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Phage Therapy: A Promising Tool to Mitigate Bacterial Blight Disease in Pomegranate Cultivation

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Abstract

Classification of bacteriophages is based on their life cycle as they are specific infectious agents for different bacteria. Lytic phages, which possess the ability to kill their host cells, can be utilized to selectively eliminate pathogenic bacteria. Phage therapy was extensively utilized until the Second World War but declined in use with the advent of antibiotics, as documented over a century ago. However, there has been a resurgence in this traditional method in recent years because of the increased expenses of developing new antibiotics and the rapid increase of bacteria that are resistant to multiple drugs. As a result, the use of phage-based treatment has been authorized currently. This article presents a comprehensive review of the utilization of phage therapy to manage bacterial blight disease in pomegranate cultivation.

Keywords- Bacteriophage, Phage Therapy, *Xanthomonas axonopodis pv punicae*, Bacterial Blight Disease

Introduction

After a decade-long decline, the prevalence of world hunger has increased, impacting almost 10% of the global population, as stated in the report on the Global Hunger Crisis 2023. As per this report, from 2019 to 2022, the number of individuals suffering from undernourishment increased by 150 million. The current state of affairs can be mainly traced back to causes including conflict, climate change, and the unprecedented COVID-19 pandemic. Climate change intensifies the probability of outbreak occurrences by modifying the evolution of pathogens and the interactions between hosts and pathogens, ultimately enabling the emergence of novel pathogenic strains. Besides, a shift in pathogen range might occur, which could result in an escalation in the transmission of plant diseases in previously unimpacted regions (Singh, 2023).

The United Nations has declared the year 2020 as the International Year of Plant Health (Food and Agriculture Organization of the United Nations 2019). Fedoroff, 2015 anticipated that to sustain a population of 10 billion on Earth by 2050, there will be a need for a 60% rise in food production. To satisfy the worldwide requirement, it is imperative to increase production capacity whilst simultaneously mitigating the loss of food attributed to pathogens as well as pests, in addition to minimizing food waste (Savary, 2019; Delgado, 2021). Phytopathogenic bacteria have been identified as the cause of economically devastating crop diseases that are prevalent. Various methods are used to treat plant phytopathogens, including bactericides with different chemical compositions, such as organic compounds containing metals or plant-based extracts. Despite the efforts, there are still technical or economic challenges associated with these approaches, and the search for efficient and eco-friendly means of protecting plants from phytopathogenic bacteria remains ongoing.

To fulfill the rising demand for food across the globe due to the surge in human population, it is of utmost importance to mitigate the crop yield losses caused by plant infections. There is a well-established requirement to develop novel strategies for environmentally friendly control of bacterial crop diseases. Due to bacterial resistance, traditional chemical and antibiotic control methods are becoming less effective. Moreover, it is widely accepted that these measures harm the environment. In recent years, bacteriophages, viruses of bacteria, have gained significant attention as a viable and eco-friendly solution for managing bacterial diseases. Bacteriophages, which are viruses with a specific target for bacteria are self-reproducible and are self-eliminating. Shortly after their discovery, the proposal to employ bacteriophages as agents for biocontrol was put forth (Twort 1915; D'Herelle 1917).

Utilization of bacteriophages appears to be a feasible strategy for managing numerous deleterious bacterial crop ailments, as evidenced by the presence of certain phage-derived products in the marketplace. Phage Biocontrol offers an advantage over traditional chemical control methods due to the possibility of customizing phage cocktails to target specific bacteria responsible for diseases and being able to modify them easily to avoid bacterial resistance. The objective of this review is to investigate the possibility of using phage therapy to treat bacterial blight disease in pomegranates.; the Indian pomegranate industry has experienced significant yield losses of 60-80% due to bacterial blight disease induced by *Xanthomonas axonopodis pv. punicae* (Ramesh, 1993; Hingorani, 1952).

Pomegranate Plant

Pomegranate (*Punica granatum L.*) holds great importance in tropical and subtropical regions worldwide. It is commonly referred to as Anar, Dalimb, or Matulum. The pomegranate's roots can be dated back to Iran, during the year 2000 B.C., where it was cultivated initially. Presently, it is significantly grown in several countries, including China, Egypt, India, Iran, Japan Morocco, Pakistan, Russia, Spain, and the USA. The pomegranate is identified as the 18th most significant fruit crop in terms of production globally. Commercial cultivation of pomegranates in India is mainly concentrated in arid and semi-arid regions including Andhra Pradesh, Gujarat, Karnataka, Maharashtra, and Tamil Nadu despite the fruit's presence in other parts of the country. The total area of pomegranate cultivation in India is 278000 Ha and annual production is 318000 Mt (Indian Horticulture database 2021-22).

