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The Use of Bio-Agents for Management of Potato Diseases

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Abstract

Potato is an important food crop in the world as well as in India. It is being affected by different pathogens, viz. fungi, bacteria, viruses and nematodes. These pathogens may cause significant yield losses of the crop, if proper protection measures have not been applied. Among potato pathogens, *Phytophthora infestans*, *Alternaria solani*, *Rhizoctonia solani* and *Fusarium* spp. are the major pathogens in the fungal group, whereas *Ralstonia solanacearum*, *Pectobacterium* spp. and *Streptomyces* spp. are in the bacterial group. For management of these pathogens, various methods, that is, chemical control, biological control, resistant varieties, cultural control and physical control, are applied. Resistant varieties are the best and cheapest method for managing the diseases. However resistant varieties are break down their resistant over the years and moreover against some pathogen absolute resistant are not available. Chemical management is the second best option for managing the diseases, due to continuous and irrational use of the chemicals; pathogens have developed resistance against certain class of fungicides/bactericides. Moreover, these chemicals also assist in environmental pollution and toxicity in the produce. Bio-agents are naturally occurring living organisms, which are found in rhizosphere, phylloplane, etc. These bio-agents help in not only managing the diseases but also increasing the crop yield. Therefore, the use of bio-agents for biological management of potato crops is the focused research area worldwide.

Keywords: Bioagents, potato, diseases, management, bacteria, fungi

1. Introduction

Potato originated in the hills of Andes and Bolivia in South America. It was introduced into Europe by Spaniards in the second half of the sixteenth century, from there it spreads throughout Europe and rest of the world in the mid-seventeenth to mid-eighteenth century. In India, it was introduced by Portuguese in the seventeenth century. Potato is the most important crop in the world. It is affected by various diseases and pests. Diseases are the major cause of concern for reducing the economic yield and affecting status of the potato growers. Major diseases of potato are late blight, early blight, black scurf, dry rot, etc. in the fungal group, whereas bacterial wilt, soft rot/blackleg of potato and common scab in the bacterial group. Sometime these diseases may cause losses up to 75%. Potato diseases can be managed by various methods, viz. chemical control, cultural control, biological control, physical and resistant varieties. Generally, chemical control is used for managing the diseases at large scale. Due to use of chemicals (fungicides/antibiotics) for longer periods for managing the disease, it was observed that pathogens have developed resistance against certain chemicals, besides also enhanced the toxicity in the environment. To avoid development of resistance in pathogens and toxicity in the environment, the use of bio-agents/biological control is the best option. In a simple way, biological control can be defined as the partial or total inhibition or destruction of pathogen population by other microorganisms. Broader way, Baker and Cook (1974) defined this as the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of environment, host or antagonist or by mass introduction of one or more antagonist [1]. The first experiment in biological control with antagonists was conducted by GB Sandford in Canada [2]. Different mechanisms of biological control of pathogenic fungi have been suggested, including microbial competition, antibiosis, hyperparasitism and induction of systemic acquired resistance in the host plants [3]. Bio-agents have remarkable capacity of multiplication; thus, when applied they multiply in exponential ratio and even can overcome stress conditions by forming thick-walled spores [4]. Recent years have witnessed the increasing popularity of biological control agents as an alternative to fungicides [5]. *Trichoderma* species as biocontrol agents (BCAs) was recognized for the first time by Weindling [6]. *Bacillus* spp. and *Pseudomonas* spp. have been used in biological management of the potato diseases. Bio-agents are effective against seed and soil-borne plant pathogens. The biological control of soil-borne plant pathogens has drawn much attention in the past few decades and is currently considered as a promising alternative to synthetic pesticides because of its safety for the environment and the human health [7]. Plant growth promoting rhizobacteria (PGPR) and vesicular-arbuscular mycorrhizae (VAM) are known to minimize plant diseases and increase crop yield. Biocontrol applications on potato plants require a better knowledge of its beneficial fungal partners. This kind of microbial community has been poorly studied, particularly because in vitro cultivation of mycorrhizae remains difficult [8]. Biocontrol agents are an important component especially in the organic cultivation of potato. Biological control of major fungal and bacterial diseases of potato is discussed in the following sections:

