0.007 ± 0.003 ^{b}	0.45 ± 1.89 ^{f}	99.54
T_2	0.003 ± 0.003 ^{cd}	4.513 ± 2.89 ^{b}	72.83 ± 6.33 ^{b}	26.57
T_3	0.010 ± 0.01 ^{b}	0.197 ± 0.07 ^{b}	8.36 ± 0.6 ^{ef}	91.57
T_4	0.000 ± 0.00 ^{d}	0.147 ± 0.06 ^{b}	8.07 ± 1.50 ^{ef}	91.85

Table 5. *Cont.*

Treatments	% Severity			% Reduction of Late Blight Severity over Control at 71 DAP
	Days after Planting			
	48	59	71	
T_5	0.000 ± 0.00 ^{d}	1.817 ± 1.75 ^{b}	8.07 ± 1.62 ^{ef}	91.87
T_6	0.000 ± 0.00 ^{d}	0.683 ± 0.36 ^{b}	2.76 ± 1.62 ^{f}	97.20
T_7	0.011 ± 0.006 ^{b}	1.400 ± 0.29 ^{b}	64.17 ± 4.32 ^{b}	35.34
T_8	0.010 ± 0.01 ^{b}	1.343 ± 0.19 ^{b}	19.27 ± 7.18 ^{de}	80.63
T_9	0.000 ± 0.00 ^{d}	1.100 ± 0.10 ^{b}	18.83 ± 0.67 ^{de}	76.65
T_{10}	0.000 ± 0.00 ^{d}	0.170 ± 0.08 ^{b}	9.00 ± 0.58 ^{ef}	76.42
T_{11}	0.010 ± 0.006 ^{b}	0.183 ± 0.00 ^{b}	5.26 ± 3.0 ^{ef}	94.69
T_{12}	0.000 ± 0.00 ^{d}	0.773 ± 0.36 ^{b}	53.33 ± 0.83 ^{c}	46.21
T_{13}	0.007 ± 0.007 ^{bc}	0.050 ± 0.01 ^{b}	30.07 ± 9.93 ^{d}	69.64
T_{14}	0.000 ± 0.00 ^{d}	0.390 ± 0.16 ^{b}	25.17 ± 9.23 ^{d}	74.72
T_{15}	0.003 ± 0.003 ^{cd}	0.140 ± 0.04 ^{b}	6.07 ± 3.47 ^{ef}	97.38
T_{16}	0.000 ± 0.00 ^{d}	0.083 ± 0.04 ^{b}	1.86 ± 0.52 ^{f}	98.13
Level of significance	**	**	**	-
CV (%)	232.72	263.53	34.16	-

Data are the averages of three replications. Values with same letters in the same column are statistically similar and ** indicates the means were significant at 1% level of probability. T_0 = water (negative control); T_1 = eight sprays of Curzate M8 (positive control);; T_2 = eight sprays of formulated *P. putida* + *B. subtilis*; T_3 = T_2 + one spray of Curzate M8; T_4 = T_2 + two sprays of Curzate M8; T_5 = T_2 + three sprays of Curzate M_8; T_6 = T_2 + four sprays of Curzate M8; T_7 = eight sprays of formulated *T. erinaceum*; T_8 = T_7 + one spray of Curzate M8; T_9 = T_7 + two sprays of Curzate M8; T_{10} = T_7 + three sprays of Curzate M8; T_{11} = T_7 + four sprays of Curzate M8; T_{12} = eight sprays of formulated *P. putida*, *B. subtilis*, and *T. erinaceum*; T_{13} = T_{12} + one spray of Curzate M8; T_{14} = T_{12} + two sprays of Curzate M8; T_{15} = T_{12} + three sprays of Curzate M8; and T_{16} = T_{12} + four sprays of Curzate M8.

Table 6. Efficacy of formulated two bacterial and one fungal bioagents in reducing the frequency of application of chemical fungicides for controlling potato late blight under artificial inoculation conditions during 2020–2021.