In Maharashtra, the production of pomegranate is primarily concentrated within two regions, namely Western Maharashtra and Marathwada. The commercial cultivation of fruit occurs in multiple districts of Maharashtra, including Ahmednagar, Aurangabad, Dhule, Latur, Nashik, Osmanabad, Pune, Satara, and Solapur. The popular pomegranate varieties in this region consist of Bhagwa, Super Bhagwa, Arakta, Ganesh, Mrudula, and Dholka. The pomegranate is a fruit that is rich in vital minerals namely phosphorus, iron, and calcium, in addition to vitamins 'B' and 'C'. Its popularity stems from its cool and refreshing juice, as well as its therapeutic properties. The bark and peel of the fruit are frequently utilized in traditional medicine to manage diarrhea and dysentery. Furthermore, fruit juice has been used as a remedy for leprosy (Jadhav, 2023).

Successful cultivation of pomegranates primarily relies on the dry and semi-arid climatic conditions, characterized by cold winters and hot, arid summers that facilitate fruit production. Pomegranate plants exhibit a considerable degree of frost tolerance and are often deemed drought-resistant. The ideal temperature range for optimal fruit development typically spans between 35-38°C. (The Ultimate Guide for Pomegranate Farming, 2023).

Historical background of Pomegranate diseases

The history of plant diseases that affect pomegranates can be traced back several centuries, with a multitude of pathogens and disorders having detrimental impacts on the health and productivity of these plants.

1. Pomegranate Wilt -

Pomegranate wilt is a devastating disease that is widely prevalent and was initially documented in Nashik, Maharashtra in 1978 by Somasekhara (1999). The extent of wilt damage ranges from 5 to 65.0% and is based on the ailment's severity. Visible symptoms of wilt include branch foliage yellowing, followed by tree-wide foliage wilting and death. The primary cause of pomegranate wilt has been observed to be *Ceratocystis fimbriata* (Somasekhara, 1999; Sharma et al., 2010). In addition to *C. fimbriata*, various pathogens, such as *Fusarium spp.* (*Fusarium oxysporum* as noted by Chavan and Dake in 2001), *Macrophomina phaseolina*, Root-knot nematode (*Meloidogyne incognita*), *Rhizoctonia bataticola*, Stem borer (*Celosterna spinator*), and Shot hole borer (*Xyleborus fornicatus*), have been observed to induce wilt infections.

2. Alternaria black spot -

The quality of pomegranate production is affected by primary pathogens, namely *Alternaria* and *Colletotrichum* species, leading to the onset of the disease (Munhuweyi, 2016). The symptoms include the emergence of brown, circular, diminutive, and reddish blemishes on the surface of fruits. *A. alternata* has been observed to induce lesions on young fruits, flowers, and leaves (Pala et al. 2009). In advanced infection stages, these markings merge to produce larger patches, initiating the process of decay within the fruit. Furthermore, the affected arils undergo a transition in hue from red to pallid yellow, rendering them unsuitable for consumption. To reduce the proliferation of this disease, it is advised that contaminated fruit be gathered and destroyed. (Indian Horticulture Database, 2012).

3. Anthracnose-

Anthracnose, a fungal disease, displays a range of symptoms such as blighted shoots, defoliation, blotches, distortion, leaf spots, dieback, and twig canker. Different species of *Colletotrichum* cause the disease, and it infects the entire plant, producing symptoms that vary depending on the cultivar. Various symptoms, such as stem rot and blighted shoots, are identified as characteristics (Pasin et al., 2009). Fruit affected by anthracnose exhibit symptoms of small, water-drenched, and depressed with margins that are light brown. Lesions expand into necrosis in the advanced stages of infection. (Pujari et al., 2013; Robert et al., 2001).

4. Phytophthora fruit rot -

P. palmivora and *P. nicotianae var. nicotianae* are responsible for fruit rot in pomegranate, with greater severity in nursery plants where initial symptoms are lesions on stems and foliage leading to damping off symptoms. Lesions on leaves become necrotic, eventually covering the entire leaf surface, while brown fruit rot occurs from July to August and results in fruit drop at a rate of 15-20% on fruits. The progression of the disease is facilitated by precipitation and a temperature range of 20-22^{o}C (Sushma, 2006).