2. Late blight of potato

The late blight disease caused by oomycete has a great importance in the history of plant pathology. Initially, its causal organism was reported *Botrytis infestans* in 1845 by C. Montagne, a retired French army doctor who had devoted much of his life to the study of fungi. About 30 years later, German scientist Anton de Bary renamed it as *Phytophthora infestans* (Mont.) de Bary [9]. During 1844–1845, the entire crop across Europe, especially in Ireland, was destroyed prematurely leading to worst ever famine the ‘Irish Potato Famine’ [10]. One million people died of starvation and another million migrated to USA and other parts of the world. The late blight fungus co-evolved with potato in Central and South America and subsequently spread to other parts of the world mainly through infected seed tubers. Late blight was recorded in India for the first time between 1870 and 1880 in the Nilgiri Hills [11]. Under subtropical plains, it was first observed in 1898–1900 in Hooghly district of West Bengal [12]. In the northern part, it appeared for the first time in 1883 in Darjeeling and spread rapidly to adjoining hills [13]. *Phytophthora infestans* caused late blight diseases in potato and tomato crops worldwide. It not only caused economic losses of yield but also the quality and quantity of the crop. Recently, reduction in 10–15% yield was expected at national level (India) due to occurrence of late blight disease [14]. *Phytophthora infestans* is highly researchable pathogen in plant diseases. The worldwide late blight disease is re-emerging; therefore, this disease is constantly observed by the late blight researchers [15]. Late blight affects all plant parts, especially leaves, stem and tubers. Whitish mycelium appears on lower leaves under humid conditions and is the most important symptom. Light brown lesions develop on stem and petioles, and rusty brown discolouration of the flesh is the typical symptom of late blights on potato tubers. The pathogen is mainly seed borne in nature but also soil borne in some cases. Management of late blight through eco-friendly means of applying botanicals has been initiated in European and American countries during the past years of the twentieth century [16, 17]. Of 100 species in 54 plant families tested, leaf extracts from onions, garlic, *Malus toringo*, *Reynoutria japonica* and *Rheum coreanum* inhibited mycelial growth of *P. infestans*. *M. toringo* extracts strongly inhibited *P. infestans* and was effective in controlling late blight also [18]. Some antifungal compounds reported from botanicals against late blight of potato [19]. The antagonist *Bacillus subtilis* B5 was found effective in inhibiting the growth of *P. infestans* [20]. Integrated management of late blight, using two sprays of *Bacillus subtilis*+ *Trichoderma viride* and one spray of fungicides, at the onset of disease is found to be effective for managing late blight of potato [21]. Rhamnolipid is a class of glycolipids, which is produced by bacteria. Rhamnolipid-based formulation (0.25%) from *Pseudomonas* spp. was tested under field trials at three different locations. The terminal disease severity in rhamnolipid formulation was 45% (compared to 100% in control), 47.5% (against 92.5%) and 59.2% (as against 76.64%) at Modipuram, Lavad, (Meerut) and Jalandhar, respectively [22]. Certain microorganisms in the phyllosphere were antagonistic to *P. infestans*, which included the yeasts *Sporobolomyces* spp., *Acetobacter* spp., isolates of *Pseudomonas* spp. and *Bacillus* spp. [23, 24]. *Bacillus* sp. inhibited mycelial growth of seven plant pathogenic fungi *in vitro* and *in vivo*, and the same bacterium protected tomato plants against *P. infestans* [25]. Various bio-agents, including a bacterium (*Serratia* sp.) and four fungi (*Trichoderma* sp., *Fusarium* sp. and two *Penicillium* spp.), were evaluated against *P. infestans* on

tomatoes under field conditions at Costa Rica, and it was reported that *Penicillium* reduced the lesion area/plant between 8 and 40% [26]. One hundred and twenty-two microorganisms isolated from the phyllosphere of potatoes and only 23 were effective microorganisms (spore-forming and non-spore-forming bacteria, yeasts and fungi) in dual cultures with different patterns of inhibition of *P. infestans* [27]. Various naturally occurring microorganisms, that is, *T. viride*, *Penicillium viridicatum*, *Penicillium aurantiogriseum*, *Chaetomium brasiliense* [28], *Acremonium strictum* [29], *Myrothecium verrucaria* and *P. aurantiogriseum* [30], showed antagonistic effect against *P. infestans*. The antagonistic activities of *Pseudomonas fluorescens*, *Pseudomonas* sp. *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp., *Trichoderma virens* and *Trichoderma harzianum* showed positive inhibition of mycelial growth of *P. infestans*, *Fusarium* spp. and *Rhizoctonia solani* under *in vitro* conditions **Table 1** [31]. One hundred fifty-two endophytic fungi were isolated from healthy tissues of vegetable plants, and only 23 (15%) isolates showed anti-oomycete activity against tomato late blight and *in vivo* [32].