Treatment	% Severity (0.1–100)		% Reduction of Late Blight Severity over Control at 65 DAP
	Days after Planting (DAP)		
	61	65	
T_0	88.33 ± 6.67 ^{a}	100.00 ± 0.00 ^{a}	0.00
T_1	3.67 ± 1.33 ^{e}	11.67 ± 6.67 ^{f}	88.33
T_2	58.33± 8.33 ^{b}	81.67 ± 6.67 ^{b}	18.33
T_3	8.33 ± 1.67 ^{e}	41.67 ± 8.33 ^{de}	58.33
T_4	8.33 ± 1.67 ^{e}	21.67 ± 1.67 ^{ef}	78.33
T_5	6.67 ± 1.67 ^{e}	20.00 ± 5.00 ^{ef}	80.00
T_6	6.67 ± 1.67 ^{e}	15.00 ± 5.00 ^{f}	85.00
T_7	50.00 ± 8.33 ^{c}	66.67 ± 8.33 ^{bc}	41.67
T_8	33.33 ± 8.33 ^{d}	58.33 ± 8.33 ^{bcd}	41.67
T_9	3.67 ± 1.33 ^{e}	23.33 ± 1.67 ^{ef}	76.67
T_{10}	2.33 ± 1.33 ^{e}	18.33 ± 6.67 ^{ef}	81.67

Table 6. *Cont.*

Treatment	% Severity (0.1–100)		% Reduction of Late Blight Severity over Control at 65 DAP
	Days after Planting (DAP)		
	61	65	
T_{11}	2.03 ± 1.51 ^{e}	13.33 ± 1.67 ^{ef}	86.67
T_{12}	33.33 ± 8.33 ^{d}	40.00 ± 10.00 ^{cd}	60.00
T_{13}	15.00 ± 5.00 ^{e}	33.33 ± 8.33 ^{de}	66.67
T_{14}	2.33 ± 1.33 ^{e}	11.67 ± 6.67 ^{f}	88.33
T_{15}	1.00 ± 0.00 ^{e}	8.33 ± 1.67 ^{f}	91.67
T_{16}	0.70 ± 0.30 ^{e}	6.67 ± 1.67 ^{f}	93.33
Level of significance	**	**	-
CV (%)	32.70	33.79	-

Data are the averages of three replications. Values with same letters in the same column are statistically similar and ** indicates the means were significant at 1% level of probability. T_0 = water (negative control); T_1 = eight sprays of Curzate M8 (positive control); T_2 = eight sprays of formulated *P. putida* + *B. subtilis*, T_3 = T_2 + one spray of Curzate M8; T_4 = T_2 + two sprays of Curzate M8; T_5 = T_2 + three sprays of Curzate M8; T_6 = T_2 + four sprays of Curzate M8; T_7 = eight sprays of formulated *T. erinaceum*; T_8 = T_7 + one spray of Curzate M8;; T_9 = T_7 + two sprays of Curzate M8; T_{10} = T_7 + three sprays of Curzate M8; T_{11} = T_7 + four sprays of Curzate M8; T_{12} = eight sprays of formulated *P. putida*, *B. subtilis*, and *T. erinaceum*; T_{13} = T_{12} + one spray of Curzate M8; T_{14} = T_{12} + two sprays of Curzate M8; T_{15} = T_{12} + three sprays of Curzate M8; and T_{16} = T_{12} + four sprays of Curzate M8.

During 2020–2021, at 59 DAP, the lowest severity was recorded in T_1(0.03%), followed by T_{16} (0.16%), T_{15} (0.37%), T_6 (0.40%), and T_{11} (0.43%), and at 71 DAP, minimum severity (0.89%) was calculated in T_1 followed by T_{16} (3.80%), T_6 (5.27%), T_{11} (6.07%), and T_{15} (12.87%), showing statistically identical data, while maximum severity (0.020%, 44.93% and 99.16%) was found in T_0 at 48, 59, and 71 DAP, respectively. With regards to the percent reduction of late blight severity over the control at 71 DAP, the highest percent reduction of late blight was observed in T_1 (99.10%), followed by T_{16} (96.17%), T_6 (94.68%), and T_{11} (93.88%), compared to the rest of the treatments (Table 7 and Supplementary Figure S2). Overall, in these two years, treatments $T_{16,}$ $T_{15,}$ $T_{11,}$ $T_{6,}$ and T_1 performed better considering all of the parameters at 48, 59, and 71 DAP.

