Efficacy of Bacterial Isolates against Causal Agent of Late Blight of Potato, *Phytophthora infestans*

Anju Rani¹, Sushil Kumar Upadhyay², Gyanika Shukla³, Chhaya Singh⁴, Raj Singh²

**ABSTRACT**

Background: Potato (*Solanum tuberosum*) is a main crop in subtropical and temperate regions as a vegetable crop. It is affected by *Phytophthora infestans* causing a devastating disease late blight. The chemical pesticides used against this disease in this present scenario cause pollution (soil as well as water) and adversely affect beneficial soil micro-flora, grazing animals and human beings.

Methods: An experiment was designed at the Department of Botany, K.V. Subharti College of Science, Swami Vivekanand Subharti University, Meerut (UP), India during 2017-2018. Three bacterial bioagents, *Pseudomonas* isolate 1 and 2 and *Bacillus* species was isolated from infected phyloplane and used against late blight pathogen, *P. infestans*. The efficacy of antagonistic bio-agents tested against mycelial growth, zoospore and zoosporangial germination of *P. infestans* by dual culture method and detached leaf method.

Result: The present study focused on use of bio-agents or antagonistic bacteria to control late blight pathogen showed concentration biased potential inhibitory activity against mycelial growth, zoospore and zoosporangial germination. It means higher the concentration of bacterial isolate, greater the inhibitory effect on various tested parameters. Among the three tested antagonistic bioagents, *Bacillus* showed maximum zone of inhibition to late blight pathogen. The proved that the *Pseudomoanas* and *Bacillus* as potential antagonistic agent for ecofriendly sustainable management of pathogenic mycoflora, *P. infestans*.

Key words: Antagonist, *Bacillus*, Bioagents, *Phytophthora infestans*, *Pseudomonas*, *Solanum tuberosum*.

**INTRODUCTION**

Late blight caused by the fungus *Phytophthora infestans*, has historically been an important disease of potato and tomato worldwide. In the mid 1800, the late blight caused widespread crop failures throughout Northern Europe including Ireland where it was responsible for the Irish famine (Saville *et al.*, 2016). Modern agro-ecosystems are dominated by genetically homogenous crop genotype, which favours the rapid development of plant diseases. Historically, such crop homogeneity led to the epidemics such as late blight that ravaged Irish potato crops in year 1884 and destruction of Texas male sterile cytoplasm genotypes by *Cochliobolus heterotrophus* in maize during 1970 in the USA (Levings, 1990; Frades and Andreasson, 2016). Conversely, natural ecosystems are usually less prone to rapid and severe epidemics, because host genotypes are more diverse and are distributed in small patches (Jarosz and Burdon, 1991). In western Uttar Pradesh, although potato cultivars resistant to late blight are available, a highly late blight susceptible cultivar ‘Kufri Bahar’ continues to be major cultivar due to inherent liking by the local farmers and consumers. Hence, farmers currently depend on fungicides for the effective management of late blight. Since potatoes are an important food crop for small farmers, the cost of fungicides is bound to be problematic. Small farmers have traditionally used mixtures of crop genotypes, which proved useful in bringing down the disease level in susceptible cultivars (Rhoades and Bebbington, 1990). Decades of research on application of eco-friendly methods for plant disease management have not yielded the desired result of complementing or totally replacing the pesticides. However, the number of fungicides that are available for control of plant diseases is dwindling due to rapid development of resistance by pathogens. Plant pathologists are therefore left with no choice but to re-evaluate the available viable alternatives such as biocontrol, inorganic salts and botanicals, which are ecofriendly, biodegradable and less expensive. Although biocontrol is rarely used for foliar diseases, numerous organisms capable of antagonizing fruit and leaf pathogens have been reported in recent years. Formerly, little attention was given to biocontrol of foliar pathogens, principally because the foliar microflora was known to consist of relatively few organisms whose populations fluctuate dramatically according to the

1K.V. Subharti College of Science, Swami Vivekanand Subharti University, Meerut-250 005, Uttar Pradesh, India.
2Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana-133 207, Ambala, Haryana, India.
3Department of Genetics and Plant Breeding, Ch. C.S. University, Meerut-250 001, Uttar Pradesh, India.
4Government Degree College, Thalisain-246 285, Uttarakhand, India.