5. Powdery Mildew-

In 1964, powdery mildew infection on pomegranate was initially documented in Azerbaijan. The specific causal agent responsible for this occurrence was subsequently identified as *Erysiphe punicae* (Braun and Cook, 2012). Powdery Mildew, a fungal disease that manifests as white powdery growth on pomegranate stems and leaves, can be detrimental to the affected plant parts. The disease can lead to leaf curling, premature leaf drop, and reduced fruit quality (Sharma, R.R., Kumar, A., and Singh, D. 2013). The premature foliage and floral appendages were enveloped in copious quantities of the compact, ivory fungal hyphae and reproductive spore accumulations that displayed a powdery aspect.

6. Bacterial blight disease-

Bacterial blight, a devastating disease that affects pomegranate crops and causes reduced yields in major pomegranate growing regions, is considered a dreaded disease. The prevalence of disease in pomegranate cultivation has increased significantly since 1998, leading to its recognition as a major constraint, which was first identified in Delhi (1952) by Hingorani and Mehta and later in Bangalore (Hingorani and Singh, 1960). Outside of India, South Africa has also reported bacterial blight occurrence (Peterson et al., 2010). Bacterial blight can cause losses of up to 80% during epidemic circumstances. The manifestation of the disease is first observed as diminutive lesions that appear water-soaked on the leaves. The lesions can progress into necrotic circular or irregular shapes and may be accompanied by a transparent halo, leading to chlorosis in infected leaves or fruit splitting in infected fruits, and can also cause cankers in stem or twig infections (Hingorani and Mehta, 1952).

Gram-negative bacteria- *Xanthomonas axonopodis pv punicae* induces bacterial blight (Hingorani and Singh, 1959), characterized by a single polar flagellum, measuring between 0.4-0.75 x 1.0-3.0 μm and is aerobic and non-spore-forming.

The pathogen's ability to survive in buds, infected stems, and plant debris for up to 7 months (Sharma, 2010), and its transmission through multiple modes including wind, rain, insects, and human contact with farm workers, make it a significant agricultural concern (Benagi and Ravikumar, 2011). The bacterium infects plants by entering through stomata, hydathodes, wounds, and lenticels with symptoms developing within 15 days influenced by weather conditions. Blight prevalence is year-round in infected orchards, with severity increasing during summer, and rainy

months due to moderate temperatures, high relative humidity, and rainfall. The intensity of blight and level of rainfall and relative humidity have a significant positive association (Sharma, 2010; Sharma et al., 2011).

The implementation of comprehensive management strategies for blight has resulted in successful blight management in recent times (Sharma, 2011)

1. New orchards are planted with disease-free and healthy saplings either from Tissue culture or certified nurseries free from disease.
2. In areas affected by blight, rainy-season crops (Mrig bahar, June-July flowering) are avoided while autumn crops (Hastha bahar, October flowering) are regulated.
3. Various rigorous hygienic practices, including disposing of infected stems and fruits on the ground, trimming dead and infected stems/twigs, and promptly treating plants with copper oxychloride (0.2%)/Bordeaux mixture (1.0%), are implemented.
4. To decrease the growth of inoculum, the orchard undergoes a 3-4 month rest period.
5. The application of 20kg/ha of bleaching powder is conducted biannually on the orchard, with particular emphasis on the sub-canopy region.
6. Chemical sprays containing Streptocycline (500ppm) and Bactronol (500ppm) with copper oxychloride (0.2%) or carbendazim (0.1%) fungicides are applied every 15 days or 7-10 day intervals during the rainy season.