3.8. Economic Analysis of Formulated Two Bacterial and One Fungal Bioagents Used for Reducing the Application Frequency of Fungicide for Controlling Late Blight of Potato

During 2019–2020 and 2020–2021, the average cost−benefit analysis revealed that the highest (Tk. 395,111.11/ha) gross return was obtained from treatment T_{16}, followed by T_6 (Tk. 390,222.23/ha), T_1 (379,200.00/ha), and T_{11} (Tk. 374,888.89/ha). Thus, the highest BCR (0.90) was calculated from treatments T_{16} and T_6 (0.88), which performed better than T_1 (0.85). BCR results indicated a return ranging from Taka 0.85 to 0.90 over the investment of Taka 1.00 in these treatments in those two years. In both years, treatments ($T_{16,}$ $T_{6,}$ T_{11}, and T_1) performed better in the field conditions, reducing the fungicide application frequency to mitigate late blight severity, as those treatments also performed better in the cost−benefit analysis (Table 8).

3.9. Detailed Economic Analysis of the Improved Management of Late Blight Using Bioagents during 2019–2020 and 2020–2021

Bangladesh has been producing 9.7 million tonnes potato on 0.5 million hectares of land, as mentioned earlier. Farmers are spending 6500 million Tk of their total expenditures on fungicides per year with conventional approaches (eight sprays of Curzate M8 (positive control)). Conversely, if we could apply two improved management approaches with bioagents 1 ((T_2) + four sprays of Curzate M8) and 2 ((T_{12}) + four sprays of Curzate M8), then the total expenditures for fungicides could be drastically reduced to 3250 million Tk. Among the three approaches, improved management with bioagents 1 and 2 showed

better economic returns compared to the farmers' approach. Cultivation of potato with improved management approaches with bioagents 1 and 2 were satisfactory, because farmers benefited from a 7.19% and 10.98% increase in their income for one hectare of land, respectively. With regards to the country's economic impact within two years, 9361.5 million Tk was the total increase of the country's return when applying improved management with bioagents 2 and 6135 million dollars from improved management with bioagents 1. Approximately 0.3 million farm families are closely engaged with potato production. In our detailed analysis, we observed that the income of an individual farm family was raised 31.21 thousand Tk when we applied improved management with bioagents 2, which indicated that the use of bioagents with chemical fungicide to minimize the late blight severity had a tremendous economic and social impact on our country. Thus, farmers will likely be willing to accept this technology, as several factors are closely associated with their income return from one hectare of potato land (Table 9).

Table 7. Efficacy of formulated two bacterial and one fungal bioagents in reducing the frequency of fungicides application for controlling late blight severity of potato under field condition during 2020–2021.

Treatment	% Severity			% Reduction of Late Blight Severity over Control at 71 DAP
	Days after Planting			
	48	59	71	
T_0	0.030 ± 0.012 ^{a}	53.00 ± 10.77 ^{a}	99.17 ± 0.33 ^{a}	0.00
T_1	0.000 ± 0.000 ^{d}	0.03 ± 0.003 ^{b}	0.89 ± 0.14 ^{f}	99.10
T_2	0.010 ± 0.006 ^{cd}	4.24 ± 2.18 ^{b}	80.67 ± 5.47 ^{b}	18.68
T_3	0.013 ± 0.009 ^{b}	1.22 ± 0.052 ^{b}	32.03 ± 1.02 ^{d}	67.69
T_4	0.003 ± 0.003 ^{d}	0.97 ± 0.38 ^{b}	17.00 ± 1.16 ^{de}	82.85
T_5	0.000 ± 0.000 ^{d}	0.58 ± 0.79 ^{b}	14.50 ± 0.76 ^{de}	85.37
T_6	0.007 ± 0.0007 ^{d}	0.40 ± 0.27 ^{b}	5.27 ± 0.71 ^{ef}	94.68
T_7	0.013 ± 0.009 ^{b}	2.42 ± 0.210 ^{b}	51.50 ± 2.08 ^{c}	48.06
T_8	0.000 ± 0.003 ^{d}	1.71 ± 0.283 ^{b}	35.83 ± 0.83 ^{d}	63.87
T_9	0.000 ± 0.000 ^{d}	0.72 ± 0.090 ^{b}	16.67 ± 1.64 ^{de}	83.19
T_{10}	0.013 ± 0.009 ^{b}	0.46 ± 0.052 ^{b}	10.87 ± 1.27 ^{ef}	71.65
T_{11}	0.003 ± 0.003 ^{d}	0.43 ± 0.030 ^{b}	6.07 ± 0.74 ^{ef}	93.88
T_{12}	0.010 ± 0.006 ^{cd}	0.75 ± 0.038 ^{b}	45.83 ± 0.83 ^{d}	53.78
T_{13}	0.003 ± 0.003 ^{d}	1.23 ± 0.253 ^{b}	34.33 ± 1.59 ^{d}	65.38
T_{14}	0.007 ± 0.007 ^{d}	1.04 ± 0.210 ^{b}	15.00 ± 1.16 ^{de}	84.87
T_{15}	0.000 ± 0.000 ^{d}	0.37 ± 0.049 ^{b}	12.87 ± 1.27 ^{de}	87.02
T_{16}	0.000 ± 0.000 ^{d}	0.16 ± 0.006 ^{b}	3.80 ± 0.23 ^{f}	96.17
Level of significance	**	**	**	-
CV (%)	232.72	263.53	34.16	-