Corresponding Author: Raj Singh, Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana-133 207, Ambala, Haryana, India. E-mail: dr.rajsingh09@gmail.com

How to cite this article: Rani, A., Upadhyay, S.K., Shukla, G., Singh, C. and Singh, R. (2020). Efficacy of Bacterial Isolates against Causal Agent of Late Blight of Potato, *Phytophthora infestans*. Indian Journal of Agricultural Research. 10.18805/IJARe.A-5548

Submitted: 06-03-2020   Accepted: 01-09-2020   Online: 19-12-2020
environmental conditions (Wicaksono et al., 2018). The rapid growth of foliage provides better opportunities for pathogen growth rather than organisms in the phyllosphere (Lindow and Brandl, 2003). One example of biological control of an airborne foliar disease is the use of the antagonistic bacteria Pseudomonas cepacia and Bacillus subtilis to control Monilia pod rot in cocoa (Lumsden et al., 1988). El-Sheikh et al. (2004) recovered eighty-three bacterial isolates from tuber surface, root surface and rhizosphere of potato samples and reported that seven isolates of Pseudomonas sp. and 6 isolates of Bacillus sp. exhibited antagonistic effects on P. infestans in vitro. Pseudomonas treatment exhibited higher potential as biocontrol agents and suppressed late blight severity to 27.6% compared to 40% for Bacillus treatments. Stephan et al. (2002) evaluated 122 microorganisms isolated from the phyllosphere of potatoes on the development of P. infestans. They reported that when 23 effective microorganisms (spore-forming and nonspore-forming bacteria, yeasts and fungi) were tested in dual cultures, different patterns of inhibition of P. infestans were observed. Trichoderma viridis and Pseudomonas fluorescens were observed to be highly effective against Alternaria solani (Verma et al., 2018). There are many bioagents which have broad spectrum activity against phytopathogens (Daranas et al., 2019). Selection of bioagents to control the pathogen is not the easy task due to huge diversity of phytopathogen and their interaction with the host (Mota et al., 2017). To develop more sustainable agricultural practices that include alternatives to chemicals for controlling pests and diseases, is a well-known biological control method (Ab-Rahman et al., 2018).

**MATERIALS AND METHODS**

The experiment was designed at the Department of Botany, K.V. Subharti College of Science, Swami Vivekanand Subharti University, Meerut (UP), India during 2017-2018. The potential of selected bacterial isolates against Phytophthora infestans causal agent of late blight of potato was tested as per the protocol given below:

**Isolation of phylloplane microflora against P. infestans**

Three bacterial isolates Bacillus, Pseudomonas isolate 1 and Pseudomonas isolate 2 were used to check their antimicrobial activity against late blight pathogen Phytophthora infestans by using dual culture method, food poison technique, spore germination and detached leaf method. Freshly infected late blight leaves were collected in perforated polythene bags and brought to the laboratory for isolation of bacteria by serial dilution method (Aneja, 2007). Infected leaves were chopped into bits of 10mm in sterile petriplates using a sharp razor. Five infected bits were then transferred to test tubes containing 10ml sterile distilled water. Test tubes containing the leaf bits were agitated using the vortex mixer. For all five leaf bits, one ml from each of the $10^{-1}$ and $10^{-2}$ was then transferred aseptically to sterile petriplates to which approximately 20ml of King’s B agar medium was added (King et al., 1954). After solidification of the media, petridishes were incubated at 30°C. King’s B agar medium containing petri-dishes were monitored regularly for the growth of bacterial colonies. Once the bacterial colonies were detected on the King’s B agar medium, these were transferred aseptically to the sterile NAM (Nutrient Agar Medium) slants for maintenance, identification and testing. In vitro isolates of bacteria were identified at generic level by following the method of Schaad (2001). The isolated microflora consisting bacterial isolates were tested against P. infestans at the first instance by dual culture method. Bacterial isolates that showed effectiveness in dual culture method were further tested for their effectiveness against spore germination and in detached leaf. Based on the result of detached leaf test, effective isolates were further tested by whole plant method.

**Dual culture method**

Three bacterial isolates were spotted at equidistant point along the perimeter of the plate and simultaneously 6mm plug from the leading edge of 7-day-old culture of P. infestans was placed at the centre of the plate. Plates without bacteria served as control. Plates were incubated at 18°C till such time when the growth in the control covered the entire petriplate. Culture filtrate was prepared for further testing.