Bacteriophage

Biology of Bacteriophage

Phages are small genomes, typically ranging in size between 5 -50 kilobase pairs (kbp), and can be comprised of ssRNA, dsRNA, ssDNA, or dsDNA, enclosed in a protein shell, and occasionally containing a lipid envelope. The morphological variations of bacteriophages are diverse, although a majority of them exhibit the characteristics depicted in Figure 1. All phages, except for filamentous ones, possess a polyhedral capsid with an icosahedral protein shell that houses the phage genome, along with a helical protein tail that is necessary for penetrating the bacteria and injecting the genetic material into the recipient host. The presence of a contractile structure in the tail of a phage is not ubiquitous and alternate attachment mechanisms are utilized in some cases (Ackermann, 2004). Phages thrive wherever bacteria grow, with their size being approximate 1/40th that of most bacteria. Phages, being a type of virus, are limited to replication within their host, and can be classified into two main types: virulent and temperate. Virulent (Lytic) phages are a favorable choice for therapeutic purposes as they can cause the process of bacterial cell lysis occurs without the incorporation of genetic material into the recipient host's DNA, whereas temperate phages cannot replicate outside their host. The ICTV classifies phages based on their nucleic acid type and morphology into distinct groups (Ackermann, 2003).

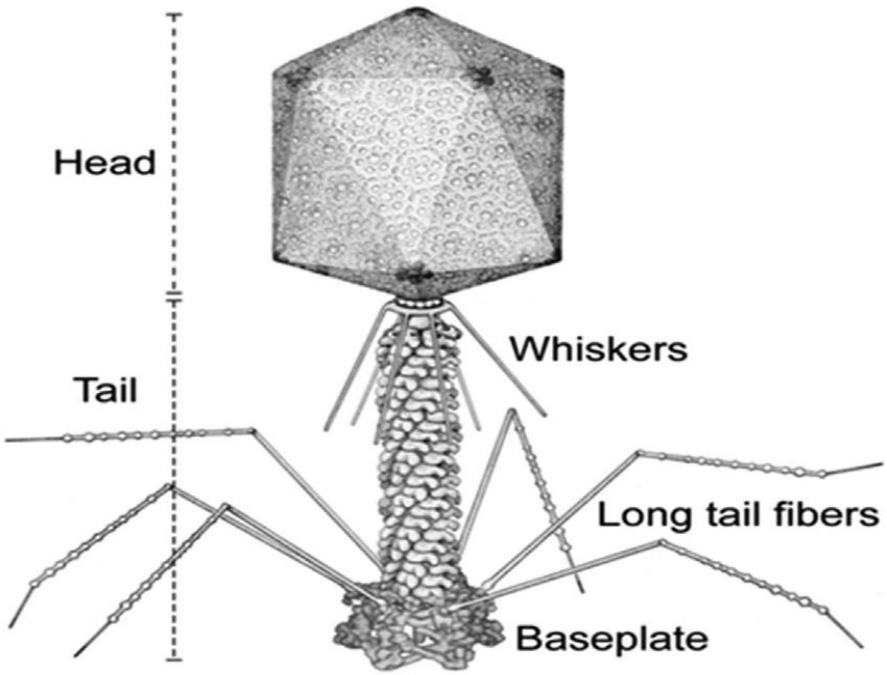

Figure 1: Structure of a typical-tailed bacteriophage
Source: (Sadeeq Ur Rahman, 2019)

The life cycle of Bacteriophage

Bacteriophages demonstrate selectivity towards various bacterial receptors and can undergo either the lytic or lysogenic cycle, as documented by Skurnik and Strauch (2006) and Sandeep (2006). In phage therapy, lytic cycle replication is employed, whereby the bacteriophage adheres to a bacterial cell to a specific receptor site and releases its DNA or RNA into the host using the phage lysozyme being discharged through its tail. A section of the cellular wall is degraded by the phage lysozyme, which results in the tail sheath contracting and propelling itself through the cell wall and plasma membrane, ultimately leading to the release of the nucleic acid. The capsid, nonetheless, persists extracellularly, whilst the process of viral nucleic acid biosynthesis commences. The viral DNA or RNA commandeers the cell by replicating with the enzymes and host nucleotides, the host DNA degrades, and protein synthesis stops. The bacteriophage then attaches to the cellular machinery to produce viral proteins, which transcribe viral RNA, instead of bacterial DNA. Upon the assembly of phage DNA/RNA and capsids, the cell synthesizes lysozyme to facilitate the breakdown of the cell wall. This process leads to the release of new viruses and the eventual death of the bacterial cell. It has been established by Thomson et al. (2004) that a single bacterial cell may release over 100 virus particles, each possessing the ability to infect a new bacterial cell.

Temperate viruses are classified as viruses that can replicate within a host cell without inducing deleterious effects on the host organism. The replication of viruses often involves both the lysogenic and lytic cycles, with the former involving the integration of phage and bacterial DNA to create a stable prophage. Upon activation of the prophage, the bacterial chromosome is detached and the lytic cycle is initiated as a result of host constraint recognition. During the final stages of reproduction, factors induced by phages assist in the breakdown of the bacterial peptidoglycan, leading to the liberation of the offspring (Wang et al., 2000).