Data are the averages of three replications. Values with same letters in the same column are statistically similar and ** indicates the means were significant at 1% level of probability. T_0 = water (negative control); T_1 = eight sprays of Curzate M8 (positive control); T_2 = eight sprays of formulated *P. putida* + *B. subtilis*; T_3 = T_2 + one spray of Curzate M8; T_4 = T_2 + two sprays of Curzate M8; T_5 = T_2 + three sprays of Curzate M8; T_6 = T_2 + four sprays of Curzate M8; T_7 = eight sprays of formulated *T. erinaceum*; T_8 = T_7 + one spray of Curzate M8; T_9 = T_7 + two sprays of Curzate M8; T_{10} = T_7 + three sprays of Curzate M8; T_{11} = T_7 + four sprays of Curzate M8; T_{12} = eight sprays of formulated *P. putida*, *B. subtilis*, and *T. erinaceum*; T_{13} = T_{12} + one spray of Curzate M8; T_{14} = T_{12} + two sprays of Curzate M8; T_{15} = T_{12} + three sprays of Curzate M8; and T_{16} = T_{12} + four sprays of Curzate M8.

Table 8. Cost−benefit analyses of formulated two bacterial and one fungal bioagents used for reducing the frequency of fungicide application in controlling late blight of potato under field condition during 2019–2020 and 2020–2021.

Treatment	Yield (t/ha)	Gross Return (Tk./ha)	Production Cost (Tk./ha)	Total Cost of the Treatment (Tk/ha)	Total Cost with Treatment (Tk/ha)	Net Return (Tk./ha)	BCR
T_0	6.42	109,083.34	192,500	0	192,500	−86,583.34	−0.45
T_1	23.70	379,200.00	192,500	13,000	205,500	173,700.00	0.85
T_2	8.61	137,777.78	192,500	9600	202,100	−64,322.23	−0.32
T_3	14.47	231,555.56	192,500	11,225	203,725	27,830.56	0.14
T_4	16.12	257,777.78	192,500	12,850	205,350	52,427.78	0.26
T_5	16.22	128,875.56	192,500	14,475	206,975	52,580.56	0.26
T_6	24.39	390,222.23	192,500	16,100	208,600	181,622.23	0.88
T_7	11.23	179,555.56	192,500	9600	202,100	−22,544.45	−0.11
T_8	12.97	207,555.56	192,500	11,225	203,725	3830.56	0.02
T_9	13.61	217,777.78	192,500	12,850	205,350	12,427.78	0.06
T_{10}	16.64	266,222.22	192,500	14,475	206,975	59,247.22	0.29
T_{11}	23.43	374,888.89	192,500	16,100	208,600	166,288.89	0.80
T_{12}	11.14	178,222.23	192,500	9600	202,100	−23,877.78	−0.12
T_{13}	12.50	200,000.00	192,500	11,225	203,725	−3725.00	−0.02
T_{14}	14.62	233,777.78	192,500	12,850	205,350	28,427.78	0.14
T_{15}	16.20	259,111.11	192,500	14,475	206,975	52,136.11	0.25
T_{16}	24.70	395,111.11	192,500	16,100	208,600	186,511.11	0.90