**Preparation of culture filtrate**

The 60ml nutrient broth were poured into conical flasks and steam sterilized at 15lbs for 15 min. For each conical flask containing nutrient broth, bacterial cultures were transferred using a sterile loop to nutrient broth. Bacterial cultures were incubated at 29°C in an incubator for seven days with occasional shaking of the flasks for 15min. After 7 days, filtrate was sterilized through 0.22µm Millipore membrane filter. The membrane filtered culture filtrates were stored in sterile vials in refrigerator till further use and tested at 5, 10, 20 and 50% concentration against the target pathogen.

**Effect of culture filtrate on mycelial growth**

Membrane filtered culture filtrate of bacterial isolates were tested against P. infestans at 1, 5 and 10% by food poison technique (Grover and Moore, 1962). Calculated volume of the culture filtrate was added to the molten Rye B medium and poured aseptically to the petriplates and allowed to solidify. The 5mm of freshly growing P. infestans culture was placed at the centre of the petridish. Non-amended medium served as the control. In all, three replications were maintained for each treatment. Radial growth of the fungi in petridishes was measured soon after the growth in control plates reached 90mm. The inhibition percentage was recorded.

**Effect of culture filtrate on zoosporangial germination**

Stock solution of each membrane filtered culture filtrate was prepared in sterile distilled water and 20µl of the stock solution was mixed gently with 20µl of the zoosporangial suspension in a cavity slide so as to get the final desired
Germ tube growth was unaffected at 5% isolate 1 and 2 showed inhibition zone up to by detached leaf. Sporangia was confirmed by microscopic studies. Effector of culture filtrate under detached leaf method Zoosporangial suspension, which was calibrated to an optimum concentration of 2.5 x 10^4/ml was allowed for germination by incubating at 12°C for one hr. The zoospores released were used for testing the effects of culture filtrate on the spore germination at various concentrations. Appropriate volume of culture filtrate and zoospores were mixed at equal proportion of 20µl and was placed in each cavity slide. Observation on zoospore germination and germ tube length were taken after five hr. At the end of the experiment, a drop of cotton blue was added to arrest the further growth of germ tube. The 20 zoospores / microscopic field and five such microscopic fields constituted one replication. In all, three replications were maintained for each treatment. Germ tube length was recorded by using a haemocytometer.

Effect of culture filtrate on zoospore germination Zoosporangial suspension, which was calibrated to an optimum concentration of 2.5 x 10^4/ml was allowed for germination by incubating at 12°C for one hr. The zoospores so released were used for testing the effects of culture filtrate on the spore germination at various concentrations. Appropriate volume of culture filtrate and zoospores were mixed at equal proportion of 20µl and was placed in each cavity slide. Observation on zoospore germination and germ tube length were taken after five hr. At the end of the experiment, a drop of cotton blue was added to arrest the further growth of germ tube. The 20 zoospores / microscopic field and five such microscopic fields constituted one replication. In all, three replications were maintained for each treatment. Germ tube length was recorded by using a haemocytometer.

Effect of culture filtrate under detached leaf method Membrane filtered culture filtrates of three bacterial isolates were again tested against P. infestans by detached leaf experiment at 25, 50 and 100% concentration. Potato plants of late blight susceptible cultivar ‘Kufri Bahar’ was raised in 20cm earthen pots in the glasshouse. Six-seven weeks old healthy plants were selected from which fully expanded leaflets were gently removed preferably from the middle of the plant, washed gently in running tap water and allowed to shade dry over a blotting paper. Leaflets were placed in plastic trays (400mm L x 300mm B x 90 mm H) on perforated plastic separators (360mm L x 260mm B - Plate 2) with 80 holes (10 x 8) and inoculated with 20µl zoosporangial suspension containing 6x10^4 zoospores/ml using auto pipette. In all, 5 replications were maintained for each treatment and three leaflets constituted one replication. Trays containing the inoculated leaflets were incubated in an environmental chamber at 18°C. At the end of the experiment on the fifth day, number of sporangia/cm^2 of leaflet and lesion area were recorded. The presence of sporangia was confirmed by microscopic studies.