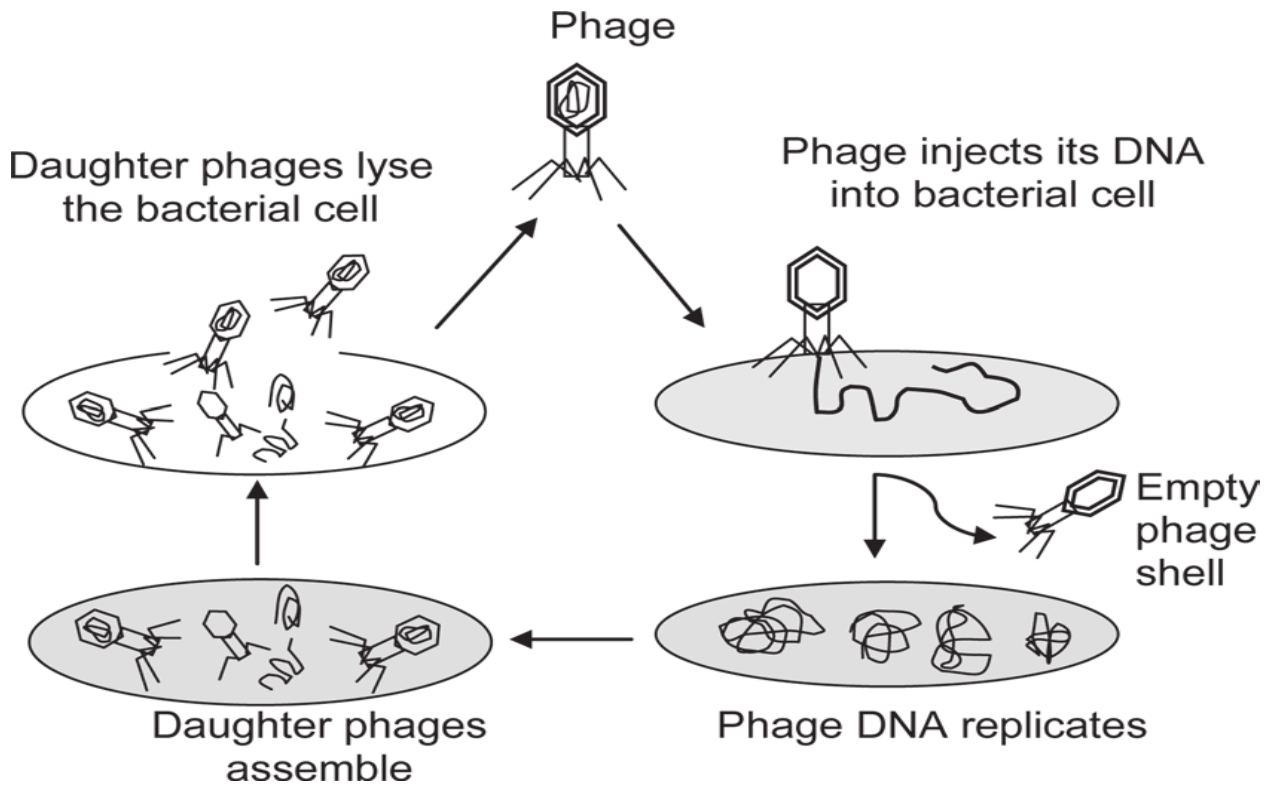

Figure 2: Typical lytic cycle of a bacteriophage
Source: (Steve Ricke, 2009)

Phage therapy

Phage therapy shows potential as a solution for antibiotic-resistant bacterial infections due to the targeted nature of phages. Phages are omnipresent, with a wide variety of habitats ranging from soil to the human gut, representing a diverse array of unique forms.

Bacteriophages, being intracellular parasites of bacteria, are obligate. In terms of abundance, they surpass bacteria by tenfold. This massive abundance remains largely unexploited, hosting a plethora of genetic diversity (Breitbart et al. 2002).

Unlike numerous antibiotics, which possess the capacity to destroy harmful bacteria whilst simultaneously causing the decimation of microbiota, consequently initiating a new set of issues, each phage has undergone evolutionary processes, enabling them to target bacterial strains or species more specifically. The specificity of phage therapy makes it an appealing choice for treating infections.