Price: potato Tk. 16.00/kg, fungicide Tk. 1625/kg, bioagents Tk. 600/kg, fungicide 8 kg/ha, bioagents 16 kg/ha. T_0 = water (negative control); T_1 = eight sprays of Curzate M8 (positive control); T_2 = eight sprays of formulated *P. putida* + *B. subtilis*; $T_3 = T_2$ + one spray of Curzate M8; $T_4 = T_2$ + two sprays of Curzate M8; $T_5 = T_2$ + three sprays of Curzate M8; $T_6 = T_2$ + four sprays of Curzate M8; T_7 = eight sprays of formulated *T. erinaceum*; $T_8 = T_7$ + one spray of Curzate M8; $T_9 = T_7$ + two sprays of Curzate M8; $T_{10} = T_7$ + three sprays of Curzate M8; $T_{11} = T_7$ + four sprays of Curzate M8; T_{12} = eight sprays of formulated *P. putida*, *B. subtilis* and *T. erinaceum*; $T_{13} = T_{12}$ + one spray of Curzate M8; $T_{14} = T_{12}$ + two sprays of Curzate M8; $T_{15} = T_{12}$ + three sprays of Curzate M8; and $T_{16} = T_{12}$ + four sprays of Curzate M8.

Table 9. Detailed Economic analysis of the improved management using bioagents based on the BCR calculated during 2019–2020 and 2020–2021.

Approaches	Total Expenditure for Fungicides Used (Million Tk)	Economic Return (Million Tk)	Percent Increase of Income/ha Compared to Conventional Practices	Total Increase of Return in the Country (Million Tk) Compared to Conventional Practices	Increase of Income per Farm Family (000′Tk) Compared to Conventional Practices
Farmers' Conventional approach	6500	85,282.5	0.00	0.00	0.00
Improved Management with Bioagents 1	3250	91,417.5	7.19	6135	20.45

Table 9. *Cont.*

Approaches	Total Expenditure for Fungicides Used (Million Tk)	Economic Return (Million Tk)	Percent Increase of Income/ha Compared to Conventional Practices	Total Increase of Return in the Country (Million Tk) Compared to Conventional Practices	Increase of Income per Farm Family (000′Tk) Compared to Conventional Practices
Improved Management with Bioagents 2	3250	94,644	10.98	9361.5	31.21

Farmers' conventional approach: T_1 ((eight sprays of Curzate M8 (Positive control)); improved management of bioagents 1: T_6 (eight sprays of formulated *P. putida* + *B. subtilis* (T_2) + four sprays of Curzate M8); improved management of bioagents 2: T_{16} (eight sprays of formulated *P. putida*, *B. subtilis*, and *T. erinaceum* (T_{12}) + four sprays of Curzate M8). Tk = Bangladeshi currency; total expenditure for fungicides used (million Tk): area (0.5 milion hectare) × 1625 × 8 kg; total expenditure for improved management with bioagents: area (0.5 milion hectare) × 1625 × 4 kg, where fungicide cost 1625 taka (Bangladeshi currency)/kg. Economic return (million Tk): ((total production cost + 13,000) × 0.5 million × BCR)/10 million. Percent increase of income/ha compared to conventional practices for improved management with bioagents: economic return − economic return of farmer's conventional approach/economic return of farmers conventional approach × 100. Total increase of return in the country (million Tk) compared to conventional practices: Economic return of improved management with bioagents − economic return of farmers' conventional approach. Increase of income per farm family (000′ Tk) compared to conventional practices: total increase of return in the country (million Tk)/0.3 million × 10 Million.