The greatest width and length of individual lesions was measured after 5 days and based on this, lesion area was calculated using the following formula

\[ \text{LA} = \pi/4ab \]

Where

\( \text{LA} \) = Lesion area; \( a \), length of lesion; \( b \), width of lesion (Singh and Bhattacharyya, 1995).

Sporulation capacity (number of zoosporangia/cm^2) was also measured after 5 days of incubation. The sporulating lesions of all the leaflets were cut and dipped in vials containing 10ml of 10% ethyl alcohol. The vials were stored at 4°C and sporangia were counted using haemocytometer. The 25ml of various concentrations of culture filtrate was prepared in sterile distilled water and sprayed on to the detached leaf till run off and shade dried. In all, 5 replications were maintained for each treatment and three leaflets constituted one replication. Inoculation of leaves, incubation and observations on lesion size and number of sporangia in different treatments were made as stated earlier.

RESULTS AND DISCUSSION

Dual culture test
Three bacterial isolates were tested against P. infestans by dual culture method. Among these bacterial isolates, Bacillus showed maximum inhibition zone of 6.0mm while Pseudomonas isolate 1 and 2 showed inhibition zone up to 5.7mm (Fig 1, Table 1). Effect of culture filtrates of three bacterial isolates (Bacillus sp., Pseudomonas isolate 1 and 2) showed > 75% inhibition and at 10% filtrate concentration these showed complete (100%) inhibition.

Effect of phylloplane microflora against zoosporangial and Zoospore germination
All the isolated phylloplane bacterial isolates were tested against the zoosporangial and zoospore germination at 5, 10, 20 and 50% concentration (Table 2). At 5% concentration, bacterial isolates showed little effect on zoosporangial germination, however at 50% concentration, the inhibition ranged between 65.7 to 86.9% (Fig 2). Similarly, zoospore germination was affected by little to severe at 5 to 50% concentration respectively with maximum inhibition (87.7%) recorded by Bacillus sp. (Fig 3). Germ tube growth was unaffected at 5% concentration, however at higher concentration of 20%, the germ tube length was reduced by 50% of the untreated control and at 50% concentration the germ tube was further reduced to 23.8 to 26.3µm compared to 90.8µm in untreated control (Fig 4).
**Table 1: In vitro effect of antagonistic bacterial isolates against Phytophthora infestans.**

| Name            | Dual culture test | Food poison technique |
|-----------------|-------------------|-----------------------|
|                 | Inhibition zone   | Mycelial inhibition % by culture filtrate |
|                 | (mm)              | 1%                    | 5%   | 10%  |
| Pseudomonas isolate 1 | 5.7*             | 0.0*                  | 81.5*| 100* |
| Pseudomonas isolate 2 | 4.0*             | 0.0*                  | 77.8*| 100* |
| Bacillus sp.      | 6.0*             | 16.7*                 | 88.9*| 100* |

*Numbers in each column followed by the same letter are not significantly different according to Duncan’s multiple range test (p=0.05).

**Fig 2: In vitro effect of bacterial isolates on the zoosporangial germination of Phytophthora infestans.**

**Table 2: Effect of bacterial isolates on the zoosporangial, zoospore and germ tube germination of Phytophthora infestans.**

| Name of isolate | Zoosporangial germination | Zoospore germination | Germ tube length (µm) |
|-----------------|---------------------------|----------------------|-----------------------|
|                 | 5%                        | 10%                  | 20%                   |
|                 | 20%                       | 50%                  | 1%                    |
|                 | 5%                        | 10%                  | 20%                   |
|                 | 50%                       |                      | 1%                    |
| Pseudomonas isolate 1 | 6.0                  | 10.9                 | 33.7                  |
| Pseudomonas isolate 2 | 13.8                | 28.0                 | 65.9                  |
| Bacillus sp.     | 5.2                      | 24.3                 | 35.2                  |
| Control          | 0.0                      | 0.0                  | 0.0                   |

*Values in parenthesis are arc sine transformed values.

**Fig 3: Effect of bacterial isolates on the zoospore germination of Phytophthora infestans.**
Efficacy of Bacterial Isolates against Causal Agent of Late Blight of Potato, *Phytophthora infestans*

**Fig 4:** *In vitro* effect of bacterial isolates on germ tube length (µm) *Phytophthora infestans* at different concentrations.