The efficacy of bacteriophages in biocontrol is influenced by environmental factors in addition to the susceptibility of the targeted bacteria. Within the phyllosphere, various factors, such as extreme temperatures, pH, desiccation, and exposure to UV radiation from sunlight may cause phage inactivation or detachment from leaves due to irrigation or rain (Suttle and Chen, 1992; Svircev et al., 2010). Furthermore, it has been suggested by Iriarte et al. (2007) that the presence of phages can be diminished by certain pesticides like copper compounds. Numerous techniques have been assessed to amplify the viability of phages on foliage, involving the implementation of safeguarding formulations, (Obradovic et al., 2004; Iriarte et al., 2007; Balogh et al., 2003), application during the early morning or evening (Flaherty et al., 2000), and the propagation of phages in the surrounding environment can be achieved through the use of carrier bacteria, according to Svircev et al. (2006). While research conducted *in vitro* has revealed that copper ions possess the ability to neutralize phages (Balogh et al., 2004), the utilization of copper-based pesticides must be implemented at a minimum of four days ahead of phage application in the field to prevent unfavorable outcomes (Balogh et al., 2008). This data implies that there is potential for the effective combination of bacteriophages and copper compounds to manage diseases.

The utilization of bacteriophages as biocontrol agents present a promising option for managing phytopathogenic infections. This has been demonstrated through various studies conducted by Adriaenssens et al. (2012), Buttimer et al. (2017), and Jones et al. (2007). Phages demonstrate exceptional selectivity towards their bacterial hosts, exclusively lysing bacterial cells, thereby offering a focused strategy for disease management.

Jones et al. (2007, 2012) have established that solely lytic bacteriophages possess applicability in bacteriophage therapy and biocontrol. Nonetheless, their inexpensive and effortless production makes them a commercially appealing option, as critiqued by Jones et al. (2007). The ability of a phage to infect a host is dependent on the presence of its receptor on the bacterial cell surface. This has consequently established itself as a critical factor in the phage typing techniques employed for the classification of bacteria as per Pitt and Gaston's 1995 study.

History of Phage Therapy

Bacteriophages, a type of virus, that target bacterial cells, can replicate solely within susceptible host bacteria, ultimately resulting in bacterial cell lysis. An elevation in phage particles in a suspension serves as proof of the existence of host bacteria in the suspension. Katznelson (1951) and Sutton (1953) and by Katznelson, Sutton, and Bayley (1954) developed a sensitive technique, called the rapid plaque count method, to detect seed-borne bacterial plant pathogens by utilizing the susceptibility of specific phages to the lysis of phytopathogenic bacteria. The plant materials were subjected to phage incubation, and the detection of increased phage particles served as an indicator of bacterial hosts within the plant material. Moreover, susceptibility to specific phages has been employed by Thomas and Thornberry et al., 1947 in the identification of certain phytopathogenic bacteria.

Certain phages exhibit obligate lytic behavior, as they are virulent and can cause the death of the infected bacterium. Phages are classified as temperate and experience a prophage cycle, during which their genome is replicated in sync with the bacterial reproduction, and then reactivated at a later point. Ackermann (2001) has classified phages according to molecular features, including replication strategies and type of RNA or DNA.

Phage therapy for the management of pomegranate bacterial blight

Experimental bacteriophages are commonly procured from environmental reservoirs that harbor their bacterial hosts and are subjected to minimal optimization procedures before their application. Nevertheless, for these bacteriophages to be effectively employed, they must undergo comprehensive selection and characterization protocols (Carlson, 2005). The typical method for propagating phages involves growing host bacteria to a significant cell density, after which the culture is infected to enable rapid phage propagation. Subsequently, purification procedures are employed to eliminate host bacteria along with bacterial remains which increases the Plaque-Forming Unit (PFU) of phage preparation. Once the purified phage preparations have been verified for sterility and stability, they are transformed into their end products.