4. Discussion

Managing late blight using eco-friendly methods is always challenging under high disease pressure in severe environments. Biological management in this country is more relevant due to the detrimental effect of chemicals on the environment and human health. In this study, it was observed that bacterial species belonging to the genera *Pseudomonas* and *Bacillus* are were able to inhibit the growth of *P. infestans* in vitro by 94% over the control. These results are in accordance with the findings of [42]. They observed the best antagonistic activity of *Pseudomonas* and *Bacillus* against *P.* infestans, as they produced a wide range of antibiotics, chemical surfactants, and biosurfactants. The antagonist *B. subtilis* B5 strain effectively inhibited *P. infestans* growth [43]. The route of action seems to be the ability of *B subtilis* strains to create mycotoxins that suppress *P. infestans* growth and stimulate peroxidase activity [44]. Elliott et al. [45] noted that Companion® and Serenade® are marketed *B. subtilis* biocontrol agents that reduce *P. infestans*. *Bacillus* strains might control *P. infestans* directly by reducing mycelial development, cyst germination, or motile zoospore swimming by creating antifungal chemicals that suppress the pathogen, or indirectly by stimulating active oxygen burst, nitrogen synthesis, callose accumulation, and lignification [64–66]. In our study, we also observed alteration of mycelia growth and morphological changes with the spore formation when formulated bioagents were applied in an in vitro condition. The metabolite of the biosurfactant producing bacterium, *P.* aeruginosa has shown high efficacy against *P.* infestans under in vitro conditions [67]. *Pseudomonas* and *Bacillus* isolates were antagonistic to *P. infestans*. Twenty-three effective microorganisms (spore-forming and non-spore-forming bacteria, yeasts, and fungi) isolated from potato phyllosphere on *P. infestans* growth were investigated in dual cultures, including their patterns of inhibition [68]. PCA (Phenazine-1-carboxylic) promotes biofilm development, allowing PCA-producing *Pseudomonas* spp. to bind to plant roots and act as biocontrol agents [69]. *Pseudomonas* biocontrol of *P. infestans* was previously shown to suppress sporangia and zoospore germination, implying the existence of several undiscovered antioomycete determinants. By up and down regulating the gene expression in *P. infestans*, Roquigny et al. [46] showed that *Pseudomonas* spp.-produced Phenazine-1-carboxylic PCA is involved in growth inhibition in *P. infestans*.

Bacterial (*P. putida* and *B. subtilis*) and fungal (*T. erinaceum*) bioagents were found to be effective at reducing late blight severity by 99% until 60 DAP, whereas these bioagents were found to be partially effective until 70 DAP, and reduced late blight severity by 46% when applied together under high disease pressure conditions. The use of these

bacterial and fungal bioagents in combination with four sprays of chemical fungicide (Curzate M8) could reduce late blight severity up to 98% and could reduce the application frequencies of fungicide by 50% in both net house and field conditions; generally, all farmers of Bangladesh have been using at least eight sprays of chemical fungicides, which might be raised up to 16 sprays depending on the weather conditions, per hectare of land, whether late blight severity is present or not, thus, we have standardized it (8 sprays) based on the field surveys in our experiment to evaluate the reduction of spray frequency of chemical fungicide with bioagents. Furthermore, the cost−benefit analysis revealed that treatments T_{10} and T_{11} showed a better performance in terms of BCR in 2018–2019, as well as treatmentsT_6 and T_{16} in 2019–2020 and 2020–2021, respectively, compared to other treatments applied. Yan et al. [70] observed that *B. velezensis* reduced late blight severity by 40.79% and 37.67% in a two-year field trial. They found that a low fungicide concentration and a high concentration of *B. velezensis* SDTB038 could reduce potato late blight. In addition, *B. velezensis* SDTB038 may successfully suppress the infection of potato leaves by *P. infestans*, making it a promising biological fungicide against potato late blight. Compared to untreated plants, the *B. subtilis* 26D strain reduced *P. infestans* mycelium growth and reduced late blight symptoms by 35%, respectively. Sorokan et al. [71] explained that *B.* strains induced systemic resistance to *P. infestans* through the activation of the transcription of *PR* genes in potato plants. The development of ectoenzymes and antifungal medicines like surfactin and iturinA gives *B. subtilis* strains a broad range of antifungal action. Antifungal metabolite-induced mycelium damage is thought to be mostly osmotic cell stress. In intimate contact with phytopathogenic fungus, the bacteria aggressively move towards fungal hyphae, kill them, and feed on them [72]. These observations are highly similar in accordance with our observations of the morphological deformation of *P. infestans* in a dual culture method. Wang et al. [73] highlighted that *B. subtilis* WL-2 and IturinA produced by *B. subtilis* WL-2 have great potential as candidates for inhibiting *P. infestans* mycelium growth and controlling potato late blight. *B. subtilis* 30B-B6 was shown to significantly decrease late blight severity [74]. As revealed by [48], *P. infestans* is very sensitive to bacterial volatiles such as 1-undecene generated by potato-associated *Pseudomonas* strains. It was shown that several potato-associated *Pseudomonas* strains could effectively suppress extremely pathogenic *P. infestans* isolates by inhibiting mycelial growth of all *P. infestans* isolates when co-cultured with the most active *Pseudomonas* strain (R47) [49]. Tomar et al. [67] in another study observed that five isolates of bacteria were found to be effective against *P. infestans* out of 95 tested as biocontrol agents. Both *P. aeruginosa*-1 and -3 had 62.22% and 46.42% inhibition after 72 h, respectively. *P. aeruginosa*-1 culture supernatant and bacterial cell suspension exhibited 10.42%, 9.94%, and 17.96% disease severity in potato plants, respectively, compared to 53.96% in the control. Zhang et al. [9] observed in greenhouse and field trials that the combined application of *Rhodopseudomonas palustris* GJ-22 and Curzate resulted in better disease control than the use of either agent alone. They highlighted the potentialities of the combined application of *R. palustris* strain GJ-22 and Curzate to control potato late blight in a more environment friendly way by a reduced level of harmful chemical fungicides application. In this study, we observed that *T. paraviridescens* and *T. erinaceum* reduced the late blight severity in both net house and field conditions. Kariukiet al. [28] observed the inhibitory action of *T. asperellum* and *T. harzianum* on the *P. infestans* mycelial growth and the suppression of late blight disease in the greenhouse experiment. Elsherbiny et al. [75] reported that *Trichoderma* VOCs suppressed the mycelial development of *P. infestans* cultured on laboratory media by 80% and on potato tubers by 93.1%. Electron microscopy demonstrated substantial morphological and ultrastructural malformations in *T. atroviride* VOC-treated hyphae, including cell deformation, collapse, and organelle disintegration. Purwantisari et al. [76] reported *T. viride* induced resistance in potato plants against late blight. Cwalina-Ambroziak et al. [77] found that using an integrated chemical and biological approach decreased the symptoms of *P. infestans* infections. *Trichoderma*'s rhizosphere competence and competitive ability could be a factor in its biocontrol roles against *P. infestans* [66]. This is because *Trichoderma* uses