Treatments	<i>Rhizoctonia solani</i>		<i>Fusarium</i> sp.		<i>Phytophthora infestans</i>	
	Inhibition of growth (%) after 3 days over control	Bell's rating	Inhibition of growth (%) after 8 days over control	Bell's rating	Inhibition of growth (%) after 12 days over control	Bell's rating
<i>Pseudomonas</i> sp.	32.22	4	47.16	3	55.68	2
<i>P. fluorescens</i>	39.25	4	53.01	2	53.40	2
<i>A. flavus</i>	39.44	3	43.77	3	59.00	2
<i>A. niger</i>	56.48	3	50.18	2	61.36	3
<i>Penicillium</i> sp.	37.22	3	63.01	2	59.00	2
<i>T. virens</i>	42.77	2	52.64	2	64.77	2
<i>T. harzianum</i>	46.11	2	57.16	2	63.63	2
CD (0.05)	8.80		5.97		2.59	

Table 1. Antagonism between bio-agents and potato pathogens.

Naturally occurring surface active compounds derived from microorganisms are called biosurfactants. These are amphiphilic biological compounds produced extracellularly as part of the cell membrane by a variety of bacteria, yeast and fungi [33]. Research on biosurfactants used as a biocontrol, particularly in potato against *P. infestans*, has initially started in India under PhytoFura network project. Biosurfactants can be used as alternatives to chemical surfactants as their capability of reducing surface and interfacial tension with low toxicity, high specificity and biodegradability make them important for inhibiting pathogens. The metabolite of biosurfactant-producing microorganism (*Pseudomonas aeruginosa*) has shown high efficacy against *P. infestans* under *in vitro* conditions [34]. Ninety-five isolates of bacteria were tested for their biosurfactant as well as biocontrol activity against *P. infestans*. Results revealed

that only 15.8% isolates showed biosurfactant activity and only five isolates were found to be effective against *P. infestans* for biocontrol properties. Amongst highest effective was *P. aeruginosa*, which was tested in different forms, viz. bacterial cells, culture filtrate and formulation against *P. infestans* on whole plant method and lowest disease severity (9.44%) recorded with culture filtrate excluding mancozeb treatment mentioned in **Figure 1** [35]. Biosurfactants produced by bacteria, yeasts and fungi can serve as green surfactants. However, large-scale production of these molecules has not been realized because of low yields in production processes and high recovery and purification costs [36]. The best antagonistic activity against *P. infestans* is observed in the genera of *Pseudomonas* and *Bacillus* as they produce a wide range of antibiotics and biosurfactants and can be used as alternatives to chemical surfactants [37].

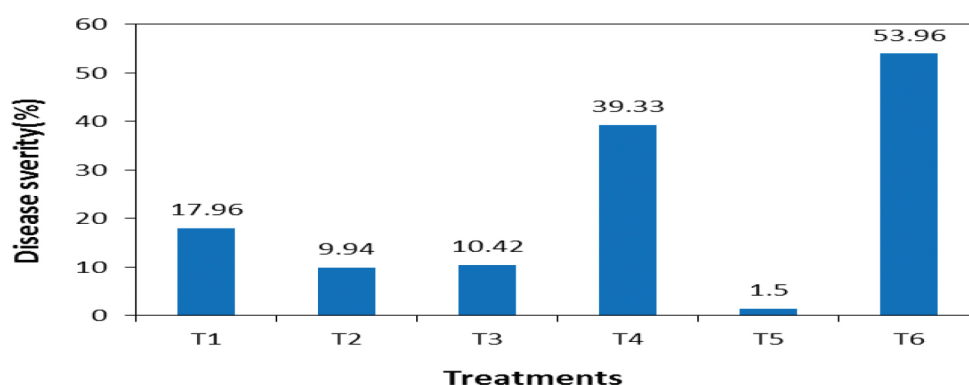


Figure 1. Effect of different forms of bio-agents on late blight development using whole plant method T1—bacterial suspension of *Pseudomonas aeruginosa* 1, T2—culture filtrate of *P. aeruginosa* 1, T3—bioformulation of *P. aeruginosa* 1, T4—Talc powder, T5—Mancozeb (0.2%) and T6—distilled water spray (control).