It is vital to protect bacteriophages from a range of external factors that could compromise their effectiveness in the field to ensure their optimal deployment as a biological control measure. Given their proteinic composition, phages are vulnerable to harsh conditions such as desiccation, heavy precipitation, pH, plant-synthesized chemicals, temperature, and UV exposure. Phages exhibit reduced efficacy when utilized on airborne surfaces of plants, as evidenced by various studies (Civerolo and Keil, 1969; McNeil et al., 2001; Goodridge, 2004; Frampton et al., 2012; Buttimer et al., 2017). Phages can be shielded from environmental factors via formulations, which can lead to increased efficiency and stability (Balogh et al., 2002). Various compounds have been documented in the literature for formulation preparation, including antioxidants, aromatic/heterocyclic amino acids, activated charcoal, alkaline gluten-based materials, casein-based materials, congo red, cascrete, dried powders of lactose, trehalose, and dextran, light adsorbing or reflecting dyes, lignin-based materials, as well as N-propyl-gallate, oxidative enzymes, pregelatinized corn flour, starch, and flour-based materials, sucrose, skim milk powder (Balogh et al., 2002, 2003; Behle et al., 1996, 1997, 2003; Ignoffo and Garcia, 1994, 1995; McGuire et al., 1990, 1994; Tamez-Guerra et al., 2000; Tewfike and Desoky, 2015; Vandenheuvel et al., 2013). Selection of a suitable stabilizing material for phages is vital to ensure that the phage is stabilized and the pathogen is not stimulated. Jones et al., 2012 demonstrated that biologically inert materials can enhance phage treatment effectiveness without promoting disease progression.

Jagdale et al. (2019) conducted a study characterizing lytic phages, namely PRϕL2 and SSϕL8. The phages, PRϕL2 and SSϕL8, belonging to various families, were employed against *Pseudomonas sp.*, which causes bacterial blight disease of pomegranate, as per prior research. The lytic activity of the phages was targeted specifically towards various pathogenic strains of *Pseudomonas sp.* and showed stability between a broad extent of the temperature range (4^{o} -50^{o} C) and pH (4-9).

Further, the study revealed that the storage life of liquid preparation, including carboxymethyl cellulose, molasses, phage broth, soluble starch, and whitening clay, showed 100% stability for phage PRϕL2 storage at 4^{o} C. There was no noteworthy variance in phage titre until the third month, but all liquid preparation exhibited a considerable contrast from fourth to the sixth month. The molasses formulation with a concentration of 0.5% demonstrated a significant reduction in Plaque forming unit over a single log cycle, whereas the remaining formulations experienced only a slight decrease. Significant phage titre difference was observed in formulations at 4^{o}C for phage SSϕL8, with stability in the first month and a considerable decline in the sixth and fourth months. The stability of active phage titre in both PRϕL2 and SSϕL8 was maintained throughout a six-month storage period for the whitening clay (0.5%) liquid preparation, as demonstrated by Jagdale et al. (2021).

The Plaque forming unit in liquid preparations of phage PRϕL2 remained stable at 30^{o}C except for those containing 0.5% molasses, but after five to six months, all formulations excluding the whitening clay at a concentration of 0.5% demonstrated a substantial reduction in phage titre. There were no noteworthy variations found in carboxymethylcellulose, soluble starch, or phage broth, and after six months, there was no considerable contrast observed in the phage titre of liquid preparations of phage SSϕL8. There was no variation in phage titre between soluble starch and whitening clay in first month, but all preparations showed a disparity in phage titre from the second to fourth month. The addition of 0.5% molasses led to a reduction in phage titre, while the inclusion of whitening clay (0.5%) in the liquid preparation resulted in consistent phage titre stability over a six-month storage period. The formulation of PRϕL2 and SSϕL8 consists of a liquid containing 0.5% whitening clay proved to be notably different from other formulations in its ability to maintain the vital phage titre (Jagdale et al., 2021).

The investigation examined the longevity of phages in a solution consisting of whitening clay (0.5%) on the epidermal surfaces of pomegranate sapling, revealing that phage titre remained stable on upper, lower, and middle leaves for up to 3 days post-spraying before decreasing by single log cycle by 18th day. The perseverance of phages on upper and lower stems was stable initially but decreased by a single log cycle at the end of the experiment. Phage titre on various plant parts experienced a slight decrease after spraying, while the persistence of certain phages in a whitening clay liquid formulation was significant and experienced a slight decrease in lower stems.

The application of phages PRϕL2 and SSϕL8 in a liquid preparation containing 0.5% whitening clay significantly reduced blight disease by 66.7% and 67.8%, respectively; whereas Streptocycline exhibited a much lower decrease, and variation was not seen between phage treatments.