**Fig 5:** Effect of culture filtrate of bacterial isolates on late blight development in detached leaves test.

**Effect of bacterial isolates against *P. infestans* in detached leaf test**

All the three selected bacterial isolates were tested by detached leaf method for lesion size and sporangial production at 25, 50 and 100% culture filtrate. Culture filtrates of three bacterial isolates *Bacillus* sp., *Pseudomonas* sp. isolates 1 and 2 were ineffective at 25% and 50% concentration while at 100% concentration, these were moderately effective in reducing the lesion size. At 25 and 50% concentration these isolates were ineffective in reducing the sporulation, however at 100% concentration, sporulation was reduced by almost 50% of the untreated control (Fig 5, Table 3).

The findings of present investigation reflected the potential antifungal activity of selected microflora and can be utilized as a biocontrol agent against the fungal pathogens of economic crops. Since biological control is the emerging strategy to control fungal phytopathogens and chances of pollution can be reduced due to the usage of chemical fungicides (Rani et al., 2017). Out of three bacterial isolates, *Bacillus* showed maximum zone of inhibition against *P. infestans* *in vitro*. Despite there are many bacterial cultures isolates that exhibited a vital range to control *in vitro* antagonistic activity of many different phytopathogenic fungi and protect the crops (Prapagdee et al., 2008; Agaras et al., 2015). The negative effect of bacterial isolate on zoosporangium and zoospore germination along with length of germ tube was remarkable and showed concentration biased inhibitory activity. It means higher the concentration of bacterial isolates, greater the inhibitory effect on germination of zoosporangium, zoospore and germ tube of *P. infestans*. Klebsiella sp., *Pseudomonas* sp. Isolates and *Streptomyces hygroscopicus* proved to be potential bioagents against many fungi like *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum* (Stockwell and Stack, 2007; Siddiqui and Khan, 2017). The inhibitory efficacy of target bacterial culture filtrate was assessed in vitro and showed moderate inhibitory effect on leaf lesion size at highest concentration. Not only bacterial isolates even many fungal isolates showed antagonistic effect against many phytopathogens like *Fusarium, Rhizoctonia, P. infestans* (Rani et al., 2006, 2007). *Streptomycyes* is a broad and highly potent inhibitory phenotype that might be competitive ‘hot spots’ to inhibited rice rhizosphere (Schlatter and Kinkel, 2014; Behie et al., 2017). Ntushelo et al. (2019) stated that *Bacillus* sp. showed insignificant antagonistic activity against *F. graminearum* and this bacterium has potential use for Bio-agriculture to supplement the despised control measures. *Pseudomonas* strains (RB15, RB30) and *B. cereus* (RB13) were reported cyanogen as well as siderophore producers along with having...
antagonistic activity against fungal phytopathogens (Rani et al., 2016). Shoot extract of *Lotus aegaeus* and *L. corniculatus* and shoot extracts of all solvents of *L. angustissimus* showed potential antibacterial activity against *Clavibacter michiganensis* and *Pseudomonas phaseolicola* respectively (Demirkol, 2018). According to Padmaja et al. (2011), lactic acid bacteria like *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Lactococcus* showed potential antimicrobial activity. Lactobacilli and Lactococci species showed maximum antagonistic activity in cabbage isolate. The *Pediococci* and *Leuconostoc* species showed maximum antimicrobial activity in banana and tomato isolates respectively. In the present scenario environmental conditions changed adversely so that bio-control method has not always produced significant results (Wicaksano et al., 2018; Iftikhar et al., 2020). The authors wish to propose the bio-control activity in the field by work on formulation as future perspective.

**CONCLUSION**

Late blight an important disease of potato crop, caused by *Phytophthora infestans*. Farmers currently depend on fungicides for the effective management of late blight. Since potatoes are an important food crop for small farmers, the cost of fungicides is bound to be problematic. However, the number of fungicides that are available for control of plant diseases is dwindling due to rapid development of resistance by pathogens. Plant pathologists are therefore left with no choice but to re-evaluate the available viable alternatives such as biocontrol, inorganic salts and botanicals, which are ecofriendly, biodegradable and less expensive. Present study focused on the use of bioagents or antagonistic microorganisms like bacteria to control late blight pathogen. Three bacterial bioagents, *Pseudomonas* isolate 1 and 2 and *Bacillus* species were used on late blight pathogen tested against mycelial growth, zoospore and zoosporangial germination and proved as significant antagonistic agent for ecofriendly sustainable management of pathogenic mycoflora.