3. Early blight of potato

Early blight of potato caused by *Alternaria solani*/*A. alternata*. The symptom of this disease is dark brown to black lesions with concentric rings, which produce a ‘target spot’ effect. Symptoms are initially observed on older leaves and weaker plants. *A. solani* is a polycyclic pathogen as many cycles of infection are possible during a season [38]. The antimicrobial activity of six plant extracts from *Ocimum basilicum* (Sweet Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander) and *Allium sativum* (Garlic) was tested for managing *Alternaria solani* *in vitro* and *in vivo*. The results revealed that the highest reduction of disease severity was achieved by *A. sativum* at 5% concentration and the lowest reduction was obtained when tomato plants were treated with *O. basilicum* at 1% and 5% concentration [39]. *T. viride* (0.5%) was found to be effective against early blight of potato for reducing disease intensity under field conditions [40]. The bio-agents *T. harzianum* and *P. fluorescens* (seed treatment + foliar spray) were effective in reducing the disease intensity of early blight of potato and also increasing tuber yield [41].

4. Black scurf of potato

Black scurf is an important disease of potato in the category of soil- and tuber-borne diseases. Infected seeds are the main sources of infection [42]. It affects roots, stolon, stems and tubers. The disease has two phases, viz. stem canker and black scurf. Stem canker phase is the girdling on the stem with brown colour and sometime upward rolling of the leaves also observed. Black scurf phase is formation of sclerotia on the surface of the tubers. This phase is more common in the field, particularly at the stage of plant senescent. *Rhizoctonia solani* has wide host range, and it is soil and seed borne in nature. Seed treatment by chemicals is effective against seed borne. However, biological control is a better option than chemical control in relation to creating pollution in the environment. The seed treatment with 1.5% boric acid followed by an application of a *T. viride* formulation containing 1×10^7 c.f.u./g @ 4.5%/kg seed tubers at planting reduced the disease to level achieved with 3% boric acid spray [43]. Out of 28 isolates, nine bacterial strains were found to be antagonistic *in vitro*, reduced the fungal growth and caused the lysis of sclerotia of *R. solani* in a dual culture assay as well as in an extracellular metabolite efficacy test. The selected antagonistic bacteria were also characterized for growth promoting attributes, that is, phosphate solubilization, nitrogen fixation and indole acetic acid production. Biocontrol efficacy and per cent yield increase by these antagonists were estimated in a greenhouse experiment, and results showed that two *Pseudomonas* spp. StT2 and StS3 were the most effective with 65.1 and 73.9% biocontrol efficacy, as well as 87.3 and 98.3% yield increase, respectively [44]. Potato seed treatment showed higher efficacy than the soil drenching when both ways (seed treatment and soil drenching) separately used with fungal and bacterial bio-agents to manage the black scurf of potato [45]. The interaction of PGPR (*Bacillus* spp.) with potato seeds or vegetative parts showed promising antagonism through producing siderophore and antibiotics against black scurf and stem canker diseases of potato caused by *R. solani*, thereby resulting in increase of potato yield. The effectiveness of PGPR strain (*Bacillus* spp.) was observed in improving the yield of potato in greenhouse and in the field conditions [46]. Seed treatment by *T. viride* showed less disease index of black scurf of potato against control [47]. Whereas, when *T. viride* including other bio-agents compared, it was found that *T. harzianum* significantly inhibiting the mycelia growth of *R. solani* [48]. Bio-agents not only reduce the disease incidence but also increase the crop yield, compared to without the use of bio-agents [49]. Sunhemp and maize green manuring reduced the disease incidence of black scurf of potato [50]. Chopped leaf matter of brassica crops and barley inhibited growth of *Rhizoctonia*, while Indian mustard almost completely inhibited the mycelial growth of *R. solani* [51]. The antagonistic effect of microorganisms was evaluated after adding rhizospheric extracts of maize, oat, barley and grass on *Rhizoctonia*. It was observed that extracts from maize and grass rhizosphere were most antagonistic [52]. The antifungal efficacy of six botanical extracts and two bio-agents, viz. *T. harzianum* and *T. viride*, were evaluated *in vitro* against sclerotial isolates of *R. solani* causing black scurf of potato through food poison and dual culture technique, increasing concentration from 5 to 15% of botanical extract suppressed the mycelial growth of all isolates. Among the tested bio-agents, mycelial growth inhibition of *R. solani* isolates was recorded in the case of *T. harzianum* (up to 72.72%) and *T. viride* (up to 56.80%) [53].