many mycoparasitic strategies, which are direct methods for biological control that work by parasitizing, detecting, growing, and colonizing pathogens. These strategies include the detection of pathogens through chemotropism; the lysis of the pathogen's cell wall, the pathogen's hyphal penetration by appresorial formation; and the production of toxins [78]. Considering the detailed economic analysis, improved management with bioagents 1 and 2 performed better compared to the farmers' conventional approach in terms of economic return, and income of per farm family was raised up to 31.21 thousand Tk as well, which indicated that using these bioagents had a positive economic impact on farmer income and on the country. Farmers benefitted while using the improved management with bioagents, which significantly focused the acceptability of these bioagents among stakeholders, consumers, and farmers. These findings support our observation on the potentiality of the combined use of bacterial and fungal bioagents with Curzate M8 to reduce late blight severity almost at the same level as the conventional eight sprays of Curzate M8 did. This was observed with either single or combined use of bacterial (*P. putida* and *B. subtilis)* and fungal *(T. erinaceum)* bioagents. Therefore, the possibility of using formulated bacterial and fungal bioagents could be an alternative for reducing the application of chemical fungicides for controlling late blight of potato and producing export quality organic potato in the country.

5. Conclusions

Bacterial (*P. putida* and *B. subtilis)* and fungal (*T. erinaceum)* bioagents were found to be effective at reducing late blight severity by 99% until 60 DAP, whereas these bioagents were found to be partially effective until 70 DAP and reduced late blight severity by 46% when applied together under field conditions. The use of these bacterial and fungal bioagents in combination with four sprays of chemical fungicide (Curzate M8) could reduce late blight severity by up to 98% and could reduce the application frequencies of fungicide by 50% in both net house and field conditions. However, the possibility of the commercial formulation and application of these bioagents needs to be investigated with a proper late blight forecasting system. Proper warning systems shed light on when and how many times chemical fungicides need to be applied in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14084383/s1. Supplementary Figure S1: Interactions effect of bacterial and fungal bioagents with Curzate M8 used for controlling late blight of potato. Supplementary Figure S2: Efficacy of some selected bacterial and fungal bioagents in reducing the frequency of fungicides application for controlling late blight severity of potato under field condition.

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