**ACKNOWLEDGEMENT**

Authors express profound gratitude to Dr. Hasrat Ali, HOD Botany, K.V. Subharti College of Science, Swami Vivekanand Subharti University, Meerut (UP) for laboratory facilities and Dr. M.N. Bhat, Principal Scientist, Plant Pathology, IARI, New Delhi, who helped a lot during current work.

**Author contributions**

Anju Rani set up whole experiment in the laboratory. Dr Sushil Kumar Upadhyay and Gyanika Shukla prepared and organized the complete manuscript content from introduction to result and discussion. The references were organized as per journal format by Chhaya Singh. Dr. Raj Singh checked and reviewed the entire manuscript critically.

**Conflict of interest statement**

The authors declare that this research was completed without any commercial or financial relationships that could be construed as a potential conflict of interest.

**REFERENCES**

Ab-Rahman, S.F.S., Singh, E., Pieterse, C.M.J., Schenk, P.M. (2018). Emerging microbial biocontrol strategies for plant pathogens. Plant Sciences. 267: 102-111.

Agaras, B.C., Scandiani, M., Luque, A., Fernández, L., Farina, F., Carmona, M., Gally, M., Romero, A., Wall, L., Valverde, C. (2015). Quantification of the potential biocontrol and direct plant growth promotion abilities based on multiple biological traits distinguish different groups of *Pseudomonas* spp. isolates. Biological Control. 90: 173-186.

Aneja, K.R. (2007). Experiments in microbiology, plant pathology and biotechnology. New Age International. 665p.

Behie, S.W., Bonet, B., Zacharia, V.M., McClung, D.J. and Traxler, M.F. (2017). Molecules to ecosystems: Actinomycete natural products in situ. Frontiers in Microbiology, 17(7): 2149. doi: 10.3389/fmicb.2016.02149.

Daranas, N., Roselló, G., Cabrera, J., Donati, I., Francés, J., Badosa, E., Spinelli, F., Montesinos, E., Bonaterra, A. (2019). Biological control of bacterial plant diseases with *Lactobacillus plantarum* strains selected for their broad spectrum activity. Annals of Applied Biology. 174(1): 92-105.

Demirkol, G. (2018). Antibacterial activity of the seeds, roots and shoots of *Lotus* populations. Legume Research: An International Journal. 41: 778-783.

El-Sheikh, M.A., El-Korany, A.E. (2004). Screening for bacteria antagonistic to *Phytophthora infestans* for the organic farming of potato. Alexandria Journal of Agricultural Research. 47(3): 169-178.
Efficacy of Bacterial Isolates against Causal Agent of Late Blight of Potato, *Phytophthora infestans*

Frades, I. andreasson, E. (2016). *Phytophthora infestans* specific phosphorylation patterns and new putative control targets. Fungal Biology. 120(4): 631-644.

Grover, R.K., Moore, J.D. (1962). Toximetric studies of fungicides against brown rot organisms. *Sclerotinia fruticola* and *s-laxa*. Phytopathology. 52(9): 876-879.

Iftikhar Y., Sajid, A., Shakeel, Q., Ahmad, Z., UlHaq, Z. (2020). Biological Antagonism: A safe and sustainable way to manage plant diseases. In: Plant disease management strategies for sustainable agriculture through traditional and modern approaches. Sustainability in Plant and Crop Protection, volume 13. Springer, Cham.

Jarosz, A.M., Burdon, J.J. (1991). Host-pathogen interactions in natural populations of *Linum arginale* and *Melamp soralinii*. II. Local and regional variation in patterns of resistance and racial structure. Evolution. 45(7): 1618-1627.

King, E.O., Ward, M.K., Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. The Journal of Laboratory and Clinical Medicine. 44(2): 301-307.

Levings, C.S. (1990). The Texas cytoplasm of maize: Cytoplasmic male sterility and disease susceptibility. Science. 250: 942-947.

Lindow, S.E., Brandl, M.T.2003. Microbiology of the phyllosphere. Applied Environmental Microbiology. 69(4): 1875-1883.