5. *Fusarium* wilt/dry rot of potato

Fusarium dry rot is an important post-harvest disease of potato tubers. This disease is distributed worldwide and occurs wherever potatoes are grown [54]. *Fusarium* spp. cause fusarium wilt in the field and under storage it causes dry rot of potato. *T. harzianum* (ANR-1) isolate was found to be effective in inhibiting the radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* (53%). Under greenhouse conditions, the application of *T. harzianum* (ANR-1) exhibited the least disease incidence (15.33%) and also found stimulatory effect on plant height (73.62 cm) and increased the dry weight (288.38 g) of tomato plants in comparison to other isolates and untreated control [55]. Immature crop plant amendments, viz. pearl millet, sesbania, sunhemp, maize and eucalyptus leaves, are used against fusarium wilt of potato. Among them, eucalyptus leaves and maize showed maximum suppressive and least was sesbania [56]. The combined effect of antagonists (*Trichoderma* and *Pseudomonas*) with modified montmorillonite particles (Mod- MMT) against *F. oxysporum* f. sp. *tuberosa* causes wilt of potato, showed less disease incidence and also enhanced plant height, fresh and dry weight, number of tubers/plant and weight of tubers [57]. Application of *Trichoderma koningii* and *Bacillus megaterium*, alone or in combination, seven days earlier than soil infestation with *F. oxysporum* and/or the mixed population of *Meloidogyne* spp., significantly reduced *Fusarium* wilt disease incidence and nematode infection on potato and improved plant growth components under greenhouse conditions. Generally, the mixture of the two biocontrol agents was more effective in controlling the plant disease and improving plant growth components than either of the two organisms used singly [58].

6. Common scab of potato

Potato common scab caused by pathogenic *Streptomyces* spp. is a serious disease in potato production worldwide. It occurs throughout the potato-cultivating regions of the world and is most prevalent in neutral or slightly alkaline soils, especially during dry years [59]. The disease symptoms are small brownish, shallow, raised or sunken and mostly appeared on tubers. The pathogen is both seed and soil borne. The pathogen is survived for longer period in the infected plant debris and soil. Biological control of common scab is one of the attractive approaches which can develop naturally in potato fields owing to antagonistic microorganisms and reduce the severity of disease [60, 61]. Three antagonistic fungi, that is, *T. harzianum*, *Penicillium digitatum* and *Aspergillus flavus*, were evaluated for biological management of common scab of potato. Results revealed that lowest disease incidence was observed with *T. harzianum* [62]. *Pseudomonas mosselii* when applied with vermicompost gave the best plant growth and yield along with maximum reduction in scab incidence and scab index [63]. Most actinomycete isolates derived from the Rice bran-amended soil showed antagonistic activity against pathogenic *Streptomyces scabiei* and *Streptomyces turgidiscabies* on R2A medium. Some of the *Streptomyces* isolates showed positive results when they were inoculated onto potato plants in a field condition. These results suggest that Rice bran amendment increases the levels of antagonistic bacteria against pathogenic strain of *Streptomyces* in the potato rhizosphere

[64]. Phage therapy is a new method to manage plant pathogens. Phage therapy has allowed disinfection of *S. scabiei*-infected seed potatoes and reduced tobacco bacterial wilt due to *R. solanacearum* by co-application with an avirulent strain of this bacterium [65, 66]. The culture broth of *Bacillus* sp. *sunhua* had a suppressive effect on common scab disease in a pot assay, decreasing the infection rate from 75 to 35% [67]. Non-virulent potato isolates of *Streptomyces* spp., with antagonistic activity higher than PonSSII, significantly reduced scab in pot experiments. Two non-pathogenic strains of *Streptomyces*, viz. *S. diastatochromogenes* strain PonII and *S. scabies* strain Pon R found to be effective against the pathogenic strain of *S. scabies* of potato in 4-year field experiments [68, 69].