The utilization of phages PRϕL2 and SSϕL8 in a liquid preparation of whitening clay (0.5%) for therapeutic purposes significantly reduced blight disease. In the application of phages PRϕL2 and SSϕL8 in a liquid preparation of whitening clay (0.5%) for effectively managing bacterial blight infection in pomegranate, insignificant differences were observed among phage treatments. Implementation of these phages as a preventive measure demonstrated superior efficacy in reducing disease in the pot assay, as opposed to a therapeutic approach (Jagdale et al., 2021)

The research examines the durability of phages PRϕL2 and SSϕL8 in different liquid preparations at varying temperatures, with the whitening clay of concentration 0.5% in the liquid formula being identified as a protective agent that preserves a high active titre. The formulation persisted on pomegranate plantlets and reduced bacterial blight disease severity.

In 2022, Karn et al., screened fifteen soil samples and isolated two bacteriophages viz., ϕS13 and ϕS14. The efficacy of phages against *Xanthomonas axonopodis pv. punicae* (Xap) was assessed using the spot assay method to determine their titre and cross-infectivity. Both phages exhibited lytic activity as indicated by the host range analysis specifically targeted towards the pathogenic *Xanthomonas axonopodis pv. punicae* whereas, both phages were unable to lyse *Xanthomonas campestris pv. campestris.* In the experiment measuring the impact of phages ϕS13, ϕS14, and a combination of both on bacterial growth, a decline in growth rate was noted, in comparison to the control group after 2 and 4 hours at three varying dilutions. Furthermore, the bacterial growth was completely prevented when the culture was kept overnight at 30°C and diluted to 10^{-4} concentration. The study reveals that the phages ϕS13 and ϕS14 belong to *Myoviridae* and *Siphoviridae* families, respectively.

The effectiveness of phages φS13 and φS14, both individually and in conjunction, was evaluated in conjunction with Xap culture under pot conditions, and the disease gravity was noted twice monthly. The usage of phages along with pathogen suspension effectively decreased the impact of bacterial blight in plants in comparison to phage treatment alone. The most effective control of disease, with a maximum percentage of 75.04%, was noted in saplings treated with a combination of ϕS13, ϕS14, and Xap, followed by treatment with ϕS13 and Xap; ϕS14, and Xap, resulting in disease control of 68.18% and 69.17% respectively.

The study examined the effect on growth parameters and found that ϕS13 + ϕS14 + Xap-95 treatment significantly increased plant height and shoot length in seedlings compared to the control. The treatment showed significant increases in comparison to pathogen along with sterile water spray control, with the amalgamation of φS13 + φS14 + Xap-95 exhibiting the highest leaf surface and longest root length. Compared to other treatments, ϕS13 + ϕS14 + Xap-95 significantly increased seedlings' root weight and dry root weight, resulting in a maximum fresh root weight of 255.44 g (Karn, 2022).

The findings of this research suggest that bacteriophages have a positive impact on survival, analyzing the growth patterns of pomegranate saplings cultivated in pots, and disease management contributing to the creation of strong plantations for sustainable output.

Limitations and Challenges

The specificity of bacteriophages probably contributes to the limited range of their cleavage spectrum. These phages tend to exert their effects on certain bacterial genera, and in some cases, a limited number of species, thereby rendering them incapable of targeting pathogenic variants belonging to a particular species (Hyman, 2010). Even though bacteriophages have the potential for treating mono-bacterial diseases, the prevalence of infections caused by diverse pathogenic bacteria is higher. Consequently, achieving the desired therapeutic effect can prove challenging for specific bacteriophages (Gill, 2010).

Conclusion

Bacteriophages have been identified as a feasible approach for eradicating pathogenic bacteria in their early stages. However, the implementation of phages in therapy was postponed due to the effective and widespread utilization of chemical bactericides in managing bacterial infections and the challenges surrounding the attainment of purified phage preparations. The effectiveness of bactericides in treating bacterial infections has been overshadowed by the significant increase in antibiotic-resistant bacteria. Due to the rise of antibiotic-resistant bacteria, global efforts have been undertaken to develop innovative alternative antibacterial agents, among which bacteriophages show great promise.

Nevertheless, additional research endeavors are warranted to examine the prospect of its utilization in field settings and integrated disease management schemes, in conjunction with other biocontrol agents. Additional investigations are necessary to comprehend the liquid formulations' appropriate amounts for field usage, which will encourage a safe and environmentally sound setting.

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