Lumsden, R.D., Rishbeth, J., Gibbs, J.N., Hamilton, W.D., Cook, R.J. (1988). Biological control of air-borne pathogens: Discussion. Philosophical Transactions of the Royal Society of London Series B. 318: 280-281.

Mota, M.S., Gomes, C.B., Souza Júnior, I.T., Moura, A.B. (2017). Bacterial selection for biological control of plant disease: criterion determination and validation. Brazilian Journal of Microbiology. 48(1): 62–70.

Ntushelo, K., Ledwaba, L.K., Rauwane, M.E., Adebo, O.A., Njobeh, P.B. (2019). The mode of action of *Bacillus* species against *Fusarium graminearum*, tools for investigation and future prospects. Toxins. 11(10): 606.

Padmaja, G.A., Ramachandra, B., Manjunath, H., Prabha, R., Krishna, R., Shankar, P.A. (2011). Characterization of lactic acid bacteria isolated from fruits and vegetables for their antibacterial activity. Asian Journal of Dairy and Food Research. 30: 85-89.

Prapagdee, B., Kuekulvong, C., Mongkolsuk, S. (2008). Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. International Journal of Biological Sciences. 4(5): 330-337.

Rani, A., Bhat, M.N., Singh, B.P. (2007). Effect of potato phylloplane fungi on potato late blight pathogen *Phytophthora infestans*. Journal of Mycology Plant Pathology. 37: 413-417.

Rani, A., Singh, R., Kumar, P., Shukla, G., Singh, C. (2017). Eco-friendly management of late blight of potato caused by *Phytophthora infestans* (L). International Journal of Pharmaceutical Research. 9(3): 21-27.

Rani, A., Bhat, M.N., Singh, B.P. (2006). Efficacy of neem formulations against late blight of potato in sub-tropical plains. Crop Research. 31(1): 179-180.

Rani, U., Choudhary, P., Rao, A.S. (2016). Antifungal properties exhibited by bacteria isolated from agriculturally cultivable soils and their antagonistic nature towards fungal phytopathogen suppression. Agricultural Science Digest. (36): 17-23.

Rhoades, R.E., Bebbington, A.J. (1990). Mixing it up: Variations in Andean farmers’ rationales for intercropping of potatoes. Field Crops Research. 25(1-2): 145-156.

Saville, A.C., Martin, M.D., Ristaino, J.B. (2016). Historic late blight outbreaks caused by a widespread dominant lineage of *Phytophthora infestans* (Mont.) de Bary. PloS One. 11(12): e0168381.

Schaad, N.W., Jones, J.B, Chun, W. (2001). Laboratory guide for the identification of plant pathogenic bacteria (edition, 3). American Phytopathological Society. 454p.

Schlatter, D.C., Kinkel, L.L. (2014). Global biogeography of *Streptomyces* antibiotic inhibition, resistance and resource use. FEMS Microbiology Ecology. 88(2): 386-397.

Siddiqui, Z. A., Khan, M. (2017). Biofilm formation by *Pseudomonas* spp. and their significance as a biocontrol agent. Biofilms in Plant and Soil Health. doi.org/10.1002/9781119246329. ch5.

Singh, B.P., Bhattachayya, S.K. (1995). Field resistance to late blight of four Indian potato cultivar. Potato Reseach. 38: 171-178.

Stephan, D., Koch, E. (2002). Screening of plant extracts, micro-organisms and commercial preparations for biocontrol of *Phytophthora infestans* on detached potato leaves. IOBC WPRS Bulletin. 25(10): 391-394.

Stockwell, V.O., Stack, J.P. (2007). Using *Pseudomonas* spp. for integrated biological control. Phytopathology. 97(2): 244-249.

Verma, A., Kumar, S., Harshita, A.S., Jaiswal, S. (2018). Evaluate the efficacy of bio-control agents and botanicals against early blight of potato caused by *Alternaria solani*. The Pharma Innovation Journal. 7(3): 28-30.

Wicaksono, W.A., Jones, E., Casonato, S., Monk, J., Ridgway, H.J. (2018). Biological control of *Pseudomonas syringae pv. actinidiae* (Ps), the causal agent of bacterial canker of kiwifruit, using endophytic bacteria recovered from a medicinal plant. Biological Control. 116: 103-112.