7. Black leg of potato

Black leg of potato caused by different species of bacteria, viz. *Pectobacterium* spp. (*Erwinia* spp.) and *Dickeya* spp. [70]. Both are pectinolytic in nature and represent a significant threat for seed potato production in Europe. *Dickeya* spp. induce various symptoms such as plant wilting, stem rot (blackleg) and tuber soft rot [71]. The bacteria live over in soil in decaying plant debris and sometimes in seed tubers. *Pseudomonas* spp. and *Bacillus* spp. were evaluated against *Pectobacterium* spp. The antagonistic properties of different *Pseudomonas* spp. strains, such as iron competition, 2,4-diacetylphloroglucinol (DAPG) antibiotic synthesis via pyoverdine and pseudobactin production and their related receptors, were found to be the means of protection [72, 73] against *Pectobacterium* spp. *Bacillus subtilis* strains were tested for the control of potato diseases caused by *Pectobacterium* spp., and results revealed reduced maceration symptoms in planta [74]. A bacteriocin-like substance produced by *Bacillus licheniformis* P40 was bactericidal to *Pectobacterium carotovorum* subsp. *carotovorum*. This substance interacted with cell membrane lipids, provoking lysis of *P. carotovorum* subsp. *carotovorum* cells. It was also effective in protecting potato tubers against soft rot under standard storage conditions [75]. Different strains of *P. fluorescens* were used to protect wounds and cracks on tubers from colonization by *Pectobacterium atrosepticum*. Application of individual and combinations of strains reduced the contamination of potato tuber peel by 85% and 60–70%, respectively, indicating the potential of *Pseudomonas* spp. for controlling soft rot caused by *Pectobacterium atrosepticum* [76]. The bacteria are able to degrade quorum-sensing signal molecules produced by *Pectobacterium* spp. and *Dickeya* spp., which is a useful and effective strategy for the control of the bacteria by preventing the secretion of large quantities of pectolytic enzymes to macerate tuber tissues [77].

8. Bacterial wilt of potato

Bacterial wilt caused by *R. solanacearum* (Smith) Yabuuchi *et al.* is one of the most important and destructive bacterial diseases, widely distributed in tropical, subtropical and some warm temperate regions of the world [78]. This disease affects the potato crop in 3.75 million acres in about 80 countries with global damage estimate exceeding \$ 950 million per year. It damages

the crop in two ways: first way, premature wilting and death of plants and second way, causing rot of tubers in storage and transit [79]. The pathogen is soil and seed borne in nature. Bacterial wilt has become a limiting factor in potato cultivation that may cause yield loss to the tune of 30–70 % in India [80]. Avirulent strains of *R. solanacearum*, *Pseudomonas* spp., *Bacillus* spp. and *Streptomyces* spp. are well-known biocontrol agents (BCAs). New or uncommon BCAs have also been identified, such as *Acinetobacter* sp., *Burkholderia* sp. and *Paenibacillus* sp. [81]. Vesicular-arbuscular mycorrhizae (VAM) is known to reduce disease incidence and enhance plant growth. The potential of vesicular-arbuscular mycorrhizae was evaluated for protection of plants from bacterial wilt in the Philippines; VAM increased growth and yield of tomatoes and reduced infection by *R. solanacearum*. This may be due to competition or the mechanical barrier in the form of VAM vesicles and hyphae that inhibit the bacterial pathogen from deeper penetration into host tissues [82]. Treatment of tubers with avirulent strain of *R. solanacearum* and strain of *P. fluorescens* caused a significant reduction in disease severity of bacterial wilt of potato [83].

9. Conclusion

Different bio-agents including fungal and bacterial were reported by various researchers for management of potato diseases. Efficacy of bio-agents is varied from lab to field conditions. It might be due to non-synchrony environment between lab and field. Some *Trichoderma* spp, *Pseudomonas* spp and *Bacillus* spp exhibited significant result to reduce the incidence of potato diseases under both lab and field. These bio-agents must be applied at larger scale. Moreover, new bio-agents with a wider range of adoptability still require to be explored. A bio-agent should be applied for specific disease where it performs highest efficacy and in particular regions. It is the important constituent of organic potato production system.

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